Casuarina Improvement for Securing Rural Livelihoods

Proceedings of the Fifth International Casuarina Workshop

Chennai, India, 03 – 07 February 2014

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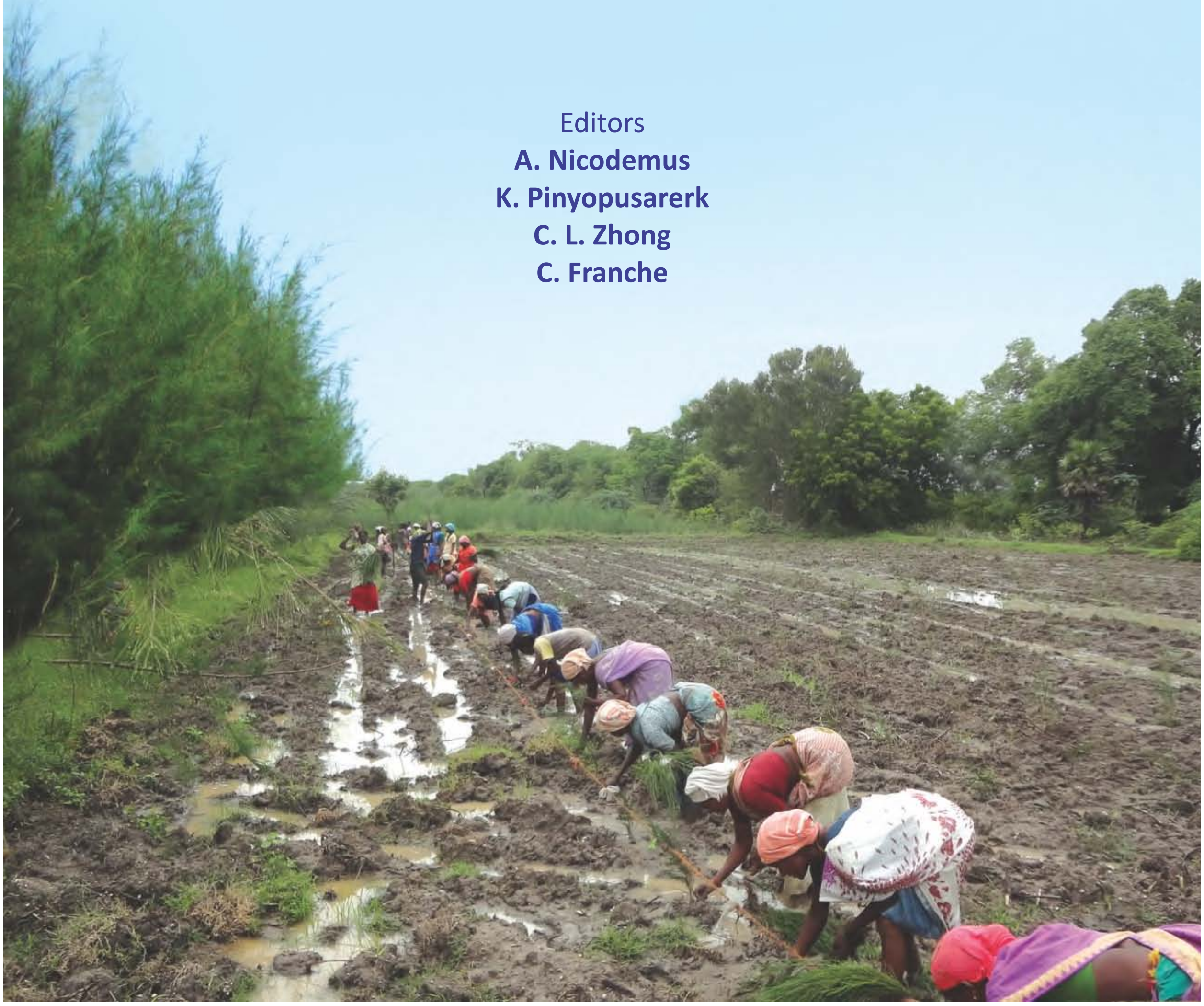

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IUFRO Working Party 2.08.02 Improvement and Culture of Nitrogen-fixing Trees

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FOREWORD

The versatile and truly multipurpose Casuarina trees have been helping people by providing industrial, livelihood and environmental products and services. They have been widely introduced outside their natural distribution range in the tropics and have become indigenized and well integrated into the local farming systems. More than 150 years of cultivation in diverse climatic and soil conditions helped in perfecting the cultivation techniques and identifying site and end use appropriate taxa. The existence of large variation in terms of adaptability, growth attributes and resilience to pest attack across species and provenances offers guaranteed selection for almost any given planting environment and utility. This variation proved to be useful to expand the cultivation from the traditional coastal areas to areas away from the coast including land affected by salt, mining and water-logging. Fortunately an active working group exists for research and development of Casuarina for over 30 years since the first Casuarina Workshop was held in Canberra during 1981. This group is also effectively aligned with the Working Party on Improvement and Culture of Nitrogen-fixing Trees of the International Union of Forestry Research Organizations.

The Fifth International Casuarina Workshop brought together nearly one hundred delegates involved in various aspects of Casuarina research and improvement for discussing the latest developments in their respective areas of research. This workshop was organized by the Institute of Forest Genetics and Tree Breeding and I am glad that IFGTB is also bringing out the proceedings of the Workshop. The articles included in this publication document the current knowledge on Casuarina and provide insights to carry forward its improvement for the socio-economic benefit of farmers and industries and agencies involved in environmental protection. I hope this publication will be widely used by all stakeholders and help in sustaining the Casuarina working group's contribution for securing rural livelihoods in the future.

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Preface

Over two million hectares of Casuarina plantations raised throughout the tropics and sub-tropics provide several socio-economic, environmental and ecological services. Their fast growth, adaptability to a range of edaphic and climatic conditions, multiple end uses and the symbiotic nitrogen fixing ability make them a highly preferred group of trees for farmers. Casuarinas are known to protect human habitats and agricultural fields through shelterbelts and windbreaks, help in reclaiming degraded sites and meet industrial raw material requirements for papermaking, plywood making and biomass-based energy generation. Nursery development, cultivation and harvesting of Casuarina generate livelihood opportunities for farmers and agriculture-dependent labour force in rural areas.

Following previous four successful international Casuarina meetings in Canberra (1981), Cairo (1990), Da Nang (1996) and Haikou (2010), the fifth meeting was held in Chennai, Republic of India during 3-7 February 2014 and the theme was *Casuarina Improvement for securing rural livelihoods*. The objectives were to bring together researchers and others interested in development of Casuarinas, and to update and compile the knowledge on this important group of species so that the results could be effectively applied to improve livelihood opportunities in rural areas leading to enhanced wood production and farm income.

A total of 82 participants from 10 countries attended this meeting and presented 80 papers categorized into the following sessions: genetics and breeding; silviculture, agroforestry, pest and diseases; nitrogen fixation and biotechnology; and industrial application, environmental services and policy issues. Outputs from this meeting are presented in this proceedings. A few research papers not presented are also included. The contents and views expressed in the papers are those of the authors. Editing of the original papers has been made to maintain the uniformity of the proceedings.

We thank all sponsors of the Workshop and those involved in organizing the Workhop and publication of this proceedings. We hope this publication will be useful to all people involved in Casuarina cultivation and improvement and also help in guiding future research and development activities for securing rural livelihoods.

A. Nicodemus
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CONTENTS

Tree Improvement and Biotechnology

Summary of Discussions and Recommendations

Following the presentations, the participants formed four broad groups and discussed the recent developments in the respective disciplines, to identify the gaps in existing knowledge and to draw recommendations for future research and development in Casuarina. The following are the four groups:

1. Silviculture, Agroforestry, Pest and Diseases
2. Nitrogen Fixation and Biotechnology
3. Genetics and Breeding
4. Industrial Application, Environmental Services and Policy Issues

The following are the major recommendations drawn from the discussions held by the different groups.

Group 1 Silviculture, Agroforestry, Pest and Diseases

1.1 Silviculture and Agroforestry

- Develop nursery techniques for different kinds of planting stock like seedlings and clones. Evaluate their field performance in terms of early establishment and productivity under different site conditions.
- Develop methods to improve nursery hygiene for pest and disease management. Standardize methods to introduce *Frankia* and mycorrhiza under nursery conditions to improve seedling quality and reduce susceptibility to diseases.
- Optimize seed use efficiency and planting density and cost-effectiveness of planting materials to keep the overall cultivation costs low. Standardize planting density for different rotation periods, end uses and planting sites and cultural regimes. Study the effects of spacing adopted on productivity and wood quality.
- Develop clone-specific silviculture practices to realize the potential of clone and planting site. Evolve silvicultural practices for *C. junghuhniana* particularly for cultivating it as a coppice crop.
- Develop suitable agroforestry models under different site conditions and in combination with different agricultural / horticultural crops. Develop ideotypes with narrow crown, upright branches and desirable root system suitable for integrating in agroforestry models.

1.2 Pests and Diseases

- Study interaction between different cultural practices and pest and disease incidence.
- Conduct detailed studies including molecular aspects on the major threats to cultivation, blister bark disease caused by *Trichosporium*, bacterial wilt by *Ralstonia solanacearum* and infestation of bark-eating caterpillar, *Indarbela quadrinotata*.
- Monitor incidence of insect and disease damage under changing climatic conditions and study changes in pest behaviour and host-plant interactions.
- Characterize available germplasm pool for resistance / tolerance to major insect pests and diseases and other characters of economic importance. Develop new varieties with pest resistance through breeding for resistance involving selected accessions.

- Detailed study on seed pest of Casuarina and develop methods to prevent damage in plantations and storage conditions.

Group 2 Nitrogen Fixation and Biotechnology

2.1 Studies on Frankia

- Study the biodiversity of *Frankia* strains.
- Study their contribution to improve abiotic stress tolerance of the host plant.
- Make a data base available with the following information on the actinomycete:
  - Host specificity
  - Effectiveness of nitrogen fixation
  - Molecular characterization
  - Environmental adaptation (salt, soil, acidity and alkalinity and heavy metal)
  - Studies on *Frankia* signalling molecules and their capacity to optimize root system architecture and functioning
  - Seed coating with *Frankia* signalling molecules to enhance plant growth under stress and mineral nutrient-limiting conditions.

2.2 Studies on mycorrhizal fungi and Plant Growth Promoting Rhizobacteria (PGPR)

- Biodiversity studies and molecular tools for quantification.
- Make a data base with the characterization of fungi and PGPR.
- Bacteria-host specificity studies.
- Identification of the best microbial combinations (*Frankia*-mycorrhiza-PGPR) for optimal tree growth and protection against abiotic stress.
- Promote locally adapted inoculum.

2.3 Metagenomic

- Use metagenomic approach to provide a broad view of the microorganisms associated with different *Casuarina* species and adapted to different environments.

2.4 Characterization of valuable traits

Studies on vegetative propagation traits, wood properties, nodulation ability, disease and pest resistance (*Trichosporium*, *Ralstonia, Indarbela*) and abiotic stress tolerance (salinity and heavy metal).

- Develop high-throughput phenomic platforms.
- Develop transgenic tools for functional analyses of valuable candidate genes.
- Develop new genome editing approaches (CRISP/Cas9) to obtain targeted gene mutations, knock-out mutants or gene replacement.

2.5 Sequence genomes of Casuarina species

- Choose with the scientific community the most valuable species among *C. glauca, C. cunninghamiana* and *C. equisetifolia.*
- Develop RNA deep sequencing on contrasted phenotypes (plus trees, salt tolerance, cold tolerance, gender determination).
- Provide an open access web data base for genomic resources.
- Form an international Casuarina consortium to share the data.

2.6 Develop molecular breeding approaches

- Use new breeding approaches such as genome-wide association studies (GWAS), genomic selection (GS) and marker-assisted recurrent selection (MARS) for developing superior trees.

Group 3 Genetics and Breeding

- Strengthen the ongoing breeding programs in India and China by adding infusion populations. The infusion can happen through collection from its native range and/or through seed exchange programmes between the countries implementing breeding programmes.
- Reset breeding objectives in advance generation breeding programmes to focus on wood quality for different end uses. Identify the desirable wood properties for preventing damage by wood-boring insects and pathogens. Study the relationship between bark and root characters and drought tolerance to make selections for planting in dry areas.
- Produce inter-provenance and inter-specific hybrids involving *C. equisetifolia, C. junghuhniana, C. cunninghamiana* and possibly *C. cristata* for improving the yield and also to face new challenges like salinity, new and early pest attack and climate change.
- The germplasm base for *C. cunninghamiana* and *C. cristata* needs to be widened particularly with introductions of provenances from the northernmost distribution range of these species.
- Evolve control pollination techniques for the important taxa by understanding the reproductive biology especially for species other than *C. equisetifolia*.
- Develop end-use specific hybrid clones to meet the requirement of various stakeholders and cost-effective vegetative propagation strategies.
- Create adequate seed production systems to meet the huge demand for quality seeds through strategies like community seed orchards and industry-owned orchards to increase productivity of plantations. Improve the accessibility and affordability of smallholding farmers to genetically improved planting material.

Group 4 Industrial Application, Environmental Services and Policy Issues

4.1 Industrial Requirements

- Evolve methods for sustaining high productivity without depleting soil nutrient status to overcome shortage of land area for cultivation.
- Improve wood quality to suit industrial requirements particularly pulp yield and other papermaking characters. Research on new uses for Casuarina wood and other plant parts like plywood, biomass for energy generation, crafts, and other wood products.

- Develop silvicultural practices suitable for mechanized harvesting of Casuarina plantations.
- Improve wood quality (e.g. low lignin) to reduce pollution load during conversion into products.

4.2 Environmental Services

- Develop methods to improve the efficiency of Casuarina in shelterbelts, windbreaks, reclamation of salt-affected and mined areas. Improve the use of Casuarina to protect vulnerable land conditions and human habitations.
- Develop / match suitable planting material for protecting human habitations and agricultural crops from natural disasters like Tsunami and cyclones.
- Study the impact of Casuarina on other flora and fauna diversity where it is planted as an exotic.
- Study the effectiveness of Casuarina plantations in mitigating climate change. Evaluate the carbon sequestration potential of different *Casuarina* species and provenances.

4.3 Policy

- Facilitate free exchange of genetic materials across nations and organizations involved in research and development through the Casuarina Network.
- Share expertise across Governments, Industry, Planters and Environmental stakeholders through the Casuarina Network. Forming cooperatives with industries and research organizations for optimal use of resources to achieve common goals.
- Conduct socio-economic studies to quantify the direct and indirect benefits of Casuarina to different stakeholders especially smallholding farmers so that policy makers are convinced for increased investment in Casuarina improvement.

Casuarina Improvement for Securing Rural Livelihoods in India

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Abstract

Casuarina is essentially a farmers' crop in India meeting several industrial, environmental and livelihood requirements both directly and indirectly. It has been well-integrated in the farmlands, shelterbelts, and windbreaks providing fuel, poles and pulpwood apart from protecting human habitations, agricultural crops and enriching soil through nitrogen fixation. The multiple end uses of Casuarina provide a stable market for the farmers who depend on tree crops for sustenance while facing problems with agricultural crops. All activities connected with Casuarina cultivation also provide employment opportunities to landless labourers in the rural areas. The direct interactions between farmers, industries and research organizations facilitated tree improvement activities leading to substantial increase in wood production from plantations. Genetically improved seeds from seed orchards and fast-growing clones tested in diverse locations have been deployed in plantations leading to increased farm income to tree farmers and raw material availability to industries. Decentralized seed production systems like community seed orchards involving stakeholders were started to improve the accessibility and affordability of superior planting material. Future research and development on Casuarina improvement needs to be focussed on expanding its cultivation in dry areas away from the coast where large areas remain either fallow or of low productivity. Infusion of new germplasm from the natural distribution range particularly for species other than *Casuarina equisetifolia* is essential to meet the present and future challenges in meeting livelihood needs. A strong international cooperation will facilitate exchange of planting material and sharing of results from improvement programmes for efficient utilization of available resources.

1 Introduction

Casuarina's potential as a truly multipurpose species is utilized for many industrial, environmental and livelihood requirements in India. The first introduction of *Casuarina equisetifolia* in Karwar during 1868 itself was to fulfil wide-ranging needs like fuel wood, preventing sand movement in the coast and enhance aesthetics through beach and avenue planting (Kaikini, 1937). Since then this versatile tree has had a remarkable journey of nearly 150 years evolving into a dependable crop for wood production, environmental services and livelihood improvement. India is the largest Casuarina-growing country in the world with an estimated half a million hectares of plantations mainly concentrated in the States of Andhra Pradesh, Odisha, Tamil Nadu and Union Territory of Puducherry in the east coast. It is also one of the five most extensively planted tree crops in the country and is harvested in short rotations of two to five years.

The last 25 years have seen substantial changes in the cultivation, genetic improvement, end uses and services of Casuarina in India. The value of the tree crop is significantly increased when paper industries started using Casuarina as a pulp wood raw material. The lucrative prices offered for the wood has motivated the farmers to adopt new cultivation techniques and use of fast-growing planting material. This value addition also prompted research institutions to begin systematic tree improvement programmes to achieve increased wood production and help farmers and industries meeting their needs. At the international level too, the socio-economic and environmental importance of Casuarina was realized and the international Casuarina Workshops were begun to be conducted. Based on the recommendations that emerged from the first two Workshops (Midgley et al., 1983; El-Lakany et al., 1991), an international series of provenance testing programme was successfully coordinated by the CSIRO Australian Tree Seed Centre immensely benefitting many countries particularly India (Pinyopusarerk et al., 1996; 2004). Results from the testing conducted in India paved way for initiating a systematic long-term breeding programme in the country (Pinyopusarerk, 1996). All these activities contributed to realizing the potential of Casuarina as well as the land area where it is cultivated leading to positive changes in the livelihoods of people associated with this crop. This paper discusses the livelihood benefits of Casuarina in India in the context of its industrial and environmental uses.

2 Historical Patterns of Cultivation and End Uses

2.1 Trends in cultivation methods

During the first half a century after its introduction in India, Casuarina was predominantly planted by the Government agencies adopting long rotations of 15 to 30 years with one or two thinnings (Kaikini, 1937). When farmers started cultivating the tree, they found that it was amenable for high density planting with irrigation and fertilizer applications. Gradually the rotation period was reduced to a maximum of 5 years with high stocking (up to 10,000 stems per ha) so that cash flow to farmers is improved. Farmers' intervention also resulted in simple and cost-effective nursery, field planting, tending, pruning and harvesting practices making the overall cost of cultivation low. Intercropping with legumes like peanut not only provides interim income but also helps in weed management and improving soil fertility. With the availability of genetically improved planting material like seeds from seed orchards and clones from fast-growing provenances, farmers tend to adopt wider spacing and intensive cultural practices to optimize productivity.

2.2 Multiple end-uses

Historically, the two main reasons for Casuarina cultivation throughout the tropics are the excellent fuel wood qualities particularly for domestic use when modern fuels were not available and the tree's ability to grow in nutrient-poor sandy coasts withstanding salt spray from the sea. Till today Casuarina remains the most suitable species for these two requirements throughout the world. In India it continues to be a popular fire wood particularly in the rural areas for domestic cooking, baking bricks and charcoal making. Casuarina litter comprising the deciduous branchlets ('needles') and fruiting material ('cones') is still swept from plantations and used as fuel by the poor. Its poles provide a highly cost-effective construction material for rural housing and used as scaffolds for construction in urban areas. It finds uses ranging from making many agricultural implements like ploughs to oars and other accessories for fishing. Most of the plantations raised by the Government agencies are targeted to protect the coastal hamlets and agricultural fields from sand movement in the sea coast and to reduce damage caused by high-speed winds. The devastation caused by the Tsunami in 2006 and the cyclone Thane in 2011 further encouraged these agencies to strengthen the shelterbelt and windbreak planting along the coast. Casuarina is also an important pioneer species for reclaiming mine overburdens and salt-affected areas.

The industrial use of Casuarina wood for papermaking has made it a cash crop for the farmers, resulting not only in the expansion of area under cultivation but also brought a stable and organized market for the crop. Since the wood-based industries have to meet their wood requirement outside the forests, they undertake active farm forestry programmes to increase pulpwood availability. They also strive to make available high quality seedlings at subsidized price to farmers so that the plantation productivity is increased benefitting both the farmers and the industry. Industries also function as a bridge between research institutions and farmers for effective transfer of new varieties and technologies to the field. The recent emergence of biomass-based power generation as a Clean Development Mechanism is expected to broaden the industrial use of Casuarina wood and have positive impact on its cultivation.

2.3 Employment generation in rural areas

Cultivation of Casuarina provides round the year employ ment to agricultural labourers in the rural areas. Nursery development, land preparation, field planting, tending activities like irrigation, pruning and fertilizer application and harvesting of the final crop are still undertaken with skilled labours in India. The workers involved in such activities are dependent on the farming activities in the rural areas for their livelihoods. If the employment opportunities come down they tend to migrate to the cities in search for work. Farmers usually time the activities related to Casuarina cultivation when labour requirement for other agricultural crops is low. This arrangement benefits farmers through availability work force and provides employment to workers throughout the year.

3 Genetic Improvement of Casuarina

IFGTB has been implementing breeding programmes for *C. equisetifolia* and *C. junghuhniana* since 1995 (Pinyopusarerk, 1996). Large breeding populations were established using a broad genetic base of around 250 open-pollinated families drawn from more than 40 provenances and landraces of Africa, Asia and Australia-Pacific (Pinyopusarerk, 1996) They were assessed for growth and form traits at four to five years age and poor-performing families and inferior trees of others were thinned out and converted into seedling seed orchards (SSO). A subset of 100 outstanding male and female clones were selected from the breeding populations and deployed in multilocation clonal tests. The best 10% of these clones were selected for large scale commercial plantation. Clonal tests were converted into clonal seed orchards (CSO) by removing the inferior clones. Seeds from SSO and CSO are supplied to farmers, industries and forest departments to raise plantations in different States. On-farm genetic gain tests showed that the orchard seeds produced up to 28% more wood compared to the locally available seed sources. Clones of *C. equisetifolia* and *C. junghuhniana* have been hybridized through control pollination and inter-specific hybrid clones selected from the full-sib progeny tests. Outstanding inter-specific hybrid clones selected through multilocation testing are being deployed in commercial plantations.

3.1 Need for improved planting material

The demand for seed to raise new plantations in the coastal States is huge, estimated to be around two tonnes a year. The quantity of seed supplied from existing seed orchards is only a fraction of the demand from farmers and industries. Industries are encouraged to produce their own improved seeds to reduce the cost of seeds and also to get guaranteed seed supply every year. During the last 10 years many seed orchards have been established by the industries in Andhra Pradesh, Maharashtra, Odisha and Tamil Nadu States in collaboration with IFGTB. Some of these orchards have already started producing seeds for the farm forestry programmes of the respective industry. Other paper industries have also expressed their willingness to establish and manage their own orchards with technical support from IFGTB. Planting stock and orchard designs have also been made available to State Forest Departments of Andhra Pradesh and Gujarat for establishing seed orchards.

In general, only about 10% of farmers raise the seedlings themselves by obtaining seeds from known sources. Usually their landholding is large and possess the level of awareness to access institutions for various planting material needs. The remaining 90% depend on sources which supply seedlings for raising plantations. The following are the main sources from which farmers obtain their seedlings for plantation establishment:

(i) Traditional nursery hubs are the major sources of planting material to farmers, especially those with low buying capacity preferring to procure seedlings close to their farms to avoid transportation costs. These nurseries are managed by landless labourers on rented land. They are low input nurseries but the highly skilful nursery operators produce seedlings with high physical quality. Since the seeds come from unimproved sources they end up yielding sub-optimal returns from plantations.

(ii) Nurseries managed by industries either at the mill site or in places where tree cultivation is extensive to cater to the farmers' demand for seedlings next to traditional nurseries. Usually the cost of seedlings sold by industries is higher than that charged by local nurseries. Some industries provide subsidy in the cost of seedlings if the farmers enter into buy-back agreement. Industries produce some quantity of seeds from their own orchards and the rest is outsourced from IFGTB and forest departments.

(iii) Forest Departments and research and academic institutions like IFGTB and Tamil Nadu Agricultural University also supply a limited quantity of seedlings to farmers. Since institutions primarily raise seedlings for their own programmes, only surplus seedlings are made available to farmers covering only a small portion of the prevailing demand. Currently the Tamil Nadu Forest Department is implementing a programme called “Tree Cultivation in Private Lands” in which good quality seedlings are provided to farmers with a cash incentive to those who show good survival and growth one year after planting.

3.2 Community seed orchards

In India, the average landholding is less than one hectare per family. Rapid industrialization, declining returns from agriculture and division of landholding among family members further reduce the individual farm size. The current policy of Government is that wood requirements of industries have to be met from sources outside the natural forests. At the same time industries cannot own large areas of agricultural land. In such a situation, the only way industries can meet their raw material need is to encourage and support tree cultivation on farmlands. Many farmers, despite their small landholding, are willing to raise tree crops at least in a portion of their farm as an 'insurance' against the risks associated with agriculture. It is common to find paddy, sugarcane, banana and tree crops like Casuarina and Eucalyptus are grown together in alternating small blocks. It is really a challenge to reach any new planting material/ technique to these farms considering the diversity among the farmers in terms of literacy, economic status and willingness to adopt new practices. Increasing plantation productivity is possible only if the organizations involved in breeding, plantation / green cover development, industries and grass root government organizations join together and work with farmers.

Through research collaboration with CSIRO, Australia and the Tamil Nadu Forest Department, IFGTB has introduced a new concept of developing "Community Seed Orchards" through which farmers and self-help group members are encouraged to establish their own seed orchards in government / village land with inputs from IFGTB in the form of planting material and technical support. The cost of maintaining the orchards for two years is also taken care of by the Institute in addition to capacity building of farmers in orchard management. The seeds produced will be used by the farmers themselves and any surplus will be sold to others like wood-based industries with quality assurance from IFGTB. Model orchards have been established in Tamil Nadu and Puducherry using site-specific fast-growing clones developed through the ongoing breeding programme.

It is the long term goal of IFGTB to use community seed orchards and the genetic gain from their seed output as a means of enhancing livelihood opportunities for traditional nursery operators. At present, the traditional low-cost nurseries are operated by landless agricultural labourers who hail from socially deprived communities with minimal livelihood security. Since they are not trained in modern nursery techniques and lack the resources to provide additional inputs in their nursery operations the planting stock is priced low and the per capita income is meagre. IFGTB is working with this highly skilful group of nursery operators to impart training in latest nursery techniques like use of genetically superior seeds, replacing chemical fertilizers with biofertilizers, pest management and clonal propagation technology. One of the community seed orchards of Casuarina is established adjacent to the nurseries at Valluvamedu in the Union Territory of Puducherry. This model of decentralized seed production and enhancing genetic quality of planting stock from traditional nurseries leading to livelihood enhancement to landless agricultural labourers is being extended to other tree species and locations in Tamil Nadu and Puducherry.

4 Expanding Casuarina Cultivation to New Areas

Casuarina is traditionally a crop of coastal areas in South India which generally receive around 1000 mm annual rainfall. These areas also benefit from a high water table. More than 90% of Casuarina plantations are located from shelterbelt areas to about 50 km from the coast. The areas away from the coast, the 'inland' region, are characterized by low rainfall (about 700 mm) and a low water table. Apart from the areas served by river-basin irrigation systems, a major part of other areas lack adequate water sources to sustain agricultural crops. They are also less industrially developed compared to the coastal region and agriculture is the major occupation for people of this region. Agriculture is predominantly rain-dependent and is restricted to one major cereal crop and in some years an additional secondary crop like pulses and vegetables is also grown depending on the quantum and pattern of rainfall.

4.1 Casuarina cultivation in dry areas

Currently the cultivation of Casuarina as a sole crop is mostly restricted to the areas where irrigation source is available at least for a few months in a year. Although availability of irrigation water provide scope for growing a wide variety of agriculture crops, farmers opt for tree crops due to shortage of labour force and the unattractive and unstable prices obtained for agriculture crops. Eucalyptus is the predominantly planted tree crop in the dry areas. The recent incidence of gall wasp in Eucalyptus plantations has made farmers and industries to look for alternatives suitable for cultivation under rain-fed conditions. Nearly two decades of field testing and cultivation of *C. junghuhniana* since its first introduction during 1995 has shown that it is more drought-tolerant than *C. equisetifolia* particularly in the inland areas. Even in interspecific hybrid combinations, *C. junghuhniana* has performed better in inland conditions especially when it is deployed as the maternal parent (Nicodemus et al., 2011). It is also free from the blister bark disease which causes severe mortality of *C. equisetifolia* when grown under moisture-stress. Paper industries, currently facing acute shortage of pulpwood raw material are keen to expand cultivation of Casuarina in the interior dry areas and found *C. junghuhniana* eminently suitable for this purpose.

4.1 Casuarina improvement for dry areas

In the beginning, the same seed sources were used for planting in both coastal and inland areas resulting in highly variable growth of trees across different locations. Extensive field testing during the implementation of breeding programme showed a clear coast to inland trend for growth, stem form and wood properties (Varghese et al., 2001; Nicodemus et al., 2011). Growth of *C. equisetifolia* was faster- in coastal areas compared to inland region but the wood density showed the opposite trend. Growth and stem straightness of *C. junghuhniana* was found to be better in inland region compared to coastal region. These results indicate that a separate genetic improvement programme for cultivation of Casuarina in the inland area almost under rain-fed conditions is needed with *C. junghuhniana* as the main species. A revisit of results obtained from the international provenance testing of *C. equisetifolia* is likely to provide information on drought-tolerant provenance and landrace accessions. Growth of a limited number of *C. cristata* provenances was found to be comparable to that of local seed source of *C. equisetifolia* under rain-fed conditions with desirable pulping traits (Nicodemus et al., 2015). Except *C. equisetifolia,* no other species showed incidence of blister-bark disease so far. Fresh collections of these promising germplasm from the natural distribution range need to be obtained and subjected to multilocation testing along with the selections from earlier introductions. Expanding Casuarina cultivation in inland areas and increasing the productivity will have direct impact on the livelihood improvement of people in these areas.

5 Priorities for the Future

It is almost 150 years since the first introduction of Casuarina in India and the tree is now well regarded as one with substantial socio-economic and environmental importance. It is necessary that the direct and indirect benefits of this multipurpose tree is evaluated through detailed socio-economic studies so that investments in its improvement programmes can be justified. India being the largest Casuarina-growing country with direct livelihood requirements, the existing genetic base has to be conserved and systematically widened to meet the needs of the future and face challenges like pest epidemic and impacts from climate change. In particular new introduction and testing of germplasm is necessary for promoting Casuarina cultivation in dry areas to overcome water deficit and pest attack. India should continue to be involved in forging a strong international cooperation for Casuarina improvement to secure rural livelihoods.

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Constraints in Casuarina Cultivation in Southern Coastal Regions of China

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Abstract

Casuarinas have been cultivated in southern coastal provinces of China for more than a century. Casuarinas are very important to the coastal ecosystems and livelihood of local communities. The planted area reached one million hectares by late 1970s but has declined to about 300,000 hectares at present due to infrastructure development and economic expansion. Many government agencies in China have been conducting research to support the establishment and management of Casuarinas. These include genetic improvement, plantation silviculture, biotechnology, *Frankia* and mycorrhizal fungal association, and pests and diseases. However, current Casuarina planting is facing many constraints. (1) Decline in plantation productivity. Plantations established over the past 20 years are clonal and only a few clones have been used. New plantations have shown considerable decline in the growth probably due to degradation in the productivity of clones. (2) Decline in ecological function, particularly wind protection efficiency. Many shelterbelt plantations are over mature and have lost wind protection efficiency. Clonal plantations have been observed to be less resistant to typhoon winds. In addition, most shelterbelt plantations are pure stand and even aged. Such simple structure may also contribute to less wind protection efficiency. (3) Excessive and uncontrolled exploitation. Large areas of Casuarina shelterbelt plantations are seriously damaged by fish and shrimp farms, mining operations and development of tourist resorts. There continues to be a contradiction between coastal environment protection and economic development. (4) Bacterial wilt *Ralstonia solanacearum*. This disease continues to cause serious problem. The mortality appears to be associated with frequent typhoon winds uprooting the trees. Measures and recommendations to address the above constraints are discussed in this paper.

1 Introduction

The family Casuarinaceae includes four genera and 99 species and subspecies of trees and shrubs that have the capacity to enter different symbiotic interactions with soil microbes including the nitrogen-fixing actinomycete *Frankia*, ectomycorrhizal and endomycorrhizal fungi (El-Lakany et al., 1990; Pinyopusarerk et al., 1993; Zhong et al., 2011). Many species are economically and ecologically important as they provide a wide range of goods and services. The wood is a main source of fuelwood and charcoal, and is also used for general construction and other wood-based industries, e.g. woodchips for paper pulp and veneer for plyboard. In the southern coast of China, Casuarinas are planted to stabilize the moving sand, in agroforestry systems, and for rehabilitation programmes. Very few species can replace Casuarinas at the foreshores (Zhong et al., 2011). Casuarinas were first introduced to China in 1897 (Yang et al., 1995), and currently cover about 300,000 hectares mostly in the coastal areas. Large-scale shelterbelt planting commenced in the 1950s with a specific objective to stabilize the moving sands, provide windbreaks for protection of agricultural lands and water resources, and as a source of fuel wood and timber. Among 23 species of Casuarinas introduced, *Casuarina equisetifolia* is the most extensively planted while *C. cunninghamiana* and *C. glauca* are more suitable in the cooler regions. However, current Casuarina planting is facing many constraints. The main issues are (1) decline in plantation productivity; (2) decline in ecological function, particularly wind protection efficiency; (3) excessive and uncontrolled exploitation; and (4) damage by bacterial wilt disease *Ralstonia solanacearum.* In this paper we describe the constraints and the research carried out to address the problems.

2 Decline in Plantation Productivity

Casuarina plantations established over the past 20 years are mainly clonal and only a few clones have been used. For instance, Clone Pingtan-2 and Huian-1 are widely planted in Fujian. Clone A8 and A13 are planted in Guangdong while Clone Bao 9 and A8 are most popular in Hainan. Decline in the plantation yield has been observed in the third rotation, which is likely due to degradation in the productivity of clones after long-term and repeated use on the same sites. According to the data obtained in Hainan province in 2008, there were 43,000 ha of Casuarina plantations for a total wood volume of 1.29 million m^3, an average of 30 m^3 per ha (Li and Huang, 2011).

Table 1. Growth and quality traits of *Casuarin*a species/seedlots at 5 years' age in Hainan, China.

Species	Seedlot	Height (m)	DBH (cm)	Single tree volume (10^{-3} m^3)	Survival (%)	Tree Form[1]	Typhoon resistance[2] (%)
C. junghuhniana	13950	8.89	9.34	25.89	88.6	4.30	100
C. cunninghamiana	13520	5.62	5.53	5.85	84.4	3.60	100
	15003	4.80	4.80	4.09	66.7	2.81	97.6
	13515	6.98	6.37	9.79	88.9	4.59	100
	Qionghai	6.95	6.37	9.82	77.8	3.33	96.9
C. equisetifolia	14505	3.77	3.16	1.37	77.8	3.67	96.9
	14492	4.93	3.75	2.48	71.1	2.83	100
	14196	4.40	3.05	1.39	44.4	2.92	100
	Wenchang	7.56	6.65	11.35	88.9	4.01	100
C. glauca	13141	4.89	5.15	4.38	84.4	2.58	85.7
	15218	4.27	4.00	2.25	86.7	2.55	92.6
	13146	4.74	4.64	3.6	68.9	2.67	82.6
	Leizhou	6.02	6.20	7.88	77.8	3.97	86.6
Allocasuarina littoralis	13986	5.65	5.07	4.90	68.9	2.62	75.6

[1] Stem form score from 1 = worst to 6 = best straightness
[2] Typhoon resistant rate (TR,%) = (fallen tree / total observed trees) x 100.

New improved genetic resources are essential to increase Casuarina productivity. Species and provenances tested in Hainan showed significant differences in growth and quality traits in *C. equisetifolia*, *C. cunninghamiana*, *C. glauca*, and *C. junghuhniana* (Table 1). These trials will soon function as important sources for selection of new genetic material which will be developed further for plantation establishment.

Intra- and interspecific hybrids are of particular interest to southern China. From 2007 to 2010, 136 crosses involving the priority species were performed through controlled pollination, and seeds were harvested from 64 successful crosses. Progeny tests of these families are currently underway in Hainan and Fujian provinces.

Clonal tests with 55 outstanding individuals selected from provenance and progeny trial and hybridization programs were carried out in 5 sites in Hainan and Guangdong provinces. Twelve fast-growing clones with typhoon and/or disease resistance have been identified (Zhang et al., 2011) (Table 2). These clones will be released after confirmation of field performance.

Associations between *Frankia* isolates and mycorrhizal fungi and Casuarina genotypes have been studied in glasshouse, nursery and field. Results showed that symbiotic *Frankia* and mycorrhizal fungi can improve growth and survival of Casuarinas, particularly in the field where native isolates are not present (Zhang et al., 2006; Yang et al., 2007). Survey and collection of ecto- and endomycorrhizal fungal resources and *Frankia* strains have been emphasized (Zhong, 2000) with a view to use them to improve plantation productivity.

3 Decline in Ecological Function

Many shelterbelt plantations are over mature and have lost wind protection efficiency. In addition, more than 80% of shelterbelt plantations are pure, even-aged forests of a single clone. The simple structure, low biodiversity, and lack of natural regeneration have contributed to low protection efficiency against typhoons and storms (Li, 2007; Ye et al., 2008).

A trial was conducted for 9 years with 2 types of structure treatments: plantation of mixed clones and plantation of single clone. Mixed clones were found to be more efficient. After two typhoons (wind speed over 42 m/s), mixed-clone plantation survived 72% compared to 33% of single-clone plantation.

Mixed species shelterbelt plantations (Casuarina mixed with Acacia, *Calophyllum inophyllum,* Coconut, *Heritiera littoralis,* and Casuarina mixed with Acacia) have been studied in Hainan and found to function better than pure species shelterbelts in wind reduction and resistance against typhoons (Table 3). Mixed-species shelterbelts decrease wind velocity by 88–98 % (Li, 2007). There is also evidence of reduction in pests and diseases in mixed species shelterbelts (Liao, 2007). This system should be adopted more elsewhere.

4 Excessive and Uncontrolled Exploitation

Large areas of Casuarina shelterbelt plantations are affected by high population and economic growth. Many Casuarina shelterbelts have been cleared for agricultural crop production. In areas near the foreshores, numerous shrimp and fish ponds are being expanded. In the prime real estate area, Casuarina plantations have been cleared

Table 2. Growth and typhoon resistance of 14 clones of Casuarina at 6 years' age in Southern China

Clone Number	Height (m)	DBH (cm)	Typhoon Resistance (TR)*
4	15.6	14.3	5.5
7	13.9	12.6	5.4
14	14.6	12.1	5.2
16	15.5	13.6	5.4
17	16.2	14.2	5.6
20	16.3	13.7	5.6
21	16.3	14.4	5.4
27	15.3	13.7	4.3
31	15.2	13.0	5.1
33	13.9	10.6	5.4
34	15.7	14.2	5.6
36	15.5	13.4	5.0
A8 (CK1)	13.8	11.6	3.5
38(CK2)	13.0	11.4	4.5

Note: Data collected from 5 sites of clonal trials in Hainan and Guangdong;

CK1 and CK2 are control clones.

*TR has 6 classes damaged by typhoon:

class 1, worst, tree fall down, angle between tree stem pole and ground is less than 10°;

class 2, bad, broken stem is under middle of the tree stem or the angle is 10- 30°;

class 3, middle, top of stem is broken or the angle is 30 - 45°;

class 4, normal, damage only on branches or the angle is 45- 60°;

class 5, better, no damage on stem or the angle 60 - 80°;

class 6, best, no damage on stem and branchlets or the angle is over 80°.

and developed into tourist resorts and residential estates. An activity which causes very serious damage to the landscape is mining. Although replanting with Casuarinas on the mined areas has been carried out, it will take many years for the ecosystem to return, if ever, to its original conditions.

5 Impact of Wilt Disease *Ralstonia solanacearum*

Many pests and diseases have been found to damage Casuarina plantations in China, but the most serious one is the bacterial wilt caused by *Ralstonia solanacearum.* The biology of this disease has been extensively studied (Liang and Cen, 1982; Sun et al., 2013). The mortality appears to be associated with frequent typhoon winds uprooting the trees. Die-back symptom is observed in plantation area along the typhoon path. The wilt and loss of foliage occurs within days of infection and affected trees subsequently die. In China, Casuarina clones are selected based on their ability to withstand typhoon winds and storms, however, the problem still cannot be overcome. Mixed-species planting as discussed above seems to lessen the disease incidence but tree farmers prefer simple management of single species.

Cash crops such as chilli, egg plant and sweet potato are often interplanted in new Casuarina plantations and they may provide a breeding ground for *R. solanacearum*. These crops appear to be good host of the bacterial wilt disease. Thus the short-term economic gain from cash crop cultivation also results in long-term loss from Casuarina plantations.

Pruning can increase growth and reduce disease damage. Results showed that pruning at 1 and 2 m from ground in 3-year-old plantation improved height growth by 0.5-0.6 m and 0.9-1.1 m respectively, and increased survival by 14 %, compared with no pruning treatment (Table 4). Pruned trees also appeared to be less affected by typhoon winds and thus less damage by bacterial wilt. Light crown canopy after pruning seems to minimize direct impact from the winds.

Table 3. Eco-function at different planting models involving Casuarina in Southern China

Location	Planting Models	Wind speed (m/s)		Reduce wind speed rate (%)	Sand in forest (g/mth)		
		Front	Inside		Inside	front	Fixed sand rate (%)
Dachang,	Pure casuarinas	6.0	3.6	41.0	247	5730	95.7
Haikou	*Cas.+Calophyllum inophyllum*	5.8	3.2	44.8	231	95.9	
	Cas.+ Acacia auriculiformis	5.7	2.1	63.2	132	97.7	
Changsa,	*Cas.+ A. auriculiformis*	6.2	2.0	67.7	126	7150	98.2
Wenchang	*Cas.+Heritiera littoralis*	6.6	3.5	43.6	224		96.8
	Cas.+ Coco nut + *A. auriculiformis + Calophyllum inophyllum*	6.4	1.4	78.1	80		98.8

Note: Data provided by Fazhi Fang and Yu Chen of the Hainan Forestry Research Institute, Haikou , China (unpublished).

6 Conclusion

China has been successful in planting Casuarinas to protect and improve its environment and coastal ecosystems. But many obstacles prevent achieving this objective. Casuarinas will continue to be an important part of the landscape in the southern coastal regions of China. Technical support from national research institutes and provincial agencies is necessary in order for county and local governments to achieve the goals.

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Growth and Development of *Casuarina equisetifolia* in the Open Sandy Sea Coasts of Cox's Bazar, Bangladesh

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Key words: Cox's Bazar, Sandy beach, Casuarina, Climate resilient, Livelihood

Abstract

Bangladesh is a pioneer in coastal afforestation programmes that reduce the losses of frequent cyclones, tidal surges and tsunami. Keora (*Sonneratia apetala*) and baen (*Avicennia officinalis*) are the principal species for plantation in the newly accreted muddy charlands; whereas, Jhau (*Casuarina equisetifolia*) has proved to be the only suitable, climate resilient and promising species in the coastal sandy beaches of the open coast and off-shore islands. The open coast of Cox's Bazar sea beach, the longest uninterrupted beach (125 km) of the world is facing the damages of frequent cyclones and tidal surges. Establishment of a green-belt of *C. equisetifolia* appears as the most suitable device to reduce the losses and damages of the natural disasters. A total of 415 ha *C. equisetifolia* plantations were established in three forest ranges along the sea beach during 1997-2012. The survival varies from 53 to 81%. The maximum height (19.2 m) and diameter (19.6 cm) were found for a 16-year-old plantation at Kosturaghat. This paper assesses the survival, growth and development of Casuarina plantations in stabilizing the sandy beaches and environmental amelioration. Establishment of plantations is encouraged for further development of coastal green-belt to maximize resilience of the coastal communities.

1 Introduction

Bangladesh is one of the coastal marginal countries of the Bay of Bengal situated between latitude 20° 34' to 26° 38' N and longitude 88° 01' to 92° 42' E (Rashid, 1991). Geographically coastal area represents an area of 47,211 km^2 (32% of the country) with 35 million people (28% of the total population) living with multiple vulnerabilities and opportunities (Islam, 2007, Hossain et al., 2008).The tidal floodplains are less than 1 m above the mean sea level (msl) and the main river and estuarine flood plains are 1-3m above msl (Rashid, 1991). The 710-km long coastline in Bangladesh is divided into three distinct geo-morphological regions, viz., western zone, central zone, and eastern zone (Siddiqi, 2001).The western and central zones are very flat and low, whereas the eastern coastal zone boasts the beautiful tourist spot of Cox's Bazar, which is world famous for its uninterrupted longest sandy sea beaches (Siddiqi, 2003; Banglapedia, 2006). The main attraction of Cox's Bazar is the long sandy beach and is one of the most-visited tourist destinations in Bangladesh. Bangladesh is one of the most vulnerable countries to the impacts of global climate change and these impacts are becoming ever more visible (IPCC, 2007). The sandy beaches of Cox's Bazar are becoming susceptible to a variety of natural hazards including flood, cyclones, storm surge, sea level rise, coastal erosion and landslides (Hossain, 2010). Recent studies reveal that an annual increase of 7.8 mm sea level rise was recorded from Cox's Bazar coastal station (Alam, 2003). A total of 14 super cyclones along with tidal surges have attacked the coast of Bangladesh since 1960 (Al-Hossaini et al., 2005). After the 1960 cyclone, the Forest Department has taken massive initiatives for planting and managing both the mangrove and non-mangrove plantation on newly accreted charland and in the coastal beaches of Bangladesh through a number of projects (Table 1) and a total of 192,395 ha coastal plantations have been established in Bangladesh (Hasan, 2013).

Keora (*Sonneratia apetala*) and baen (*Avicennia officinalis*) are the principle species for coastal plantation in the newly accreted muddy charlands (Siddiqi, 2001); whereas Jhau (*Casuarina equisetifolia*) has proved to be the only suitable, climate resilient and promising species in the coastal sandy beaches of the open coast and off-shore islands (Hossain et al., 2008; Hossain, 2010). *C. equisetifolia* is thought to be indigenous in the coasts of Bangladesh (Troup, 1921; Parrotta, 1993) and also a priority plantation species in the sandy beaches, off-shore islands, roadside and coastal homesteads (Serajuddoula et al., 1995; Nandy et al., 2002; Islam, 2003; Jashimuddin et al., 2006). The species is also suitable for industrial raw material and fuelwood production, conservation of coastal ecosystems and the environment, protection of wildlife and aquatic resources, agricultural land protection against salinity intrusion and attracting the tourists (Hossain et al., 1998; Bhuiyan et al., 2000; Hossain and Khan, 2005; Chowdhury et al., 2009). Established *C. equisetifolia* plantations in the sandy beaches are able to halt and reduce these climate change

Table 1. Projects for coastal forests executed by the Forest Department, Bangladesh

1.	Afforestation in the coastal belt and offshore islands (1960-61 to 1964-65).
2.	Afforestation in the coastal belt and offshore islands (1965-66 to 1969-70).
3.	Afforestation project in the coastal regions of Chittgong, Noakhali, Barisal and Patuakhali (1974-75 to 1979-80).
4.	Mangrove Afforestation project (1980-81 to 1984-85).
5.	Second Forestry project (1985-86 to 1991-92).
6.	Forest Resources Management project (1992-93 to2001-2002).
7.	Extended Forest Resources Management project (2002-03 to 2003-04).
8.	Coastal Green Belt project (1995-96 to 2001-02).
9.	Coastal Char Land Afforestation project (2005-06 to 2009-10).
10.	Management Support project for Sundarbans Reserve Forest (2005-06 to 2009-10).
11.	Afforestation Project in the Coastal Areas to Mitigate Adverse Effects of Climate Change (2010-11 to 2012-2013).
12.	Community Based Adaptation to Climate Change through Coastal Afforestation in Bangladesh (CBACC-CF) Project (2009 to 2014).
13.	Afforestation in the Coastal Embankment of Water Development Board and Adjacent Char Areas 2009-10 to 2011-12).
14.	Climate Resilient Participatory Afforestation and Re-forestation Project (2012-13 to 2015-2016).

induced impacts and also provide benefits to the local livelihoods through aesthetic views to tourists, litters and thinned forest produces. Shoreline forests are recognized as a buffer against the actions of wind, waves and water under tidal influence as a protection measure (Lyche, 1991; Tanaka, 2009). Therefore, the present study was undertaken to assess the status, growth and development of Casuarina plantations along with the environmental amelioration and dependency of coastal communities for livelihoods on the established plantations.

2 Materials and Methods

The study was conducted in the 415 ha coastal Casuarina plantations of Cox's Bazar, Inani and Teknaf Ranges of Chittagong Coastal Forest Division extending from Kosturaghat to Shah Parir Dweep (Fig. 1). The land surface of the coast is flat to gentle slope and sandy in nature. Sandy beaches colonized with *Ipomoea pes-caprae* are considered suitable for Casuarina plantations. Plantations were established with polybag-raised seedlings (Hossain et al., 1998) with a spacing of 2 x 2 m. Most of the area is inundated by the sea water during rainy season. The plantations are in the form of line or block plantings depending on the availability of lands

2.1 Sampling procedure

A reconnaissance survey was conducted to investigate the overall physical condition of the study area. There are three Ranges and seven Beats. Three cross sections of each year plantation consisting of 100 m length were selected in each forest beat. Each cross section was 100 m apart from one to another. Thus, 21 sections were created in three different beats. From each section of each beat 5 plots of size 10 m × 10 m (100 m^2) were selected randomly for growth measurement.

2.2 Data collection and analysis

Data were collected from randomly selected representative plots of different year's plantations. The parameters measured were total number of trees, diameter at breast height (dbh in cm) and total height (in m) of the trees. Each of the selected sample plots was laid out by measuring tape. Trees on the outer edge were marked with paint. Within the selected plot, total number of trees, total height and dbh of all trees were recorded. Soil erosion measurement was based on ocular estimation and root exposure was based on the percent of exposure of roots. The secondary data and related information was collected from the office of the Chittagong Coastal Forest Division and Cox's Bazar South Forest Division.

3 Results and Discussion

Jhau (*C. equisetifolia*) was planted in the open sandy beaches during 1996-97. All the plantations were raised by seedlings grwon in and most of the plantations are subjected to inundation by sea water at least in the rainy season. In extreme cases, washout of the young seedlings through sand erosion is also common. The growth performance varies in all the Ranges, Beats and with age of the plantations (Table 2). Among all the plantations, the best performing plantation was a 16-year-old plantation at Kosturaghat beat with an average height of 19.2 m and dbh of 19.6 cm. One-year-old plantation was found in Shah Parir Dweep beat with an average height and collar diameter of 1.6 m and 3.7 cm respectively. The height and diameter growth of the plantations are promising in comparison to similar jhau plantations at Char Mohiuddin and Char Nangulia of Noakhali Coastal Forest Division (Siddiqi et al., 1993). Survival varies considerably from 53 % to 81 % among all the plantations. The highest survival percentage was found in the 14-year-old plantation at Kosturaghat beat, whereas, 7-year-old plantation showed the lowest

Table 2. Growth performance of *Casuarina equisetifolia* in the open sandy sea coasts of Cox's Bazar

Forest Range	Beat	Age (Year)	Year of Planting	Area (ha)	Dbh (cm)	Height (m)	Survival (%)
Cox's Bazar	Kosturaghat	16	1996-97	60	19.6	19.2	69
	Kolatoli	16	1996-97	40	18.9	19.0	72
	Kosturaghat	15	1997-98	25	17.9	18.0	72
	Kosturaghat	14	1998-99	5	16.4	17.2	81
	Himchari	11	2001-02	20	15.7	16.8	65
	Himchari	10	2002-03	40	14.1	16.3	67
	Kolatoli	10	2002-03	32	15.0	17.1	80
	Kosturaghat	10	2002-03	8	14.6	16.7	75
	Kosturaghat	2	2010-11	5	2.6*	1.4	72
Inani	RejuTahol Fari	11	2001-02	20	16.2	17.1	78
	Inani	10	2002-03	30	14.3	16.1	69
Teknaf coastal	Shah Parir Dweep	7	2005-06	20	16.5	13.2	59
	Teknaf Sadar	7	2005-06	20	16.7	14.1	53
	Teknaf Sadar	5	2007-08	40	14.1	12.7	61
	Teknaf Sadar	4	2008-09	40	12.3	11.5	71
	Shah Parir Dweep	1	2011-12	10	3.7*	1.6	56
	Total			415			

*Collar diameter (cm)

survival at Teknafsadar beat (Table 2). Low survival in some plantations is because of both the anthropogenic and natural calamities like washout of young plantations by tidal surges.

Most of the Casuarina plantations are inundated by sea water during rainy season, but during winter season the plantations are 6 to 244 m away from the low tide levels (Table 3). However, plantations of Himchari, Kosturaghat and Shah Parir Dweep are very close to sea water during high tides.

Soil erosion is extremely associated with the Casuarina plantations in the sea coasts of the Cox's Bazar in Bangladesh. Severe soil erosion was observed only in the first row of Casuarina plantations from sea-side at Reju Tahol Fari and Shah Parir Dweep beats. Low (10 %) and moderate (55 %) soil erosion was observed at Kolatoli and Kosturaghat beats. Severe soil erosion was observed in Himchari, Reju Tahol Fari, Inani and Shah Parir Dweep. However, there were some depositions of sand dunes in few established plantations. There was no soil erosion in the 2-, 10- and 14- year-old plantations at Kolatoli and Kosturaghat beats (Table 3). Severe soil erosion occurred due to tidal surges and wave action of the Bay of Bengal, particularly in the rainy season. Root exposure of *C. equisetifolia* occurred due to the severe soil erosion. Severe exposure of root was observed in the first row of Casuarina plantations from seaside at Himchari, Reju Tahol Fari, Teknaf and Shah Parir Dweep beats. In case of 2nd and 3rd row of plantations, root exposure was gradually reduced to inward Casuarina plantations (Table 3).

It was also observed that the soil erosion and exposure of roots were observed mostly in a few rows of the seaside plantations, but the plantations at inner side gradually stabilized and supported dense ground vegetation. This indicates the gradual transformation of sandy sites suitable for planting mainland species. Ground vegetation under any plantation plays a very significant role for the improvement of soil fertility. In most of the Casuarina plantations of the studied area, there was an existence of different types of ground vegetation. In the 15- and 16-year-old plantations at Kosturaghat beat, luxuriant ground vegetation was established. *Ipomoea pes-caprae* was present in the young plantations at Kosturaghat, Teknafsadar and Shah Parir Dweep beats. Mixed ground vegetation (*Vitex negundo, Lantana camara* and *Clerodendrum* sp.) was found in a number of plantations at different forest beats. Soil formation and stabilization were found variable in the Casuarina plantations. Loose to moderately compact, stabilized soil was found in the established Casuarina plantations. In general, loose soil is common in the younger plantations, whereas moderately compact soil was found in the old mature plantations.

Some landless poor Climate refugees (from other parts of the country) and Rohinga refugees (from Myanmar) are living inside the established Casuarina plantations and use produces by collecting small poles, posts for making houses, fishing posts and leaf-litters and twigs for cooking (Table 4). A survey of 45 households (large, medium and small depending on family member) living in and around the jhau plantations indicate their family size; duration of living in the area shows that Kosturaghat was the oldest and the Shah Parir Dweep was recent habitation. All the

Table 3. *Casuarina equisetifolia* plantations from the Cox's Bazar sea coast including the status of erosion, root exposure and ground vegetation

Beat	Age (year)	Distance (m) of plantations from sea		Soil erosion	Root exposure (%)			Ground vegetation	Soil form
		High tide	Low tide		R^1	R^2	R^3		
Kosturaghat	16	152	244	Low	80	50	40	Grass	L
Kolatoli	16	137	213	Low	80	50	40	Mixed	MC
Kosturaghat	15	122	213	Low	20	A	A	Grass	MC
Kosturaghat	14	107	198	No	A	A	A	Grass	C,MC
Himchari	11	0	06	Severe	100	90	50	Mixed	L
Himchari	10	0	07	Severe	100	80	50	Poor grass	MC
Kolatoli	10	122	213	No	A	A	A	Mixed	MC
Kosturaghat	10	90	183	No	A	A	A	Grass	L
Kosturaghat	2	0	30	No	A	A	A	*Ipomea*	L
RejuTaholFari	11	15	61	Severe	100	A	A	Mixed	L,MC
Inani	10	12	45	Severe	100	A	A	Mixed	MC
Shah ParirDweep	7	0	40	Severe	100	55	A	*Ipomea*	L,MC
Teknafsadar	7	09	30	Moderate	80	50	50	*Ipomoea*	L
Teknafsadar	5	12	37	Moderate	80	50	50	*Ipomoea*	L
Teknafsadar	4	12	45	Moderate	100	70	40	*Mixed*	L
Shah ParirDweep	1	0	17	Moderate	50	A	A	*Ipomea*	L,MC

A-Absent, L- Loose, MC- Moderately compact, C- Compact.

inhabitants use the leaf-litters as fuel and take shelter and protection in the plantations from the cyclone, erosion and strong winds. Livelihood status of these Climate and Rohinga refugees were greatly dependent on the Casuarina plantations in the sea coasts of Cox's Bazar. The majority of the Rohinga refugees were involved with fishing (80-90 %). The rest were involved in occupations like boating, day labor and rickshaw pulling (Table 4). Rohinga refugees living inside the Casuarina plantations were continuously getting numerous tangible and intangible supports for their livelihood from the Casuarina plantations. They were also using the leaf litter, twigs, branches and fruits to meet their fuel demand for cooking. Maximum amount (90%) of leaf litter use as fuel was found at Kosturaghat beat. In most cases, they have to brush the forest floor for litter collection.

Illicit felling is another concern in the established Casuarina plantations in the coastal areas. The problem is pronounced in the older plantations at Kosturaghat and Shah Parir Dweep beats respectively. At present, livelihood support through the establishment of social forestry plantations is a successful program in Bangladesh (Khan et al., 2004). Casuarina plantations are established as protection forests for conserving the environment and reducing the losses from cyclones and tidal surges. However, the inhabitants are deriving benefits from the leaf-litter, twigs and branches for cooking. Seized poles or posts of Casuarina are also used for piling, house posts and fishing posts and sold at the rate of 8.75 – 10 US $ per piece (Table 5). Seized or uprooted trees are generally sold by the Forest Department and used for this purpose.

Casuarina plantations are playing a vital role to halt or minimize the damages of natural calamities and disasters at a certain degree (Forbes and Broadhead, 2007; Zoysa, 2008). The established plantations already gave sufficient protection to the adjacent agricultural crops and local communities in the beach area. Casuarina plantations along the sea coasts are able to act as a natural barrier to reduce the severity of cyclone, soil erosion, salinity intrusion and strong wind. In addition, they provide wood and wood fuels to local people, harbour biodiversity and increase the marine fish population (Fig. 2). The species is now also considered and planted in different projects as one of the climate resilient species in coastal areas of Bangladesh. Since the species is suitable for reclamation of unstable coastal ecosystems (Pinyopusarerk et al., 2004), it has been rightly used in the plantations in Cox's Bazar sea coasts since 1997 to protect the sandy beaches along with the inland habitation and agricultural crops.

4 Conclusion

Casuarina plantations are able to protect the lives and properties of the people of Cox's Bazar sea coast from the damages of natural disaster like cyclones, floods and salinity intrusion. The stabilized plantations protect the vulnerable coastal environment of Cox's Bazar and also increase the aesthetic view of this tourist attraction. However, the remaining vacant coastal areas need to be filled up with Casuarina plantations immediately to reduce the damages of future natural calamities that are expecting to be severe due to climate change induced catastrophes. The expectation is also that the coming projects target the development of participatory Casuarina plantations that will not only improve the environment, but also provide pole, posts for community uses. Further Casuarina species and provenance trials should be established in Bangladesh with regional and international cooperation to find out the superior site-adaptive genetic resources for maximizing the production from Casuarina plantations.

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Results of the 2013 Survey of Casuarinaceae Populations in he Philippines

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Abstract

A survey conducted in 2013 to determine the occurrence of Casuarinaceae populations across the Philippine archipelago resulted in locating a total of 44 natural and planted populations. Of these, seven natural and 21 planted populations were of *Casuarina equisetifolia* Linn. and five other populations highly resembled this species which are yet to be identified. Eight natural and one planted populations were identified to be that of *Gymnostoma rumphianum* (Miq.) LAS Johnson (ex *C. rumphiana* Miq.). There were also two other natural populations of Casuarinaceae located more than 1,000 masl in Mindanao highlands which are being investigated. These populations have not been included in earlier Casuarina seed collection and domestication programs. Collection of seeds and specimen from these populations are being organised by the Department of Environment and Natural Resources (DENR) and University of the Philippines Los Baños (UPLB) for taxonomic identification and for deployment as new breeding populations as well as for exchange and infusion to current breeding programs.

1 Introduction

The Philippines recognized the economic value of the family Casuarinaceae since it was first used in reforestation and rehabilitation plantings in the 1970s. Casuarinas were earlier mainly used for handicrafts and for household fuel. More recently, Casuarinas have been very successfully used in rehabilitation of disturbed land, including mining, and for shelterbelts. The increasing demand for fuel wood of 30 to 40 million tonnes annually (DENR, 1989) alone highlighted the need for the expansion of fuel wood plantations of which Casuarinas are prime resources.

The growing interest on the development of more Casuarina plantations to support programs for industry development and land management has also been highlighted in discussions both at the local and national context. Prompted by the growing interest on the development of more Casuarina plantations in the country, we conducted a nationwide survey of available Casuarina populations. Results of the survey were also intended to guide a nationwide collection of Casuarina seeds for the purposes of field trialling and infusion in current plantation and breeding programs. This paper reports on the results of a survey investigation we conducted in April to December 2013 to locate and describe the distribution of Casuarinas in the Philippines.

2 Survey Method

We conducted a review of literature and herbarium specimen collections to determine the known species under the family Casuarinaceae in the Philippines. The review of literature and herbaria collections provided information to guide us in designing a survey of planted and natural Casuarina populations across the Philippine archipelago.

A survey questionnaire was sent to all 15 Department of Environment and Natural Resources (DENR) regional administrative units in April 2013. The survey questionnaire was designed to locate Casuarina populations – both planted and natural – that are known to forest managers in each administrative region to have potential for producing good quantities of Casuarina seeds.

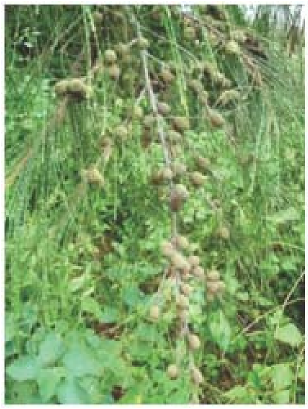
Completed questionnaires were collated immediately as they were submitted. Initial results of the survey indicated difficulties by respondents to differentiate the identities of trees within their jurisdictions, as well as between natural and planted populations. This prompted us to directly communicate (by phone calls and/or e-mail correspondence) with respondents and to request the collection of herbarium specimen and/or digital photos of sample trees in cases of difficult or uncertain identity of trees in reported populations where possible. (collection of herbarium specimen and digital photos are underway and will be presented in a separate report)

3 Results

3.1 *Casuarina* species in the Philippines

Merrill (1923) listed three species belonging to the family Casuarinaceae that were found in the Philippines, namely, *Casuarina equisetifolia* Linn., *C. rumphiana* Miq. and *C. sumatrana* Jungh. Rojo (1999) reported four species under Casuarinaceae namely: *Casuarina equisetifolia* L., *Ceusthostoma palawanense* L. Johnson, *Gymnostoma rumphianum* (Miq.) L. Johnson and *Gymnostoma*

Fig. 2. A natural population (A) and a fruit-bearing branch (B) of *Casuarina equisetifolia* in Zambales, Philippines.



sumatranum (Jung. ex de Vriese) L. Johnson. There are currently three known species of the family Casuarinaceae that are occurring naturally in the Philippines, namely *Casuarina equisetifolia* L., *Gymnostoma rumphianum* (Miq.) LAS Johnson (ex *C. rumphiana* Miq.) and *Ceuthostoma palawanense* LAS Johnson (Madulid, 2002).

At the time of writing this report we have so far identified and located natural and planted populations of *C.*

Table 1. Details of natural and planted populations of *Casuarina equisetifolia* located in the Philippines during the 2013 survey

S. No.	Province	Number of populations		Latitude	Longitude	Altitude
		Natural	Plantation	(N)	(E)	
1	Abra		1	17° 35'	120° 45'	40
2	Albay	1		13° 13'	123° 44'	145
3	Benguet		2	16° 30'	120° 40'	1300
4	Cebu		1	10.28	123° 55'	647
5	Cotabato	1		7°13′	124° 14′	1250
6	Davao	1		6° 55'	125° 20'	1104
7	Davao Oriental	1		7° 19'	125° 45'	191
8	Ifugao		2	16° 50′	121° 10′	1000
9	Ilocos Norte		5	18° 10′	120° 45′	300
10	Leyte		2	10° 50′	124° 50′	305
11	Mt Province		2	17° 05'	121° 10′	800
12	Nueva Ecija		1	15° 35'	121° 00′	232
13	Pangasinan		1	15° 55'	120° 20′	83
14	Tarlac		1	15° 30'	120° 30′	308
15	Zambales	1	2	15° 15'	120° 01'	56
16	Mindoro	1		13° 06'	121° 15'	365
17	Aklan		1	11° 40'	122° 20′	300
18	Palawan	1		8° 35'	117° 25'	47
19	Cagayan*	3		19° 16'	121° 29'	191
20	Quezon*	2		13° 58'	122° 32'	86
	Total	12	21			

* Showed close resemblance with *C. equisetifolia* but further verification is needed to confirm species identity.

Table 2. Natural and planted populations of *Gymnostoma rumphianum* in the Philippines located during the 2013 survey

S. No.	Provinces	Number of populations	Latitude (N)	Longitude (E)	Elevation (masl)
1	Albay	1	13° 13'	123 °43' "	352
2	Zambales	2	15° 09'	120° 04'	28
3	Cebu	1	10° 28'	123° 55'	647
4	Davao	2	7° 28'	125° 14'	1,220
5	Davao Oriental	1	6° 42'	126° 09'	500
6	Eastern Samar *	1	11° 40'	125° 30′	350
7	Leyte	2	10° 50′	124° 50′	800
	Total	9			

* Plantations

equisetifolia and *G. rumphianum*. Information regarding the current occurrence of *C. palawanense* has not been captured perhaps due to its sparse distribution in its habitat range along the Palawan group of islands. We rely on the information that *Ceuthostoma palawanense* are still occurring in the Palawan group of islands based on word-of-mouth information from indigenous people and on herbarium specimen collected recently from the area.

3.2 *Casuarina equisetifolia*

Casuarina equisetifolia is the most common species of *Casuarina* in the Philippines. Table 1 enumerates the natural and planted populations identified during the survey and Fig. 1 shows their relative locations in the Philippines archipelago. The survey indicated 21 plantations in 12 provinces and seven natural populations of *C. equisetifolia* across the archipelago. There were five populations which showed close resemblance with *C. equisetifolia* but need further verification to establish their identity. Specimens and photos are being collected to confirm their identities in comparison with existing specimen in herbaria collections. Fig. 2 shows a natural population and fruit-bearing branch of *C. equisetifolia* in Zambales region.

3.3 *Gymnostoma rumphianum* (Miq.) LAS Johnson

The survey found 9 natural populations and one plantation of *G. rumphianum* occurring in seven provinces across the country (Table 2 and Fig. 3). The habit and morphology of needles and cones are shown in Fig. 4.

3.4 Observations on the occurrence of Casuarina in areas more than 1,000 masl in the Philippines

The identities of two Casuarina-like populations (Table 3) observed at more than 1,000 masl at Mt Apo National Park and Mt Kitanglad National Park are being investigated. From submitted photos (Fig. 5), we suspect they could be *G. rumphianum*. Specimens and more detailed photos are being collected for comparison with collections at the National Herbarium in Manila.

4 Discussion

4.1 Growing national interest on Casuarina in the Philippines

Clearly the evident successes of Casuarina-based industries among countries in the region, such as China, Vietnam and India, have been noticed in the Philippines. With its fast-growing population comes a clear need to also increase its production of wood especially for home for construction, furniture, and other wood-based products.

Fig. 1. Location of natural and planted populations of *Casuarina equisetifolia* in the Philippines (numbers refer to locations given in Table 1)

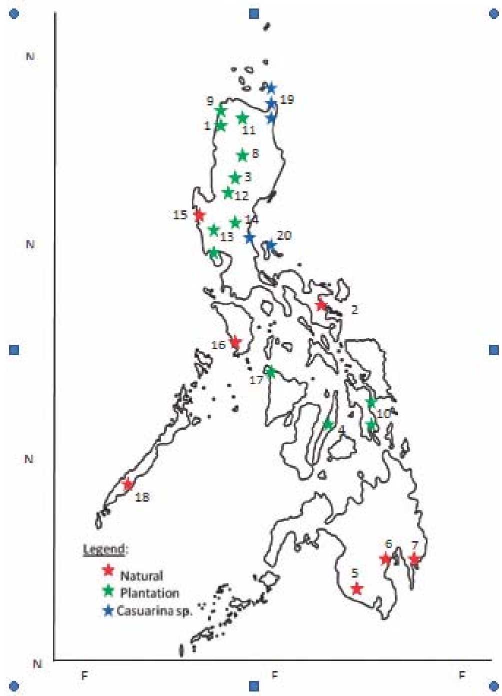
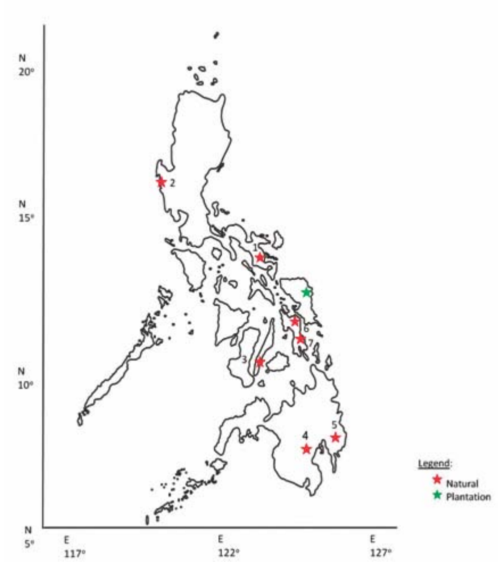


Fig. 3. Location of natural and planted populations of *Gymnostoma rumphianum* in the Philippines (numbers refer to locations given in Table 2)



According to the Philippines National Statistics Office (PNSO), 44% of the 8.9 million households in the country still use wood for cooking. The annual demand for fuel wood is about 35.46 million cubic meters with an estimated value of around P11.34 billion (US$253.41 million). This included demands from industries such as tobacco flue curing, potteries, brick making and sugar production. To meet this demand will require an annual harvest of 300,000 hectares fuel wood plantations. We believe this demand will increase further as the population reaches around 150 million by 2030 according to PNSO 2013 projections.

The development of renewable energy resources is being given focus in order to minimize the country's carbon footprint. The development of wood energy plantations is a very significant part of the current National Greening Program being implemented by the DENR. The San Carlos Bioenergy Inc. (SCBI) project in central Philippines is also a key example of such effort from the private sector. SCBI has been building its wood energy plantations in recent years which include Casuarinas to supply a 10MW initial energy production target capacity.

We found that *C. equisetifolia* is one of the most successful species being used in the rehabilitation of around seven

Fig. 4. Tree habit and morphology of fruit-bearing branches of *Gymnostoma rumphianum* in Philippines.

million hectares of the country's forest land. It has been widely used in sand dune and beachfront stabilisation projects across the country. The La Paz Sand Dune Stabilization Project in Northern Philippines has been a prime learning project on the use of *C. equisetifolia* since 1970s. Along with the national efforts to improve the resilience of areas vulnerable to impacts of climate change (e.g., rising sea levels, storm surges, heavy rainfall, prolonged drought), the planting of more *C. equisetifolia* is anticipated to accelerate over the coming years as initiated by the National Greening Program.

Six key mine sites we visited during the survey were all using *C. equisetifolia* for restoring tree cover in very difficult areas disturbed during mining operations. Although *C. equisetifolia* has been successful in harsh conditions brought about by mining, we also found their limiting impact on colonization of the areas by native flora surrounding the land being rehabilitated. With the anticipated contributions from development of the mining industry to the national development program, the availability of suitable species for planting in heavily disturbed areas will become more important than ever.

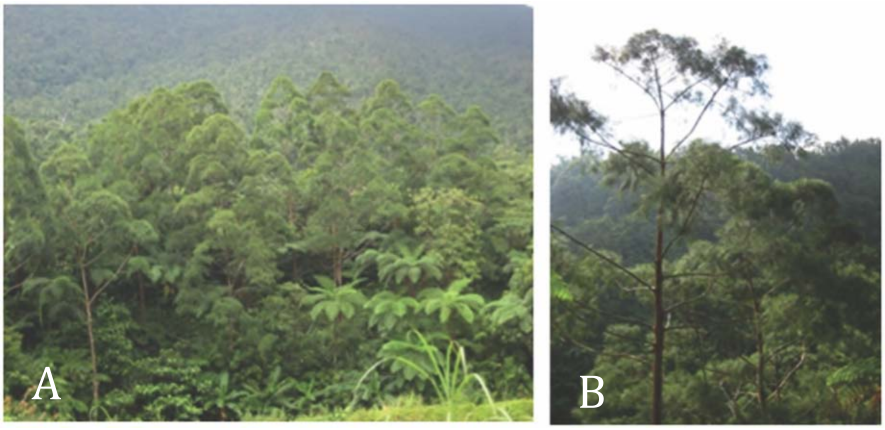
4.2 Organising fresh collection of Casuarina Seeds throughout the Philippines

Three populations from the Philippines namely, Aklan (Panay Island), Narra (Palawan Island) and San Jose (Mindoro Island) were part of the international provenance testing programme coordinated by CSIRO, Australia during

Table 3. Details of two natural populations of Casuarina located in the highlands of Mindanao, Philippines whose identities are yet to be established

S. No.	Province	Latitude (N)	Longitude (E)	Elevation (masl)
1	Mt Apo National Park, Davao	6° 55'	125° 20'	1104
2	Mt Kitanglad National Park, Bukidnon	7° 25'	125° 16'	1606

Fig. 5. A populations (A) and tree habit (B) of a yet to be identified Casuarinaceae member in the Philippines



1990s (Pinyopusarerk et al., 2004). The populations of Palawan Island and Mindoro Island were not well represented in that collection. They should be re-sampled to start new breeding populations, or to infuse into current breeding populations. The planted populations in Panay Island were part of a reforestation project area planted in 1970s. The origin of the seeds used to establish the plantation is not known. The plantation during the time of collection was not managed or thinned; the plantation is now being used to produce seeds for current planting projects, including the NGP.

Results of the 2013 survey of Casuarina populations in the Philippines will be used for organising fresh collections of Casuarina seeds in the country. This is strongly in-line with the planting material requirement of the current National Greening Program (2011-16) and future industry development.

Leading organisations, including the DENR (ERDB and FMB), the UPLB and along with other participating state universities are aiming to establish new breeding populations to support long-term development of wood industry based on Casuarinas. The collection will be coordinated from Los Baños by the ERDB and UPLB (College of Forestry and Natural Resources), with additional support from the Department of Science and Technology and the DENR lead National Greening Program 2011-16.

5 Conclusion

The identity and location of 44 natural and planted populations of the family Casuarinaceae in the Philippines were confirmed through a field survey. These populations will be used for a proposed nationwide collection of fresh Casuarina seeds to help meet the planting materials requirement for of the National Greening Program 2011-16, for use in breeding programs and plantations, for land rehabilitation and for establishing resilient coastlines against rising sea level. Identities of trees in each population will be further confirmed from specimen to be collected with the new collections of seeds. The fresh collection of seeds is being considered for exchange with other Casuarina breeding programs in the region.

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Conservation of Genetic Resources and Improvement of Agroforestry Tree Species in Sub-Saharan Africa: An Overview

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Abstract

Increased populations and pressure for social and economic development in Africa contribute to a trend of diminishing forest cover. Sub-Saharan forests are amongst the most important repositories of terrestrial diversity. This diversity includes variation at landscape, ecosystem, species, population, individual, and molecular levels of biotic organization. African forests are sources of timber, food, including fruits, fats, oils, leafy vegetables, nuts and condiments, which complement staple food crops in the local diet. Many local trees and shrubs are sources of micronutrients for communities through the consumption of fruits and vegetables. The overall goal in the management of forest genetic resources is to help ensure that forest biological diversity, at all levels, is conserved, managed and sustainably utilised in support of local and national development, including food security, poverty alleviation, environmental conservation, economic and social advancement and the maintenance of cultural and spiritual values. The exploitation, use and commercialisation of products from food tree species constitute an important activity of people living within and around forests. Forest loss and fragmentation, conversion to farmland and degradation not only cause plant biodiversity losses but also indirectly hamper regeneration rates and help accelerate species rarity, isolation and extinction rates, especially for food tree crops. There is an urgent need therefore for the conservation and management of local tree genetic resources in sub-Saharan Africa.

1 Introduction

Increased human population and pressure for social and economic development in Africa contribute to diminishing forest cover. Sub-Saharan forests are amongst the most important repositories of terrestrial diversity, notably in the Congo Basin forest which is the second largest continuous forest in the world after the Amazon. This diversity includes variation at the landscape, ecosystem, species, population, individual, and molecular levels of biotic organization, and is essential for selection and breeding to meet present and future human needs (FAO, 2007). Thus, all these levels must be considered when conservation objectives and actions are determined. African forests provide a wide range of products and services to people, especially to the rural poor communities. They are sources of food, including fruits, fats, oils, leafy vegetables, nuts and condiments, which complement staple food crops in the local diet. In addition, trees and shrubs provide numerous traditional medicines that the rural poor use for health-care. Many native trees and shrubs are sources of micronutrients for humans through the consumption of fruits and vegetables. Forests and other wooded areas not only provide goods and livelihoods but also protect soils, regulate water and absorb carbon.

Forest trees and other woody plants support many other organisms, and have developed complex mechanisms to maintain high levels of genetic diversity. This genetic variation, both inter and intra specific, of trees and shrubs serves a number of fundamentally important purposes. It allows them to adapt to changes in the environment, including those brought about by pests, diseases and climate changes. The overall goal in the management of forest genetic resources is to help ensure that forest biological diversity, at all levels, is conserved, managed and sustainably utilised in support of local and national development, including food security, poverty alleviation, environmental conservation, economic and social advancement and the maintenance of cultural and spiritual values.

Annual deforestation rates in Africa are relatively high (0.8% per year). For West and Central Africa, the deforestation rate is about 0.6% per year. Many tree species provide food to people living in and around forests in Africa. However, deforestation and forest fragmentation are reducing the habitats for valuable plants. Habitat loss is driving reduction of species' populations and extinction rates of plant species. Deforestation, forest fragmentation, conversion to farmland and degradation not only reduce plant biodiversity but also indirectly reduce natural regeneration, and accelerate species rarity, isolation and extinction rates, especially for food tree crops. Faced with such challenges, there is an urgent need for the conservation and management of genetic resources of these species in Africa. This paper gives an overview of the domestication and improvement of key food tree species and the challenges for the conservation of their diversity for future generations. We examine conservation of forest genetic resources for food tree species in protected areas and on farms, and their incorporation in domestication and tree improvement programmes.

2 Conservation of African Food Tree Genetic Resources

2.1 General considerations on conservation approaches

A major threat for African forest ecosystems is the conversion of forests to other land uses, especially for agriculture. Increasing pressure from human populations and timber industries, who want higher standards of living without concern for the sustainability of resource utilization, raises concerns. The major factors contributing to the destruction of African forests are unsustainable logging, fires, grazing, civil conflicts, and natural disasters including severe droughts. These create landscapes consisting of mosaics of different types of forest and non-forest land cover.

In many parts of the tropics, including African tropical ecosystems, we now have a landscape composed of patches of relatively undisturbed forest, forests designated for timber production, forests co-managed by rural communities, and a complex range of secondary forests and agroforests all set in matrices of pastures, croplands and plantations. Thus, forests have been transformed from large, remote wilderness areas into mosaic landscapes of agricultural, agroforestry and forestry land uses. While it is inevitable that land-use changes will occur in the future, such changes should be planned to help ensure that the complementary goals of conservation and sustainable resource management are achieved. Both conservation and development objectives have to be achieved in these landscape mosaics, and ensure that production and protection functions are optimised.

As the forests of sub-Saharan Africa are contracting and protected areas becoming difficult to manage and control, agriculture-based approaches for conserving the biodiversity are becoming increasingly important (Dawson et al., 2009). As many local food tree species are highly valued by farmers, it is crucial that their genetic resources be maintained and conserved on-farms. Simons and Leakey (2004) predict that human activity will have such a negative impact on many currently afforested regions in the tropics that in the near future it will be only possible to conserve important tree species, especially food tree species, if they are managed on-farms. The key to success for genetic conservation and utilisation of food tree species will therefore lie in the development of programmes that harmonize conservation and sustainable utilization of biological diversity through domestication and improvement within a mosaic of land-use options. This seeks to bring about agreement and consensus among the various stakeholders involved in the management of food tree species, so that needs and aspirations of all interested parties are met. Clearly there should be a close and continuing collaboration, dialogue and involvement of stakeholders in the planning and execution of related programmes.

Conservation of genetic resources of food tree species can involve *in situ*, *circa situ* and/or *ex situ* approaches. The *ex situ* method involves the transfer of plants or propagation material (seed, pollen, tissue, vegetatively propagated materials) from their original site to gene-banks, plantations and/or genetic conservation stands. Apart from the plantations or genetic conservation stands other types of *ex situ* approaches are not discussed. The *in situ* conservation method consists of conservation of genetic resources of target species on site, within the natural or original ecosystem in which they occur, or on the site formerly occupied by that ecosystem (Kanowski and Boshier, 1997). Although most frequently applied to populations regenerated naturally, *in situ* conservation may include artificial regeneration whenever planting or sowing is done without conscious selection and in the same area where the seed or other reproductive materials were collected. Thus, the approach includes nature reserves, fully protected forests in which human intervention is deliberately minimised, natural regeneration either on farms or in the forests.

Kanowski and Boshier (1997) noted that classical *in situ* conservation, which seeks to maintain populations in the natural state, has often been overemphasized at the expense of *circa situ* conservation realized within production systems (farmer-based or on-farm in more managed populations). Considering how people have impacted forests and forest genetic resources, and the pressures on forests and trees in Africa, on-farm or *circa situ* strategies are seen a better option than classical *in situ* for conservation of forest genetic resources in general and for food tree species in particular (Kanowski and Boshier , 1997; Simons and Leakey, 2004).

2.2 The ex-situ conservation approach

2.2.1 Propagation material

Experiences from the last three decades with African Seed Centres (Burkina Faso, Mali, Senegal, Zimbabwe) show that long-term seed conservation generally does not work for tree species in general and for food tree species in particular (e.g. *Adansonia* species, *Vitellaria paradoxa*). Unsuccessful storage is due to inappropriate storage conditions, poor management of stored germplasm, pests and diseases, deterioration of germplasm following power failures in cold storage facilities, the physiological state of the seed material (e.g. recalcitrant seed) and other stored plant materials. However, success stories of seed conservation like *Prosopis africana* seed collected and stored in seed banks at ICRISAT Sahelian Centre in Sadoré, Niger, and some other tropical tree species stored by CIRAD-Forêt are quite encouraging.

2.2.2 Genetic conservation stands

Genetic conservation stands include plantations to conserve a given population, arboreta, species and provenance trials, seed stands and clonal stands. To be effective, the genetic material must be representative of the genetic composition of the species. Practically, seeds or clones should be collected from at least 50 genetically unrelated trees. Various studies suggest that trees separated by at least 50 m are not genetically related, but this clearly depends on pollen and seed dispersal distances.

2.3 *In situ* conservation approach

A conservation programme for any species would be incomplete without a strategy for the protection of wild populations. One advantage of this approach is that a species is conserved together with its symbionts, pollinators and other associated species, which is difficult or impossible in other types of conservation. Conserving genetic resources in their natural environment, whether in production forests or in protected areas, has an advantage of conserving the function of an ecosystem rather than just conserving species. This means that *in situ* programmes for conservation of selected target food tree species in Africa may result in conservation of a number of valuable associated animal and plant species. *In situ* conservation implies that a given population is maintained in the environment in which it has evolved. This implies the planned and systematic management of identified target species in a network of conservation areas such as Strictly Protected Areas, managed forests and Nature Reserves.

In Africa, it is widely recognised that the Protected Area system alone is insufficient to provide the necessary geographical and biological coverage, so efforts should be deployed supporting conservation on farms. Many parks and classified forests in most African countries have been encroached, depleted and destroyed. Some of them exist only on maps and in official speeches. Unless there are drastic changes in the management of such sites, the future of such areas is uncertain. Agroforestry provides an important means for conserving biodiversity (Kanowski and Boshier, 1997; Weber et al., 2001; Tchoundjeu et al., 2006). When an active tree planting culture exists and participatory tree domestication is practiced, hundreds of native food tree species could be conserved in African tree-crop ecosystems for current and future needs.

Regarding intra-specific genetic variation, studies have confirmed similar levels of genetic variation across natural and farmland populations, and very little differentiation between unmanaged and managed stands for various species (Dawson et al., 2009). Variation has been characterised extensively in farmers' fields and natural forests for a number of commercially important food trees, including *Adansonia digitata, Irvingia gabonensis, Dacryodes edulis, Vitellaria paradoxa* and *Tamarindus indica*. Studies have also confirmed small differences in the average number of species between forest reserves and farms in the West African Sahel (Kindt et al., 2008). Therefore, incorporating conservation of genetic resources of food trees on farms will offer, in many cases, the best prospects for achieving diversification and conservation goals in Africa. Understanding the relative magnitude and geographical distribution of genetic variation within the species are essential for designing conservation plans. Molecular markers can provide information on how diversity is structured within and among natural, managed and cultivated stands, and can measure the extent to which individuals and populations are connected to each other (e.g. Dawson et al., 2009).

2.3.1 Protected forests and gene conservation areas

The ultimate expression of *in situ* conservation of forest genetic resources is the maintenance of a comprehensive network of reserves. Protected areas and their role in conservation of forest resources have been extensively discussed (Kanowski and Boshier, 1997). Protected areas are dedicated to the protection and maintenance of biological diversity and associated cultural resources, and managed through legal or other effective means. Such areas cover various situations, ranging from managed resource areas (including gene conservation areas), protected watersheds, national parks and strictly protected reserves to sacred forests. Moreover some individual taxa have been protected in gene conservation areas, e.g. *Acacia senegal* in Burkina Faso and Sudan, and *Sclerocarya birrea* in Zambia.

Although most protected areas are state managed, few have been established according to genetic principles. Reserve design and management are more often determined by political, social and economical considerations (Kanowski and Boshier, 1997). Moreover, their location on slopes and sites of lower fertility and in stands of lower economic value have biased their composition and limited their value for genetic resource conservation. In addition, because of the large areas required to maintain sufficient effective population sizes of most tree taxa, and the impracticality or undesirability of managing exclusively for conservation goals, there are limited prospects for *in situ* conservation on an adequate scale (Kanowski and Boshier, 1997).

Protected areas were established during the colonial era in most countries in sub-Saharan Africa, but today these national parks and nature reserves are very depleted and subject to disappearance. A survey of 10 developing countries with large forest resources found that only 1% of the protected forest areas are secure, with more than 20% suffering from degradation, and 60% currently secure but with threats likely in the near future. Unless there are immediate changes in the management of protected areas, today's socio-economic constraints in Africa limit the success of such ambitious schemes. The main problem in achieving conservation goals is the lack of adequate institutional and political frameworks in which land-use and operational management choices that are fair to all stakeholders, can be considered, efficiently implemented, monitored and regularly adjusted to meet new and emerging needs. Therefore forest genetic resource conservation decisions should not be made in isolation but as an integral component of national development plans and conservation programmes.

2.3.2 Conservation on farm and forest restoration and rehabilitation

Managed forests in farming systems

In the most extreme case, where forests have become highly fragmented, effective genetic conservation is likely to require both proactive ecological restoration and conservation through use by local communities (Kanowski and Boshier, 1997). The latter, *circa situ* strategy acknowledges the role which generations of rural communities have had in maintaining the genetic resources of most food trees in most African farming systems, e.g. *Adansonia digitata* (West, Central, Eastern and Southern Africa), *Dacryodes edulis* (West and Central Africa), *Detarium microcarpum* (Sahel), *Irvingia gabonensis* (West and Central Africa), *Parkia biglobosa* (Sahel), *Saba senegalensis* (Sahel), *Sclerocarya birrea* (West, Central, Eastern and Southern Africa), *Tamarindus indica* (West, Central, Eastern and Southern Africa), *Vitellaria paradoxa* (West, Central and Eastern Africa), and *Ziziphus mauritiana* (West, Central, Eastern and Southern Africa).

For tree species in general and food tree species in particular, the following case study in the West African Sahel may be scaled up in other regions for genetic resource conservation on-farm. In the West African Sahel, research suggests that provenances from drier zones are better adapted to drought than provenances from more humid zones. Considering the fact that some computer models predict a drier climate in the Sahel during the 21st century, it would be prudent for tree domestication and conservation programs to conserve and collect germplasm from the drier parts of the region for future plantings. Specifically, transfers of germplasm should only be made from the drier to the more humid parts of the region. This strategy could increase the drought tolerance/adaptation of tree populations on farms in subsequent generations as a result of gene flow from the introduced germplasm.

These on-farm plantations can have research, conservation and development objectives. There has been very little research on intra-specific genetic variation in native tree species from the West African Sahel, so the results contribute to our knowledge. The knowledge in turn is essential to design appropriate strategies for tree domestication and conservation programs. The on-farm plantations also serve as *circa situ* conservation sites for the selected germplasm, and can be managed as seed orchards for the production and sale of source-identified selected germplasm for reforestation programs in the region.

In addition, protecting and encouraging natural regeneration may be an effective conservation approach. Such silvicultural practices have been applied successfully in some forests and agroforests in the West African Sahel, and in East and Southern Africa. Restoring the vegetation by assisting natural regeneration is one urgent measure for helping to rehabilitate the environment and provide the ever growing African population with benefits provided by native tree species, especially food tree species. This also helps conserve the environment and landscape for future generations, and restore the threatened plant and animal biodiversity.

3 Food Tree Domestication, and Plant Genetic Resources Conservation and Management

Tree domestication seeks to develop tree/plant products with a view to increasing their contribution to the livelihoods of people who depend on the trees and to the GDP while conserving their productive potential. To achieve this, tree domestication programmes should manage/conserve the resources and foster and organize the marketing of tree/plant products at the local, national and international levels.

In setting priorities for domestication, the choice of species for improvement is complex in both socioeconomic and biophysical terms, and the clients consist of many individual smallholder households with differing needs, making it difficult to generalize across an ecozone. Farmers use many different species and there is little scientific knowledge about most of the species.

In the last decade, agroforestry domestication strategies, approaches and techniques, together with the marketing of agroforestry tree products (AFTPs) have become one of the pillars helping to eradicate poverty and hunger, promote social equity, environmental sustainability and combat desertification (Leakey et al., 2012). The philosophy of the agroforestry tree domestication initiatives builds on the efforts of smallholder farmers who for years cultivated tree species on their farms.

To achieve greater impact, tree domestication programmes need to consider national and regional priorities, and implement the programmes in networks with all relevant stakeholders, including farmers, national agricultural research systems (NARS), universities, extension services, non-governmental organisations (NGOs) and development projects. Tree domestication has been defined as a long-term process with different inter-linked steps (Leakey et al., 2004; 2008). For most tree species, it may simply consist of identifying suitable seed sources and developing appropriate propagation and cultural practices. In widely planted and economically important timber and fruit tree species for example, the full domestication process may involve systematic sampling and characterisation of genetic variation, development of optimal propagation and horticultural techniques and intensive breeding, including the use of molecular genetics technologies. Tree domestication seeks to bring out the maximum human benefit within a species as it becomes genetically refined from a wild tree to a cultivated plant (Simons and Leakey, 2004).

Tree domestication can be implemented on farm by the farmers, who bring the trees into cultivation themselves and/or on research stations through genetic improvement programmes. Participatory tree improvement approaches should be introduced on farms. One option in the breeding programme that will be of interest to farmers and industries is the mass vegetative propagation of the most superior individuals selected from the introduced cultivars and plus trees, or created from the breeding population by controlled pollination. Efficient techniques for vegetative propagation of local fruit trees are now well developed and widely practiced. Such techniques can facilitate the mass reproduction by farmers of individuals selected for specific combinations of traits.

There are few conclusive studies of genetic variation within the proposed priority food tree species for sub-Saharan Africa. Some preliminary results show genetic variation in both growth and fruit traits within some of the food species (e.g. *Adansonia digitata*, *Dacryodes edulis*, *Irvingia gabonensis*, *Parkia biglobosa*, *Saba senegalensis*, *Screlocarya birrea, Tamarindus indica*, *Vitellaria paradoxa* and *Ziziphus mauritiana*), thus supporting investment in further improvement through selection and breeding. Moreover, some provenance/progeny trials have been established, but results regarding food products (mostly leaf and fruit production) are very scanty. Genetic improvement using selected materials should be initiated for higher productivity and adaptation of food tree species in different landscapes. The selection of plus-trees and their clonal development may be a faster means of improvement and have greater impact than conventional breeding (Kalinganire et al., 2008). However, genetic diversity should not be reduced, especially on farms because farmers tend to use very few clones/trees to establish food tree crops. In any case, selection and breeding of food tree crops can achieve enormous gains in yield and quality.

4 Concluding Remarks

The paper presents two strategies for the better use of genetic resources of food tree species. These strategies consist of the best options for genetic conservation and for domestication. As forests are degrading, new situations require new approaches to biodiversity conservation, especially considering projected climate changes across Africa due to global warming. Tree biodiversity should be conserved not only in parks and nature reserves (and other large set-aside forests), but also on farms. Moreover, mainstreaming a broad genetic base through the conservation of a range of provenances of target species, preferably on farms and in the species' natural distribution area, is likely to be the safest available option to-date. In addition, more attention should be paid to the value of forests elsewhere in the landscape and ways for optimising production and conservation in these forests.

Food tree species are very important for the rural communities in sub-Saharan Africa, but considerable work is needed to conserve of their genetic resources and increase productivity. This requires all stakeholders working in a participatory manner with rural communities and with policy makers and shapers. Moreover, the approach that links conservation through use with development should be better understood in the context of multi-functional landscapes. The success of campaigns to promote conservation and use for native food tree species in sub-Saharan Africa could be substantially enhanced if they were accompanied by improved marketing opportunities, advances in domestication, and changes in policies that govern protected forests, limit farmers' access to forest resources and discourage farmers from planting trees.

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The Untapped Potential of *Casuarina equisetifolia* in the Kenyan Coast

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Key words: agroforestry, coastal region, community land, poverty, seedling uptake, tourism

Abstract

Casuarina equisetifolia - is one of the most preferred tree species in the coastal region of Kenya for construction poles. The species has not yet been utilized for other products commercially although the required technology and markets are available. Despite the huge environmental and economic benefits of this species, the coastal region remains poor with most farmers reluctant to embrace the growing of the species as an economic venture in the face of poor climatic conditions that have made crop farming unproductive. The objectives of the present study was to assess the trends of *C. equisetifolia* uptake and determine the factors affecting its uptake by farmers in the coastal region. The study sites were the two major *C. equisetifolia* growing counties of Kilifi and Kwale. The counties have been growing *C. equisetifolia* intensively and have diverse socio-economic conditions which allowed for comparison of trends. Data collection was done through review of secondary data mainly records kept by Kenya Forestry Research Institute, Kenya Forest Service and private nurseries. Results revealed that *C. equisetifolia* uptake varied between the sites with Jilore area in Kilifi county leading with 7,993 seedlings planted in a year, while Buda area of Kwale had the lowest uptake at 1,399 trees in a year. The level of uptake also varied between the years. Uptake in almost all the sites was lower between 2003 -2008 and highest between 2009 and 2012 for most sites except Buda whose uptake was highest between 2005 and 2006. Generally, the uptake of *C. equisetifolia* is still relatively low. In order to enhance its growing and improve farmers' livelihoods and environmental conservation, there is a need to create awareness and start value addition schemes targeting products like chip board, briquettes, timber, paper and, transmission poles as well as carbon credits to enhance co-benefits.

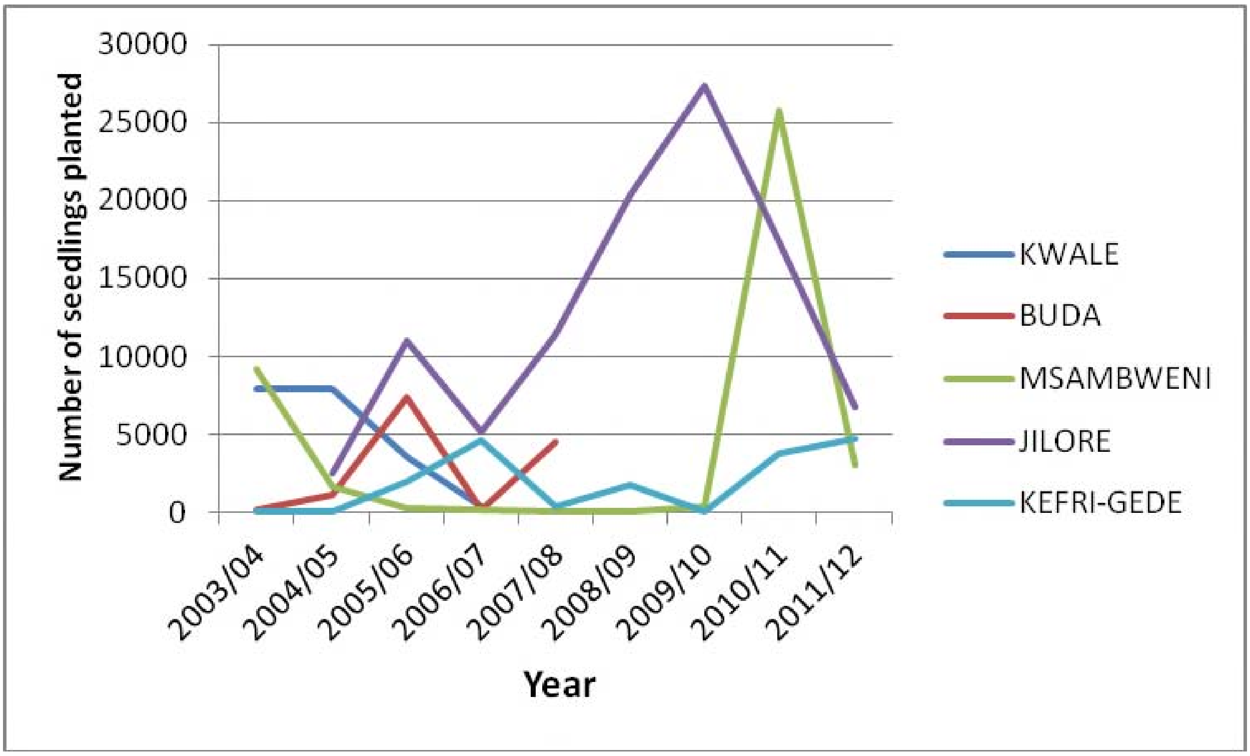
1 Introduction

According to Stiftung (2010), the overall poverty level in Coast province of Kenya is 62% and a majority of its residents depend on agriculture for their livelihoods. However, smallholder agriculture faces challenges such as low productivity, high dependency on rainfed agriculture, insecurity of traditional land tenure system and environmental degradation due to unsustainable agricultural practices resulting in low productivity and consequently high poverty levels. Agroforestry is an intensive land use management system that combines trees or shrubs with crops and/or livestock, and is generally aimed at diversifying products, markets and farm income as well as enhancing environmental conservation (Lasco, 1992). Other benefits of agroforestry include improvement of water and soil quality through replenishment of nitrogen and other trace elements, enhancement of land and aquatic habitats for fish and wildlife and improved biodiversity (Kabwe et al., 2009). It is thus an effective way of achieving more sustainable production systems while addressing environmental concerns.

Casuarina equisetifolia is a high priority agroforestry species commonly grown along the Kenyan Coast. It is an evergreen, dioecious or/and monoecious tree that grow up to 30m tall and has a finely branched crown. The trunk is straight, cylindrical, usually branchless for up to 10m, and up to 100cm in diameter, occasionally with buttresses. The bark of the tree is light grayish brown and smooth on young trunks. The species occurs naturally on tropical seashores around most coastal areas of the Pacific and Indian Ocean, and has been naturalized in Kenya (Omondi et al., 2004). It prefers a humid climate with a mean annual rainfall of between 600-3,000mm, altitude range of 0-1200m and mean annual temperature of between 10 ˚C and 35 ˚C. *C. equisetifolia* tolerates drought well for 6-7 months but is sensitive to frost. It grows on a wide range of soils from coastal and lowland lava flows, poor soil of fenland and limestone soils, but the perception that the tree is a coastal species planted to produce poles for constructing tourist hotels has largely determined its distribution along the Kenyan coast (Mbuvi, 2010).

In Kenya, the species is mainly grown in the coastal region by both small and large scale farmers with the latter accounting for 81.8% of the production (Mbuvi, 2010). Additionally, the species is an important multipurpose agro-forestry species preferred for its nitrogen fixing properties besides being used in land reclamation. For

Fig. 1. Number of seedlings produced from KFS and KEFRI nurseries for planting



instance, it has been successfully used by Bamburi Cement Company (in Kenya), to reclaim limestone quarries.

Although *C. equisetifolia* is ranked as a high priority species in the Kenyan coast, its uptake is still relatively low. The present study was undertaken to assess the trends of *C. equisetifolia* uptake and determine the factors affecting its uptake by farmers in the Coast region.

2 Materials and Methods

Data collection was done through review of secondary data mainly kept by Kenya Forestry Research Institute (KEFRI), Kenya Forest Service (KFS) and private nurseries. Nursery data for a period of at least 10 years (2003-2012) was collected and the total number of seedlings either issued free or sold each year in all the sampled nurseries were recorded and computed to determine the total number of seedlings planted. Additionally, key informant interviews were held with officials from KEFRI, KFS and private nurseries to determine the factors affecting tree uptake levels by farmers. Descriptive and inferential statistics were used to analyse the data, summaries of representative practices were produced and results presented using graphical techniques.

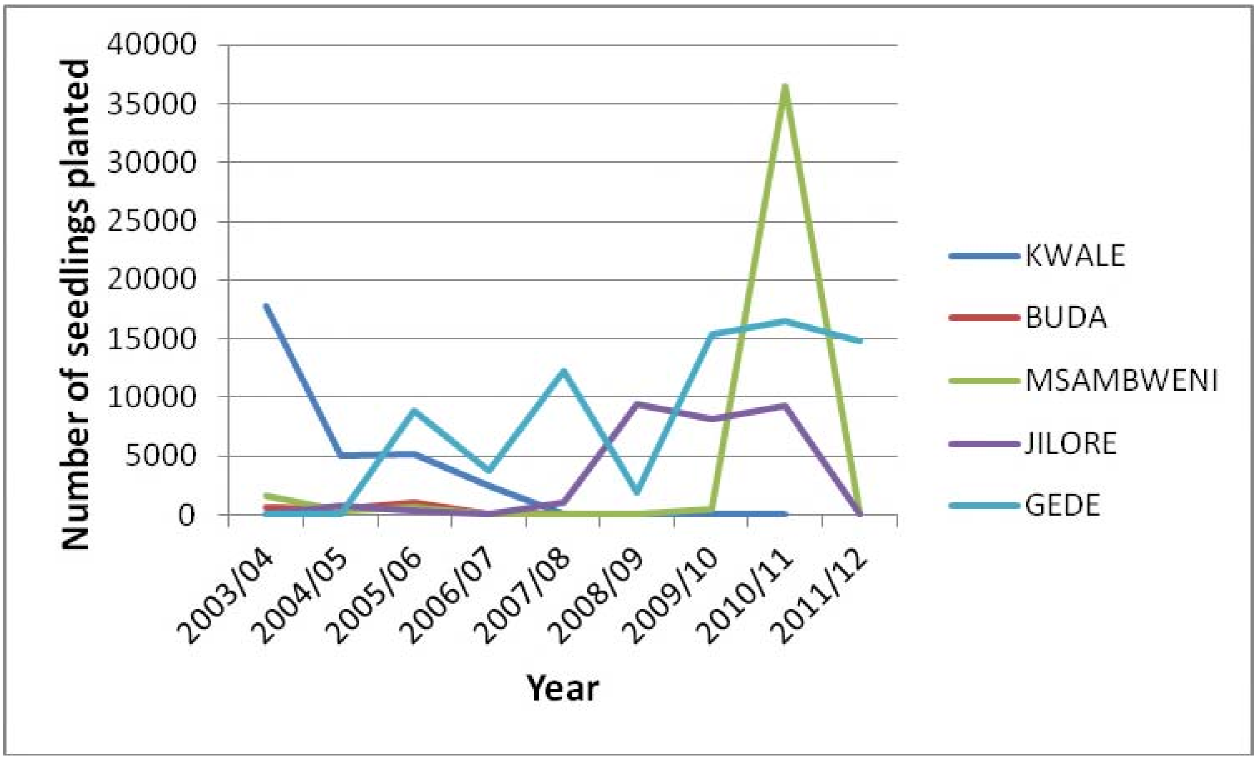
3 Results and Discussion

3.1 Trends in uptake of *C. equisetifolia*

The average number of seedlings supplied from Jilore KFS nursery between the year 2003 and 2012 was 7,993 per year (Fig. 1). This was the highest recorded number followed by Gede (5057), Msambweni (4446), Kwale (3669) and Buda (1930). The scenario in Jilore can be attributed to the fact that *C. equisetifolia* was first grown in Jilore KFS tree nursery in the late 1960s and it has over the time increased the production to meet the growing demand for *C. equisetifolia* due to increased awareness of its economic and envirnmental value to the local community.

Uptake trends also varried between the years (Fig.1 & 2). Uptake levels in almost all the sites was lower between 2001 and 2008 and highest between 2009 and 2012 for most sites except Buda where uptake was highest between 2005 and 2006. The increased uptake can be attributed to the increased levels of community awareness of the importance of *C. equisetifolia* in the recent years. This coupled with the growth of tourism and the subsequent demand for *C. equisetifolia* for construction in tourist hotels, has resulted in increased uptake of the species. There were no seedlings recorded in Buda between 2008 and 2012. This can be attributed to its interior location hence low accessiblity. Furthermore, competition from private nurseries has rendered institutional nurseries dormant since most large scale farm owners contract individuals to raise seedlings which they in turn plant on their farms. In Msambweni, uptake levels were generally low with less than 2000 seedlings planted between 2003 and 2008. This was followed by a sharp increase to over 35,000 seedlings between 2009 and 2012 and thereafter a drastic decline between 2011and 2012.

Fig. 2. Number of seedlings produced from private nurseries for planting



Of all the seedlings obtained from the private nurseries for planting, Gede recorded the highest number of seedlings per year at 8183 seedlings, followed by Msambweni (4417), Kwale (3795), Jilore (3235) and Buda (450). In areas where seedling production by instituional nurseries was low, private nurseries were producing more except in Buda. In general, private nurseries have moved in to fill the gap left by institutionl nurseries in meeting the growing demand for *C. equisetifolia* in the coastal region.

3.1.1 Relationship between tourism and *C. equisetifolia* uptake trends

Results of the present study indicates that tree uptake levels have been increasing as visitor numbers and consequently the income generated from tourism increases (Table 1). For instance, between 2009 and 2011, both uptake levels, tourism numbers and tourist revenues were at their highest. These factors can be attributed to the fact that the coastal region constitutes nearly 60% of Kenya's tourism. With the growing number of tourists visiting the Kenyan coast, more tourist resorts are being constructed and/or repaired hence a huge demand for *C. equisetifolia* poles. Consequently, farmers are growing more trees in order to meet the huge market demand and generate additional income.

3.2 Factors affecting uptake levels

3.2.1 Communal land ownership

Family controlled access of land has been a major hindrance to the species' long-term development ventures in the coastl region of Kenya. A large proportion of the land is owned jointly by families and clans while a sizeable number of residents live as squatters. Key informants were of the view that although some farmers were willing to undertake tree farming, conflicting interests among family members has made it difficult for individuals to make key investment decisions. Furthermore, lack of security of tenure was hampering female and young farmers from adopting agroforestry practices.

3.2.2 Inadequate awareness among farmers

The study revealed that most residents of the coastal region were not aware of the economic benefits of tree farming. Most residents still view tree farming as an outdated, non-profitable venture geared towards environmental conservation. Furthermore local communities are ignorant of diverse tree products and services such as chip boards and briquettes timber, paper, transmission poles as well as carbon credits that can further enhance benefits of tree farming.

3.2.3 High poverty levels

According to the key informants, majority of coastal residents are poor with Ganze District in Kilifi County being rated as one of the poorest Districts in the country. The high poverty levels have made residents prefer short term economic gains such as crop farming (currently unproductive due to the changing climatic patterns) for immediate survival as opposed to long-term high return

Table 1. A comparison between visitor arrivals, tourism earnings and *C. equisetifolia* uptake trends in coastal Kenya

Particular	2003	2004	2005	2006	2007	2008	2009	2010
C. equisetifolia uptake	34,991	19,789	40,044	16,870	29,637	33,535	52,847	110,847
Tourism earnings (Ksh. in billion)	25.8	38.5	48.9	56.2	65.2	52.7	62.5	73.7
Number of visitor arrivals ('000)	1,146	1,361	1,479	1,600	1,817	1,203	1,490	1,609

Source: KNBS (www.knbs.org.ke/tourism-summary) and field survey data; 1 USD = 102 Ksh (approx.)

economic ventures such as tree farming. Furthermore, most poor people are either squatters, live in communal land or own very small parcels of land that cannot accommodate both crop and tree farming hence making agroforestry non-viable to them.

3.2.4 Insufficient production of quality seeds

Despite being ranked as a high priority species in the Kenyan coast, production of sufficient and quality seeds is still limited thus disadvantaging interested target groups. Efforts are however being put in place by stakeholders such as KEFRI and KFS to establish seed stands in suitable sites in the coastal region with an aim to produce large quantities of high quality seeds.

4 Conclusion

C. equisetifolia benefits farmers through diversification of products, markets and farm income, improvement of soil and water quality, and improved biodiversity. However, unless farmers widely adopt the species as part of their farming system, its potential benefits on livelihoods and the environment will not be realized. There is a need to create more awareness on the importance of tree farming as an economic venture in order to change farmers' attitude towards tree farming for economic gain. Value addition schemes targeting products such as chip boards, briquettes, timber, paper and transmission poles, as well as carbon credits should be initiated so as to enhance co-benefits and maximize farmers' income from tree farming. Land tenure problems in the coastal region need to be addressed through initiatives like conversion of land held under customary tenure into individual holdings for encouraging long term investments such as tree farming.

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Promoting the Use of N_2-fixing Casuarinas for Profitable Farm-forestry in Australia: Realising the Vision

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Abstract

N_2-fixing casuarinas and acacias are well-recognised indigenous components of the Australian flora, both families making significant contributions to the nitrogen economy of natural ecosystems. For the brigalow-belah dominated areas in northern Australia, their inputs of nitrogen allowed production of wheat using the high levels of NH_4^+-saturated clay for many years. However, despite the centres of origin for both families being in Australasia, their potential in Australian farm-forestry using rotations less than five years has been little explored. Active applied research into the Casuarinaceae (*Allocasuarina, Casuarina, Gymnostoma, Ceuthostoma*) and its N_2-fixing microsymbiont *Frankia* is largely restricted to India, China and France. Farmers in India are pioneering profitable farming of casuarinas for paper manufacture in 3-year rotational cycles with rice. We propose major investment in a new rural scheme to promote the application of casuarinas on Australian farms and to develop local rural secondary industries with strong cash flow, responsive to market forces. Large scale carbon sequestration (up to 20 tonnes dm/ha/annum) can be achieved, reducing N-fertiliser use and expanding farmers' product and cash flow options, using vertically integrated production to maximise their value. These forest products include wood and fuel pellets, paper pulp, charcoal for hydrogen production, N-fertiliser and volatile chemicals, even including ammonia and hydrogen. One million ha of casuarinas could produce $50 million of C-neutral N-fertiliser per annum as by-product, or adequate ammonia as stock for electricity generation or H_2 production using the N_2 fixation reaction for heat storage. This article examines the prospect for integrated uses of these under-utilised genetic resources, planning the required applied research in profitable fire-resistant landscape design and community economic development.

1 Introduction

Casuarinas are very fast-growing, N_2-fixing trees mainly distributed in Australasian region (Ganguli and Kennedy, 2013). This article proposes a major innovative research program to make better use of this genetic resource, organised in several integrated phases. Firstly, short term (3-5 year) farm-forest rotations are identified as a significant gap in Australian farming research; their increased use in these rotations offers prospects for large scale carbon sequestration (10- 20 tonnes dm/ha/annum) with reduced fertiliser-N use, while improving farmers' product options. For best farming futures, diversity in farm design is important. The more viable choices farmers have, the better they can respond to changes in climate, commodity prices and other market conditions that define profitability.

Second, farm-forestry is increasingly regarded as having potential for providing new secondary industry options, with vertically integrated production to maximise the value of these products. However, such plans will need site specificity for both inputs and outputs, responding to terrain, soil type, available water as well as markets for secondary products. These activities will most often be on a local scale and include wood products, paper pulp (Parthiban et al., 2012), charcoal for hydrogen production, N-fertiliser and volatile chemicals, potentially including ammonia and hydrogen gas. It is claimed here that 1 million ha of Casuarinas could produce $50 million worth of C-neutral N-fertiliser per annum alone, making a strong base for profitability from several times this value of other products. Secondary chemical products and even diversified electrical power generation using solar thermo chemical energy storage for 24-hr supply, adding immensely to the future prospects of rural communities.

Thirdly, farm-forestry with frequent rotation minimising waste materials acting as fuel can be a timely response to national emergencies such as bushfire. Australia's recurrent cycle of flood and excessive vegetation and fuel generation followed by devastating fires, promoted by drought conditions of extreme low humidity has now become a chronic condition. Invariably, there is a lack of preparedness for extreme conditions and huge insurance losses are suffered on an annual basis, quite apart from loss of human life and livestock. By contrast, intelligent and strategic landscape design using all the benefits of GPS mapping could provide a new kind of water and fire management, using all the technological resources available. This future would avoid high risk monoculture stands of forest trees encouraging both disease and fire. There is much to learn about reducing the risk of fire, however at the moment far too few resources are made available to overcome this threat or reduce its intensity.

Such a program can only succeed if the rural landscape is repopulated, reversing the drift to major cities. That will require a development program involving a complete suite of disciplines, with the adequate social investment. Clearly, such a challenging task will require decades to achieve.

1.1 Research objectives

Casuarinas species are chosen as highly water-efficient nitrogen-fixers and an under-exploited natural resource in Australia. A comprehensive research project would include the following objectives:

- A significant review of previous research outputs on casuarinas, with reassessment for farm-forest rotations. This should incorporate close examination of successful research elsewhere, particularly in India (Kumaravelu et al., 2012) and in China.
- Experimental field data on farm productivity of casuarinas for carbon and nitrogen fixation in cropland and rangeland landscapes.
- Data on the suitability of farm-forest rotations in areas with steeper terrain or on land potentially needing rehabilitation, such as highly acidified areas. Within the Casuarinaceae, there are shallow and deeper-rooting species from which to choose for each site. Other species such as nitrogen-fixing acacias may also be suitable for particular sites providing biodiversity.
- Data on the potential for farm-forest rotations with deeper roots to provide better water management, remediating acidification. These may have negligible access to irrigation facilities and annual rainfall may be scanty. Managing water tables, often saline, can also be achieved by deep-rooted species - of casuarinas like *C. obesa* (Hollingworth, 2007).

A broad program of research should investigate the full economic potential of regional industrial production from farm-forest casuarina and acacia plantations, based on their capacity to fix atmospheric N_2. In principle, if the industrial production is shown to be feasible, increased local populations employed adding value to farm-forest products will be necessary, requiring new economic assessment to establish needs for investment.

An eventual requirement will be implementation of new carbon sequestering technology on farms, but focus on the preliminary research is needed showing its feasibility. Full implementation will require a new participatory action phase where the technology will be adapted regionally and climatically.

The program will also require the support of new government policy, allowing implementation of farm forestry on the large scale required. Widespread extension of necessary tools to farming and rural communities will be essential, no doubt with new legislation. Major success in the technology will require social readjustments and reintegration on a large scale, involving more jobs in factories close to farm supplies to minimise transport costs. Only high value timber and other elite forest products can be transported economically to long distances. Power generation, itself a product of the new developments would likely be localised.

Fig. 1. Alley farming of mallee eucalypts in the Narrogin area in Western Australia (Photo credit: Future Farm Industries Cooperative Research Centre).

The position paper produced in stage 1 will be a quantitative technical and economic assessment, analysing the potential for farm-forestry to enhance the productive capacity of farms normally growing other produce. The feasibility to establish an ethical supply of renewable energy able to completely replace coal will also be assessed (e.g. 7.5 million ha of casuarinas on favoured farmland, 25% of current Australian cropland, could produce 150 million tonnes of dry wood or cogeneration pellets for electricity per annum, based on recent Indian data for ethical paper pulp and paper manufacture (Jain, 2011; Parthiban et al., 2012). Other by-products could support a substantial chemical industry, including more than the entire Australian requirement for nitrogen fertiliser (Kennedy, 2012), with no carbon dioxide penalty unlike chemical fertilisers like urea (about 5 million tonnes carbon dioxide equivalents to the atmosphere per year).

The surprisingly limited previous Australian research on the indigenous Casuarinaceae has been relatively unfocused, conducted mainly in connection with international aid programs. For example, CSIRO's ACIAR work was largely related to production of fuel-wood in Africa. Recent intensive overseas research in India and China is strongly production oriented. Project outputs will include assessment of all quantitative data related to the possible economic value of forest-farming production of casuarinas, an output that would be environmentally sustainable and highly ethical as native species. This will include quantities and composition of foliage and cone fall, wood and pellet production and assessment, total carbon and nitrogen fixed and soil C and N sequestration. The expertise of Australian foresters (e.g. FORSCI – Director John Turner) as well as in institutions such as CSIRO and universities will be essential to promote the quality of this output. Given that casuarinas may require protection from fire, this would be an opportunity for landscape design on a sufficient scale to manage the fire risk. This contrasts with the current inadequate investment in this area, characterised by some fuel reduction burns but little else.

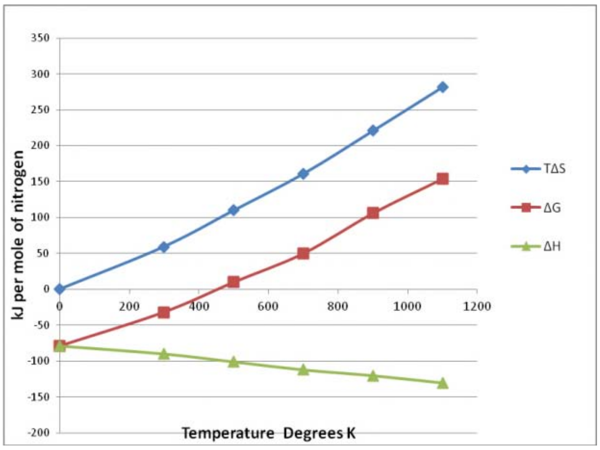
2 Project Outputs

Ultimately, the outputs of this project would be the basis for new rural industries, on the scale of millions of hectares of products, able to satisfy a significant portion of our needs for fuel (high density casuarina matches coal in calorific content, burning to a pure white ash), paper pulp, chemical products and nitrogen fertiliser. Such production, responsibly integrated for genetic diversity, site specificity and productivity, and product selection governed by current commodity markets, would be certifiable to high standards of environmental stewardship. The project would need to strongly adhere to the ethical guidelines of the Montreal Process (see www.montrealprocess/).

3 Farm Forestry

This activity will aim to establish a new farming practice and demonstrate benefits as well as expose the challenges.

Fig. 2. Thermodynamics of the nitrogen fixation reaction $3H_2 + N_2$ $2NH_3$ under standard conditions of 1 atmosphere pressure of reactants and product at temperatures shown. K_p is equal to 1.0 at 450 K (ΔG=0), where ΔH is equal to -TΔS and ΔG is equal to zero. Dinitrogen has a very stable bond energy at 298 K of 945 kJ per mole, so in the Haber process the reaction is run at higher temperature and pressure, to speed its rate and to shift the reaction towards ammonia formation. Changes in entropic energy (TΔS), in free energy (ΔG) and enthalpy (ΔH) will differ under reaction conditions, which will be discussed using newly calculated data based on the action thermodynamics method (Rose et al., 2008).

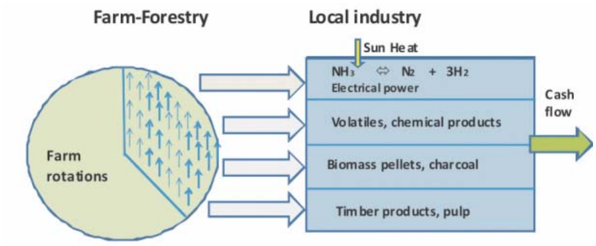


The outputs from the field trials and data analysis sought will include: (i) information on the nutrients most susceptible to depletion on any site; (ii) the age of greatest nutrient stress; (iii) the quantities of nutrients removed with different harvesting strategies; (iv) the potential cost of nutrient replacement; (v) optimum planting density; (vi) best genetics for casuarinas (Suresh et al., 2012), eventually needed for cloned production of highest productivity lines; (vii) optimal ages for harvesting for different purposes; (viii) ability to rehabilitate acidified or salinised soil; (ix) choice of species and lines to adapt to climate change.

3.1 Biological technologies – microbiology and forest genetics

At the University of Umea, a set of *Frankia* isolates have been maintained by Anita Sellstedt (Sellstedt, 1995), from a prior CSIRO collection of the 1980s. In collaborative research, these and other cultures now cultured at the University of Sydney will be characterised using molecular analysis of ribosomal DNA so that strain typing can be achieved. These techniques will then allow correlations between root-nodulating *Frankia* strains and casuarina productivity to be made, a necessary part of allowing the symbiosis to be optimised for effectiveness and to achieve maximal productivity.

Fig. 3. Integrated farm forestry- local industry would be based on an action-entropy model sustained by optimised support resources (Rose et al., 2008) allowing diversification and decentralised populations.



Amongst many research outputs from postgraduate students funded by the project will be sets of primers for PCR identification of strains of *Frankia* including those for nitrogen-fixing nif genes. PCR enables amplification of small DNA or RNA signals. These will allow confirmation of effective strains isolated from nodules and survival studies in soil. This will help ensure that the nitrogen-fixing symbiosis between *Frankia* and casuarinas are as effective as possible under field conditions. *Frankia* are difficult organisms to isolate from soil or nodules, partly because of their slow growth. Molecular techniques will allow identification of strains and also provide some measure or numbers, using real time PCR, collaborating with overseas experts in this area at the University of Umea (Anita Sellestedt) and in CIRAD, Montpellier, France (Claudine Franche).

3.2 Plant improvement by breeding and cloning

In India, large scale breeding of better clones of casuarinas selected for homogeneous and rapid growth has been achieved (Nicodemus et al., 2011; Kannan et al., 2012; Karthikeyan et al., 2013). This work will need to be repeated in Australia, with new selections to examine the range of diversity. There is much to be learnt regarding genetics and heritability of properties. At the University of Sydney, exhaustive chemical analysis of casuarina seedlings and trees can be carried out, including additions to soil carbon using CSIRO-recognised protocols. This will enable confirmation of carbon accumulation below and above ground and the spectrum of the phytochemical products (volatiles and polar compounds) as part of the general composition of trees as potential economic products to be understood. FORSCI based in Sydney has vast experience in forest nutrition and productivity and will be integral to the program. Australia has suffered a vast loss in personnel with forest expertise and this will enable a period of rebuilding that which has been lost, including education. The link of forestry with potentially all farmers in Australia will be a major stimulus to recovering this expertise.

In all this research, quantitative information will be sought, including the relative value of products such as timber, paper pulp, charcoal, biomass pellets, organic volatiles, N-fertilisers, ammonia, hydrogen and soil enrichment or depletion. The position paper will set down a technical and economic assessment, analysing the potential for farm-forestry to enhance the productive capacity of farms normally growing crops and livestock. The feasibility to establish an ethical supply of renewable energy able to complement the use of coal or even replace it eventually will be assessed. For example, 7.5 million ha of casuarinas on favoured farmlands could produce 150 million tonnes of dry wood or cogeneration pellets for electricity per annum, of the same order as the current consumption of coal in power stations. Other chemical products could support a substantial chemical industry or supply the entire Australian requirement for N-fertiliser. Companies like Australian New Energy would have a major role here. Climate change offsets might include a process that major electricity generators would be required to finance a compensating fixation of carbon dioxide so they could approach neutrality.

3.3 Secondary industry

Forest products are well recognised as supplying valuable feedstock for industry. In Western Australia some progress has been made in mallee farming in harvestable alleys (Sudmeyer et al., 2012), helping with ongoing landscape restoration (see Figure 1). This follows a period of excessive land clearing for wheat cropping that seriously failed, leading to widespread salinisation in the southwest of Western Australia. This mallee development still suffers from a lack of capital commitment at the large scale needed but it also would benefit if more integrated rural development was mandated as a national objective with a long time scale of several decades. Other technologies such as higher value pelletised densified biomass fuels and Syngas fuels as promoted by Australian New Energy (http://australiannewenergy.com.au/ contact Simon Penfold, based in Moolap, Victoria) will also be part of the program.

3.3.1 Ammonia as feedstock for H_2 generation

The root nodules of N_2–fixing casuarinas are clearly a major source of ammonia, normally used for plant growth. H_2 is often proposed as a clean fuel that would overcome many environmental problems related to pollution. But its main sources such as steam and gas are considered to be too expensive. Electrolysis of water to $2H_2$ and O_2 is usually considered as an alternative option, but uses large quantities of electricity and has only been used where cheap hydroelectric sources are available, as in Norway. However, ammonia itself can also be used as a viable fuel to directly power motor vehicles.

Less known is the fact that the Haber reaction normally used to prepare N-fertilisers can be reversed to produce hydrogen gas, consuming a considerable amount of heat (see Figure 2).

$3H_2 + N_2 \Leftrightarrow 2NH_3 + 66.8$ kJ (at 20 MPa, 293 K)

This reaction, usually catalysed using Fe-based catalysts and other metals and high pressures, is unusual in that it is poised and able to be conducted in either direction, depending on the conditions of reaction. As an exothermic reaction as written, it releases heat raising the entropy of its environment. However, in this finely poised reaction heat, coupled with adiabatic expansions can be used like a reactant, driving it towards release of N_2 and H_2 with solar heating. Technology to allow solar energy to be stored in a system operating in sunlight and then to be reversed at night has already been well developed at a pilot scale (Lovegrove et al., 2004) and is awaiting investment. This approach would be very appropriate for decentralised power generation, using portable factory units able to be installed from pre-fabricated units.

In principle, Casuarinas could offer a relatively inexpensive source of ammonia from their foliage, harvested on a continuous basis if farms operate on a 3-4 year rotation cycle. It is not unreasonable to assume that 100 kg of ammonia could easily be harvested per ha on an annual cycle without loss of the forest resource or even more using rotations 1000 ha would yield a minimum of 100 tonnes of ammonia of value $50,000 at $US50 per ha. In time, the efficiency of such a process might be increased and twice as much yield obtained, with minimum costs in fertiliser and other inputs, compared to growing grain crops.

While there is a prospect for making H_2 which is a highly desired clean fuel, ammonia itself is an excellent fuel, releasing 317 kJ per mol of ammonia burnt (ΔH = -317 kJ/mol).

$$NH_3 + 0.75O_2 \Rightarrow 0.5N_2 + 1.5H_2O + 317\,kJ$$

By comparison, the reaction of H_2 with oxygen releases 285 kJ per mole of H_2 burnt (ΔH = -285 kJ/mol). The specific energy cost in 2004 was $US13.3 per GJ (Lovegrove et al., 2004). Thus, one tonne of ammonia would yield 19.2 GJ of energy, worth $US255, or $US25.5 yield per ha as electricity, so the value as fertilizer or explosive would be greater at current market values. Ammonia can be stored at low pressures or on metal ammine cartridges for greater safety, although these take some hours to charge.

Thus, ammonia from farm-forests has the potential to be used in small scale electricity stations, forming H_2 by day while generating solar power and releasing heat by night allowing continuing power generation from steam (Lovegrove et al., 2004). The potential for alternative uses of the farm-forest product would have strong economic advantages, enabling market opportunities to provide maximised returns at short notice, even on a daily basis. Such flexibility can be contrasted with the very large economic inertia when chemical products are manufactured in super-large installations by the Haber process or power is generated in facilities able to supply whole states with millions of consumers.

4 Conclusion – a new rural vision based on partnerships

This research and development project will quantify the feasibility of new technologies related to achieving carbon-effective farming using indigenous nitrogen-fixing casuarina species for short-term agroforestry on a 3-5-year production cycle. It will generate detailed information on the potential for soil carbon sequestration from foliage-fall and nitrogen-addition to fertility of the soil in farming-forestry in a range of habitats, using versatile species of Casuarina such as *Casuarina glauca*; the many species of Casurarinas are collectively capable of growing in a range of habitats and adverse climatic conditions, often too harsh for most other plants. The potential agro-industrial impact of using these trees as an intercrop forestry system in farming landscapes, for land rehabilitation but with particular emphasis on generating cash products such as charcoal, volatile chemical by-products and nitrogen fertilizers will be investigated. In many of these areas that can be integrated the preliminary research has already been completed.

The integrated program (see Figure 3) must be linked to completed research in industrial chemistry such as for the N_2 fixation reaction as a source of electrical power using its heat-storing properties. The data thus obtained could be utilized to generate more sustainable plantations of Casuarinas, for increasing soil carbon and fertility in a given habitat in the context of local industries as by-products, similar to those being sought in mallee farming.

The study also seeks to optimize the range of suitable strains of the nitrogen-fixing endophyte *Frankia* for fully effective inoculation of Casuarinas for increasing its capacity of nitrogen fixation by this micro-symbiont, encouraging more profuse growth of these trees, which in turn aids increased soil carbon sequestration as well as increasing soil fertility.

It will also seek more rapid progress through active international cooperation with India and China where utilization of Casuarinas is already highly active for paper-making and as short-term woody products such as building materials. It will target the greening of vast marginal lands of Australia by significantly increasing the population of N_2-fixing native casuarina trees. It will aim to reduce the trend of depopulation of the countryside and even to substantially reverse it, making rural communities much more attractive places to live. The overall result would be a more resilient Australia, united in a diversity of production and rewarding activities. In that sense, the proposal is still very much a work in progress, in which the 5th International Casuarina Workshop held in Tamil Nadu State in India is one of the progressive stages in its development that are required for success.

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Selection of Potential Isolates of Ectomycorrihzal Fungi for Improving Seedling Growth of *Casuarina equisetifolia*

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Abstract

A nursery study was made to identify the potential isolates of ectomycorrhizal (ECM) fungi (*Laccaria fraterna* and *Pisolithus albus*) and develop suitable type of inoculum for quality seedling production of *Casuarina equisetifolia* and also to determine the persistence of the inoculated fungi in the roots. In general, seedling growth was higher in all treatments as compared to uninoculated (control). Among different types of inocula tested, vegetative mycelial inoculum of *P. albus* was found to be the most efficient inoculum which gave maximum percent of Mycorrhizal Inoculation Effect (MIE). Morphological and anatomical studies revealed colonization of roots in all inoculated plants by *P. albus*. The total number of myco tips (mycorrhiza colonized root tips) found in inoculated seedlings grown in sterilized potting medium was more than those grown in unsterilized potting medium. The results of the study indicated that mycorrhization of seedlings with different forms of inocula *viz.,* basidiospores, vegetative mycelial and alginate bead of *L. fraterna* and *P. albus* exhibited their potential in improving planting stock of *C. equisetifolia*. This study would enable and support organic tree husbandry and agro farming.

1 Introduction

Casuarina equisetifolia is an evergreen tree cultivated by forest departments, farmers and tree growers in coastal and inland areas in different parts of Tamil Nadu, India. It is a multipurpose and farmer-friendly tree crop providing wood for various end uses. It is also planted as a bio-shield or shelterbelt to protect the people living on the coast against cyclone or tsunami and to stabilize the coastal sand dune movement into inland. These planting programmes need a large number of high quality planting stock which can adapt to different planting environments and provide enhanced yield from plantations.

The mycorrhizal fungi play an important role in planting stock improvement of forestry crops. Among different mycorrhizal fungi, ectomycorrhizal (ECM) fungi are found in about 10% of the plant families (Wang and Qiu, 2006). The ECM fungi are essential for seedling establishment and long-term nutrition of trees (Aragon et al., 2007). Indeed, many forest trees would find difficult to survive without mycorrhizas. Sometimes host species fail to grow until mycorrhizal fungi are introduced into the ecosystem (Mikola, 1973). In general, plants with ECM associations are healthy, sturdy and more disease resistant. Therefore selection of suitable ECM fungi is important for nursery production of forestry species (Trappe, 1977).

In India, most of the research works reported is on endomycorrhizal fungi with agriculture, horticulture and a few forest crops. Limited reports are available on ECM fungal association with economically important forestry species like Acacias, Eucalypts and Pines (Last et al., 1992; Vijayakumar et al., 1999; Mohan, 2013). The objective of the present study was to screen and select suitable isolates and type of inocula of two ECM fungi *viz., Laccaria fraterna* and *Pisolithus albus* for improving seedling growth of *C. equisetifolia* in nursery.

2 Materials and Methods

2.1 Inoculum production

The ECM fungal isolates viz., *L. fraterna* (1 isolate) and *P. albus* (4 isolates) were used for the present study. Pure cultures were obtained from the spores and tissues of basidiomata of these two fungi and maintained on sterile Potato Dextrose Agar (PDA) medium for further studies.

2.1.1 Basidiospore inoculum

The fruit bodies of *L. fraterna* were collected and dried under room temperature and powdered by using a mixer grinder. Dried *P. albus* fruit bodies were collected and powdered. The basidiospore inoculum of both the fungi was maintained in refrigerator at 4 °C until used for nursery experiment.

2.1.2 Vegetative mycelial inoculum

All isolates were grown at 25±1 °C for 4 weeks in Modified Melin Norkrans (MMN) liquid medium for preparation of vegetative mycelial inoculum by using vermiculite as carrier material (Marx and Bryan, 1975).

2.1.3 Alginate bead inoculum production

Mycelium of fungal isolates was produced in 2 litre flasks, harvested aseptically and washed with sterile distilled water and kept in saline solution (0.85% NaCl) at 8±1 °C. fresh mycelium was immobilized in calcium alginate gel.

The mycelium was prepared for immobilization by fragmenting 9 g in 150 mL of sterilized distilled water for 6-7 seconds at 3600 rpm in a blender. The mycelial suspension was mixed with an equal volume of a sterilized sodium alginate solution (2%). This mixture was then dripped into a 0.1M $CaCl_2.2H_2O$ solution under mechanical shaking in order to achieve polymerization and formation of 4 mm diameter calcium alginate beads. The entire operation was performed at room temperature in aseptic condition. After complete polymerization beads were washed in sterilized distilled water to eliminate chloride residues and stored at 8±1 °C. Alginate beads can easily be dissolved in citrate or phosphate solutions to release the concentrated suspensions of immobilized mycorrhiza. Mycelium that grew from the beads after 48 hours were placed on a Petri dish containing solid culture medium (PDA or MMN) and incubated at 25±1 °C.

2.2 Nursery experiment

Potting media used in the present study were soil + sand (2:1 ratio). Both the sterilized (fumigated with formalin) and unsterilized potting media were used and nutrient parameters were analyzed through standard methods (Patiram et al., 2007). Physico-chemical properties of sterilized potting medium were: pH 8.23, EC 0.09, N, P, K were 76, 10.4 and 290 kg ha^{-1} respectively and Cu, Zn, Fe and Mn were 1.2, 1.8, 7.8 and 9.7 mg kg^{-1} respectively. Physico-chemical properties of unsterilized potting medium were: pH 7.9, EC 0.14, N,P,K were 70, 8.6 and 305 kg ha^{-1} and Cu, Zn, Fe and Mn were 1.8, 1.0, 10.8 and 8.8 mg kg^{-1} respectively.

2.2.1 Application of different types of ECM inocula

Healthy seeds of *C. equisetifolia* were obtained from Seed Technology Division, IFGTB, Coimbatore and nursery experiment was conducted at Experimental Nursery, FLUCC Division, IFGTB, Coimbatore by using standard nursery bed (10 x 1 m) for raising seedlings. Fifteen days old seedlings were used for setting up nursery experiment and sterilized and unsterilized potting media were filled in polythene bags (10 x 20 cm in size). The plants were maintained under nursery conditions for a period of 6 months. The following are details of different treatments used in the experiment and the design followed.

Number of treatments	:	16
Number of plants per treatment	:	15
Number of replicates per treatment	:	03
Design	:	Completely Rando mized Design
Dosage		
Basidiospore inoculum	:	2 g per poly bag
Vegetative mycelial inoculum	:	5 g per poly bag
Alginate bead inoculum	:	10 beads per poly bag

T1-Basidiospores inoculum of *L. fraterna*
T2-Vegetative mycelial inoculum of *L. fraterna*
T3-Alginate bead inoculum of *L. fraterna*
T4- Basidiospores inoculum of *P. albus* (Isolate No. 1)
T5- Vegetative mycelial inoculum of *P. albus* (Isolate No. 1)
T6- Alginate bead inoculum of *P. albus* (Isolate No. 1)
T7- Basidiospores inoculum of *P. albus* (Isolate No. 2)
T8- Vegetative mycelial inoculum of *P. albus* (Isolate No. 2)
T9- Alginate bead inoculum of *P. albus* (Isolate No. 2)
T10- Basidiospores inoculum of *P. albus* (Isolate No. 3)
T11- Vegetative mycelial inoculum of *P. albus* (Isolate No. 3)
T12- Alginate bead inoculum of *P. albus* (Isolate No. 3)
T13- Basidiospores inoculum of *P. albus* (Isolate No. 4)
T14- Vegetative mycelial inoculum of *P. albus* (Isolate No. 4)
T15- Alginate bead inoculum of *P. albus* (Isolate No. 4)
T16-Uninoculated (control), poly bags containing ECM fungal inocula free vermiculite and alginate beads.

2.3 Data collection and statistical analysis

Six month old seedlings were harvested and assessed for growth parameters like shoot height, collar diameter and total dry weight. Total number of mycorrhizal tips (ECM colonized roots) was also recorded by following the methods proposed by Richards and Wilson (1963) and Mohan (1991).

All data were subjected to analysis of variance and the significant difference among the means were compared by Duncan's Multiple Range Test (DMRT) at P=0.05 level using SPSS PC+ Studentware statistical software (SPSS Inc.)

3 Results

3.1 Shoot height

Among different treatments, isolate 3 of *P. albus* as vegetative mycelial (vermiculite based) inoculum gave significantly greater shoot height in sterilized (48.9 cm) and unsterilized (55.4 cm) potting media compared to other types of inocula and isolates of *P. albus* and *L. fraterna* (Table 1). It was also observed that uninoculated control plants had significantly low shoot height in sterilized (20.4 cm) and unsterilized (26 cm) potting media.

3.2 Collar diameter

Among different isolates of *P. albus*, it was found that the isolate 3 of vegetative mycelial inoculum gave significantly higher root collar diameter in both sterilized (4.45 mm) and sterilized (5.47 mm) potting media (Table 1). This is followed by isolates 4, 2 and 1 of vegetative mycelial inoculum of *P. albus* when compared to different inocula of *L. fraterna* and uninouclated control plants.

3.3 Total dry weight

Inoculation with the ECM fungi consistently increased the total dry weight of seedlings compared to uninoculated (control). Among different isolates of *P. albus*, it was observed that the isolate 3 of vegetative mycelial inoculum gave significantly higher total dry weight in sterilized (18.3 g) and unsterilized (22.8 g) potting media tested (Table 1).

Table 1. Effect of different types of ECM fungi inocula on the growh of six month old *Casuarina equisetifolia* seedlings in different potting media

Treatment	Sterilized potting media			Unsterilized potting media		
	Shoot height (cm)	Collar diameter (mm)	Total dry weight (g)	Shoot height (cm)	Collar diameter (mm)	Total dry weight (g)
T1	27.9_{b}	2.65_{b}	7.42_{b}	32.6_{b}	3.07_{a}	8.76_{b}
T2	34.4_{cd}	3.29_{cd}	9.37_{d}	39.4_{c}	4.30_{def}	11.78_{d}
T2	32.6_{c}	2.77_{b}	8.46_{c}	34.7_{b}	3.61_{b}	9.81_{c}
T4	36.7_{de}	3.44_{de}	10.62_{e}	41.3_{cd}	3.94_{c}	12.25_{e}
T5	40.2_{fg}	3.78_{f}	12.51_{g}	45.4_{ef}	4.24_{cdef}	15.88_{i}
T6	35.4_{d}	3.20_{c}	8.96_{d}	43.3_{de}	4.16_{cde}	13.77_{f}
T7	38.5_{ef}	3.81_{f}	12.56_{g}	47.1_{f}	4.21_{cdef}	16.24_{i}
T8	44.6_{h}	4.25_{gh}	15.92_{k}	54.2_{hi}	4.66_{ghi}	20.92_{m}
T9	40.4_{fg}	3.30_{cd}	13.89_{i}	50.3_{g}	4.40_{efg}	18.24_{k}
T10	42.4_{gh}	4.18_{g}	15.08_{j}	51.9_{gh}	4.78_{hi}	17.39_{j}
T11	48.9_{i}	4.45_{h}	18.37_{l}	55.4_{i}	5.37_{j}	22.82_{n}
T12	43.4_{h}	3.49_{de}	16.09_{k}	53.8_{hi}	4.85_{i}	19.18_{l}
T13	34.8_{cd}	3.59_{e}	10.81_{e}	38.7_{c}	4.03_{cd}	14.43_{g}
T14	44.5_{h}	4.34_{gh}	12.94_{h}	43.1_{de}	4.50_{fgh}	16.04_{i}
T15	39.0_{ef}	3.81_{f}	11.71_{f}	40.8_{cd}	4.31_{def}	14.96_{h}
T16	20.4_{a}	2.21_{a}	4.06_{a}	26.1_{a}	2.82_{a}	5.73_{a}

Note: Means sharing a common letter in the same column within a potting media are not significantly different at P = 0.05 level.

3.4 Total number of mycorrhizal colonized root tips

The ECM inoculated seedlings had more mycorrhizal colonized root tips in both the potting media. It was also observed that the significantly higher number of mycorrhizal colonized root tips were found in seedlings inoculated with all three inocula types of isolate 3 from *P. albus* compared to other isolates. The number of mycorrhizal colonized root tips was recorded high in seedlings grown in sterilized potting medium over unsterilized potting medium (Table 2).

4 Discussion

Many tree species, including pines will not grow and develop normally without the association of ECM fungi. Reforestation with such species requires inoculation with ECM fungi at nursery stage to equip the seedlings for early establishment and fast initial growth in unfavourable environments. Various forms of inocula of *P. tinctorius* have been successfully used by many researchers for mycorrhization of forestry species (Mohanan, 2003; Mohan and Manokaran, 2005). Encapsulation of spores and hyphal fragments of ECM fungi is a new technology applied in the mycorrhizal manipulation. Encapsulation of mycelial fragments from aseptic culture within beads of alginate gel is a more advanced form of inoculum, where fungal hyphae continue to grow within these beads. This technique allows mycelium to recover from fragmentation before application, so that the encapsulated mycelium acts as more effective propagules and become efficient in seedling mycorrhization.

The present study had shown that application of different types of inocula of selected ECM fungi can improve growth performances of *C. equisetifolia* seedlings in nursery. In general, seedling growth in terms of height increment was higher in all ECM inocula treatments than uninoculated (control) seedlings in sterilized and unsterilized potting media. The height of seedlings treated with vegetative mycelia of *P. albus* (isolate 3) increased 2-3 fold followed by basidiospore and alginate bead inoculua. This study is corroborated with the findings made by Mohanan (2003) who observed that maximum seedling height increment was recorded in *P. tinctorius* spore inoculum treatment on *A. mangium* seedlings in Kerala, India. Mohan and Manokaran (2005) investigated the effectiveness of basidiospore inoculum of *P. tinctorius* in forming mycorrhizal association and the growth improvement of *Eucalyptus globulus* seedlings in nursery. They observed that the inoculated plants had better growth by means of

Table 2. Effect of different types of ECM fungi inocula on total number of mycorrhizal colonized root tips in 6-month old *Casuarina equisetifolia* seedlings grown in different potting media

Treatment	Sterilized potting medium	Unsterilized potting medium
T1	169_{bc}	161_{b}
T2	206_{cde}	198_{d}
T2	194_{bcd}	186_{c}
T4	214_{cdef}	205_{de}
T5	252_{efghi}	243_{i}
T6	224_{defg}	213_{fg}
T7	152_{b}	210_{ef}
T8	263_{fghij}	253_{j}
T9	240_{defghi}	226_{h}
T10	275_{ghij}	261_{k}
T11	309_{j}	289_{m}
T12	292_{ij}	276_{l}
T13	231_{defgh}	218_{g}
T14	281_{hij}	263_{k}
T15	271_{ghij}	252_{j}
T16	0_{a}	0_{a}

Note: Means sharing a common letter in the same column within a potting media are not significantly different at P = 0.05 level.

total biomass, plant height and root collar diameter and the plant height was invariably increased (3 fold) on 6-month old seedlings.

Utilization of pure cultures or vegetative mycelium does not pose any problem of bulk handling and transportation as compared to natural soil inoculum. In the present study, it was observed that the seedlings inoculated with vegetative mycelial inoculum of *P. albus* (Isolate 3) resulted in significantly greater plant height, collar diameter and total dry weight in all age levels in both the potting media. Vegetative mycelial inoculum of ECM fungi has been regularly used throughout the world (Einarsson and Kristjansson, 1990). Marx (1980) reported that the mycorrhizal introduction with vegetative inoculum ensures introduction of appropriate fungi in suitable quantities in host plants. The present study is also in accordance with the findings reported for *Pinus patula* seedlings inoculated with two ECM fungi *viz., Laccaria laccta* and *Thelephora terrestris* and recorded that up to first six months, the seedlings inoculated with *T. terrestris* had better growth, mycorrhizal development and dry matter production, but at the later stage, the seedlings inoculated with *L. laccata* grew better (Natarajan and Reddy, 1994). In another study, Natarajan et al. (1995) observed positive colonization under *in vitro* mycorrhization and growth response of *Acacia nilotica* seedlings by inoculation with ECM fungi. Similarly, Natarajan and Reddy (1997) studied an *in vitro* ECM formation of different pine species such as *P. patula, P. pseudostrobus, P. oocarpa* and *P. elliotti* and found positive mycorrhizal colonization by using vegetative mycelium. In another study, Saravanan and Natarajan (1996) have observed that *P. tinctorius* inoculum produced on vermiculite and inoculum entrapped in alginate gels (mycobeads) significantly improved *Acacia nilotica* seedling growth and nitrogen fixation activity. In the present study, alginate beads showed moderate effect as compared to vegetative mycelial and basidiospore inocula of different ECM fungi. However, better growth improvement was observed on inoculated plants as compared to uninoculated (control) plants. Ayswarya et al. (2009) studied the effect of different types of inocula of ECM fungus (*P. albus*) on growth improvement of *Eucalytpus tereticornis* clones in Andhra Pradesh, India. They found that the treatments with the ECM fungus recorded significantly greater shoot height, root length, shoot and root biomass and collar diameter over uninoculated (control) plantlets. They also found that the maximum growth was recorded in the treatment with vegetative mycelium inoculum, followed by basidiospore inoculum.

During the present study, number of ECM colonized root (mycorrhizal tips) was found maximum and highly significant in *C. equisetifolia* seedlings inoculated with basidiospore inoculum of *P. albus* (isolate No. 3) followed by vegetative mycelial (vermiculite based) inoculum in all age levels. This result is in accordance with the findings made by Kidd and Reid (1979). They studied the effect of inoculation with different ECM fungi (*Cenococcum ganiforme*, *Suillus granulatus* and *P.s tinctorius*) in seedlings of different pine species such as *P. contorta, P. ponderosa, P. flexilis* and *P. aristata* and found that percent mycorrhizal short roots and mycorrhizal root numbers were significantly correlated with photosynthetic rate.

5 Conclusion

Mycorrhization of seedlings with different forms of inocula viz. basidiospores, vegetative mycelial (vermiculite based) and alginate beads of *L. fraterna* and *P. albus* showed their potential in improving planting stock of commercially important multipurpose trees like *C. equisetifolia.* Morphological and anatomical studies revealed colonization of roots of all ECM inoculated plants**.** It was also observed that total number of mycorrhizal tips is found more in ECM inoculated seedlings grown in sterilized potting medium than those grown in unsterilized potting medium at all ages. ECM application in seedling stage may assist the seedlings to withstand dry conditions and support the seedlings when out-planted under organic tree husbandry and agro farming.

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Rehabilitation of Saline Lands Using Selected Salt-tolerant Casuarina-microorganisms Combinations

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Abstract

Abiotic stresses like salt and drought adversely affect ecosystem biodiversity and productivity. Recently, global warming has accelerated yield losses in ecosystems. Concomitant to the losses in agricultural yield there is increased demand for food and wood due to rapid population growth in developing countries particularly in the sub-saharan Africa. Using salt tolerant nitrogen fixing trees like members of Casuarinaceae could alleviate this problem by increasing soil fertility, enhancing productivity and improving smallholding farmers' livelihoods and food security. These plants are naturally salt tolerant and are able to interact with arbuscular mycorrhizal fungi (AMF) that mitigate the adverse effect of salinity. The aim of this work was to select the best combination of *Casuarina* species and AMF to rehabilitate salinized lands. The impact of three AMF: *Rhizophagus irregularis*, *Glomus aggregatum* and *G. fasciculatum* on *C. equisetifolia* and *C. glauca* growth in saline condition was tested. Results showed that inoculation with *G. aggregatum* and *G. fasciculatum* increased growth of *C. equisetifolia* and *C. glauca* while *G. aggregatum* was more effective on *C. equisetifolia* in saline condition. However, at high NaCl concentration better growth was obtained with *C. glauca* plants inoculated with *G. fasciculatum*. This study shows the importance of selecting the most effective AMF to increase plant performance in degraded lands. *C. glauca/G. fasciculatum* is a highly salt tolerant combination to be used for the rehabilitation of lands affected by salt.

1 Introduction

Land salinization is a major threat to world agriculture especially in the context of climate change (Munns and Tester, 2008). Furthermore, salinization is increased by human activities such as irrigation with inappropriate water (Bohnert, 2004, Hammer et al., 2010). Approximately, 7% of the global lands are affected by salt (Evelin et al., 2009). Wang et al. (2003) estimated that salinization would lead to a 30% loss of arable lands within the next 25 years and up to 50% by the year 2050. Concomitant to the losses of agriculturally productive lands is the increased demand for food and wood due to rapid population growth in developing countries. According to Van Eeckhout (2010), 239 million people in sub-saharan Africa are hungry. Revegetation of such lands can improve soil fertility, produce forage, livestock and transform these abandoned ecosystems into productive lands. However, establishment of plants in saline areas can be very difficult. Planting salt tolerant species, particularly N_2 fixing trees is one of the most promising ways to deal with this problem (Rasmussen et al., 2009).

Casuarina equisetifolia and *C. glauca,* are N_2 fixing trees widely used in revegetation programs due to their capacity to grow in poor and disturbed soils and adaptation to abiotic stresses such as salinity (Echbab et al., 2007, Diagne et al., 2013). Apart from producing high quality wood, they

Fig. 1. Impact of *R. irregularis, G. fasciculatum* and *G. aggregatum* on *C. equisetifolia* growth under different salt concentrations.

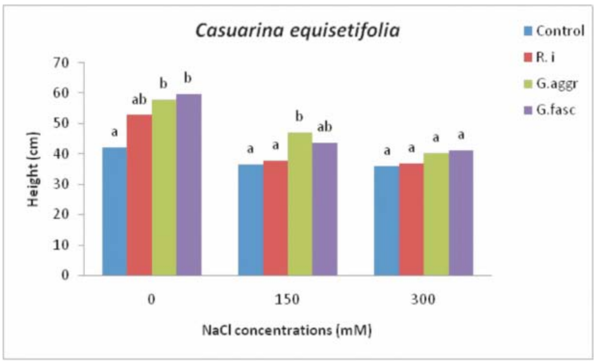
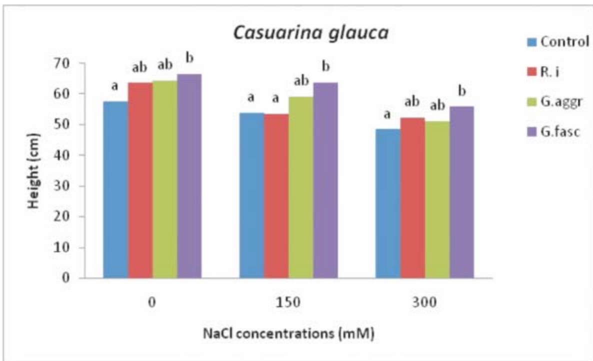


Fig. 2. Impact of *R. irregularis, G. fasciculatum* and *G. aggregatum* on *C. glauca* growth under different salt concentrations.



improve soil fertility through the relationship with nitrogen fixing bacteria and arbuscular mycorhizal fungi (AMF) which increase plant N and Pi uptake and water use efficiency (Beltrano et al., 2013).

It has been demonstrated that, the symbiotic microorganisms such as nitrogen-fixing bacteria and arbuscular mycorrhizal fungi can alleviate salt stress on plant growth (Evelin et al., 2009). The use of these biofertilizers for improving plant salt tolerance helps in minimizing the environmental hazards (Javaid, 2010, Cekic et al., 2012). Nevertheless, selection of the right symbiotic and host partners is the key for a successful rehabilitation of lands affected by salt. Thus appropriate combinations of plant/nitrogen fixing bacteria/AMF well adapted to saline lands are recommended. The objective of our study was to determine the best combination of Casuarina/Nitrogen fixing bacteria/AMF adapted to salinity to be widely used for the rehabilitation of degraded lands in order to increase soil fertility, ecosystems productivity and improve food security in arid and semi arid tropics.

2 Materials and Methods

2.1 Plant material and bacterial strains

C. glauca seeds were obtained from CSIRO, Australia, and *C. equisetifolia* seeds were from PRONASEF, Senegal. The arbuscular mycorrhizal fungi strain *Glomus intraradices* (DAOM 197198) recently reassigned to *Rhizophagus irregularis* Schenk and Smith (Stockinger et al., 2009), *G. aggregatum* and *G. fasciculatum* were used to inoculate *C. glauca* and *C. equisetifolia* plants.

2.2 Greenhouse experimentation on microbial inoculation

One month-old seedlings of *C. glauca* and *C. equisetifolia* were inoculated with any of *R. irregularis, G. aggregatum* and *G. fasciculatum* according to Plenchette et al. (1989).

The following treatments were carried out: no inoculation (control); inoculated with *R. irregularis* (R.i); inoculated with *G. aggregatum* (G.aggr) and inoculated with *G. fasciculatum* (G.fasc).

NaCl treatments

The salinity stress was applied one month after inoculation to allow symbiosis establishment. Three NaCl concentrations (0mM, 150mM and 300mM) were applied to the plants (12 plants per treatment). The soil was salinized step-wise to avoid subjecting plants to salt stress shock. NaCl concentrations were gradually increased by 50 mM at two days intervals until reaching the required salinity of each NaCl treatment.

Growth parameters

After 4 months of growing, plants were harvested and their height (cm) was recorded. Total biomass was assessed from seedling dry weights recorded after three days of oven drying at 65 °C.

Chlorophyll determination

Chlorophyll (a and b) contents of shoot system were quantified by spectrophotometry according to Makeen et al. (2007).

Statistical analysis

The data were subjected to statistical analysis with one-way analysis of variance and the means were compared using the Newman Keuls multiple range test ($P < 0.05$).

3 Results

3.1 Impact of AMF on Casuarina growth under saline conditions

At 0 mM of NaCl, AMF increased growth of both the *Casuarina* species but only *G. aggregatum* and *G. fasciculatum* significantly increased growth of *C. equisetifolia* (Fig. 1) while *C. glauca* growth was significantly stimulated by *G. fasciculatum* (Fig. 2). At 150 mM, growth was better for *C. equisetifolia* plants inoculated with *G. aggregatum* and *C. glauca* plants inoculated with *G. fasciculatum* (Fig. 1 & 2) than other combinations. Under the NaCl concentration of 300 mM, the best growth was

Fig. 3. Total biomass of *C. equisetifolia* plants inoculated with *R. irregularis, G. fasciculatum* and *G. aggregatum* under different salt concentrations.

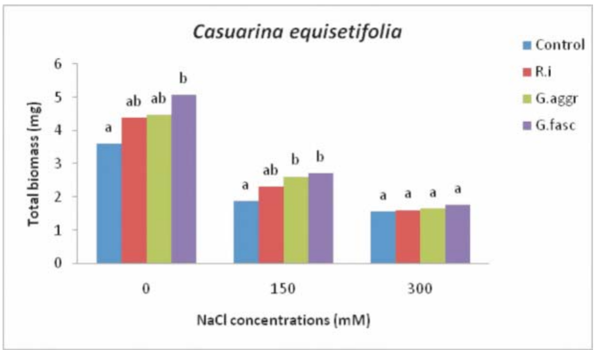
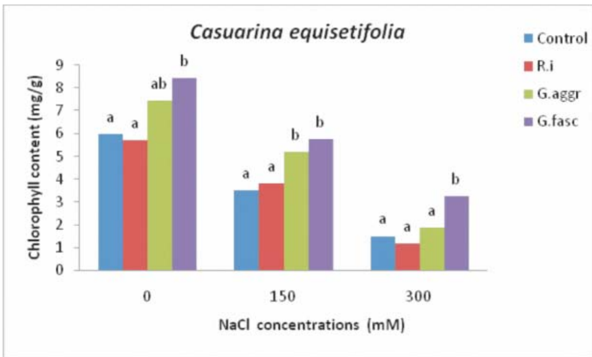


Fig. 5. Chlorophyll content of *C. equisetifolia* inoculated with *R. irregularis, G. fasciculatum* and *G. aggregatum* under different salt concentrations.



noted in *C. glauca* inoculated with *G. fasciculatum* (Fig. 2). Taken together, these results show that *G. aggregatum* is more appropiate for *C. equisetifolia* growth enhancement than *C. glauca* in high salinity condition and the combination of *C. glauca* and *G. fasciculatum* is more tolerant to high salt concentration.

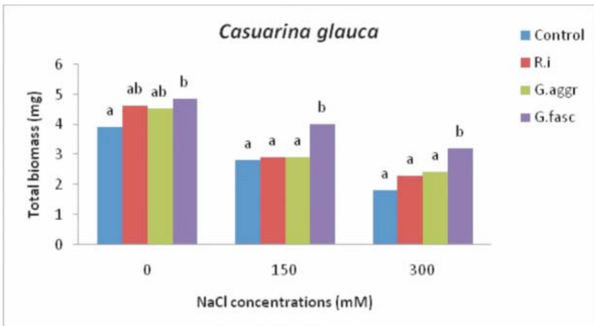
3.2 Impact of AMF on Casuarina biomass under saline conditions

In no saline condition, the *G. fasciculatum* inoculated seedlings had significantly higher total biomass compared to uninoculated plants and those inoculated with *R. irregularis or G. aggregatum* (Fig. 3 & 4). At 150 mM, inoculation with *G. aggregatum* or *G. fasciculatum* significantly increase total biomass of *C. equisetifolia* (Fig. 3). For *C. glauca* plants, the total biomass is significantly increased when plants are inoculated with *G. fasciculatum* (Fig. 4). With an increasing NaCl concentration (300 mM), the higher biomass was observed in *C. glauca* plants inoculated with *G. fasciculatum* (Fig. 4).

3.3 Impact of AMF on Casuarina chlorophyll content in saline condition

Result obtained show that salinity has a negative effect on plant chlorophyll synthesis in *C. equisetifolia* and *C. glauca.*

Fig. 4. Total biomass of *C. glauca* inoculated with *R. irregularis, G. fasciculatum* and *G. aggregatum* under different salt concentrations.

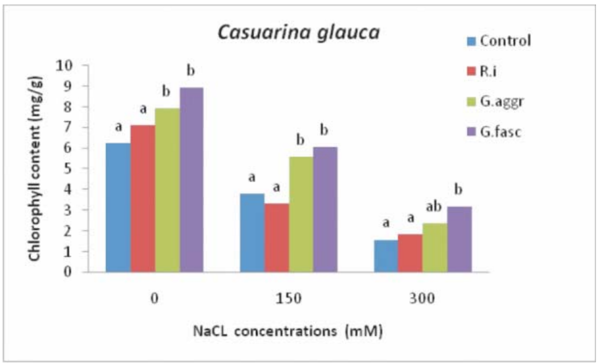


Total chlorophyll decreased with an increasing NaCl concentration (Fig. 5 & 6). However, inoculation with appropriate AMF can mitigate the negative effect of salt on plant chlorophyll synthesis. At 150 mM of salt *G. fasciculatum* and *G. aggregatum* enhance plant chlorophyll content compared to the control. With an increasing salt concentration of up to 300 mM, only *G. fasciculatum* increased chlorophyll synthesis in both the species of *Casuarina.*

4 Discussion

Inoculation with AMF increases plant tolerance to salinity (Beltrona et al., 2013; Evelin et al., 2009). Similar results were obtained in the present study showing increased growth of Casuarina plants inoculated with *G. fasciculatum* and *G. aggregatum*. However, in saline condition, the two *Casuarina* species showed difference in response to different AMF. At 150 mM. *G. aggregatum* inoculation significantly increased growth in *C. equisetifolia* whereas *C. glauca* growth was promoted by *G. fasciculatum*. At 300 mM, increase in growth was observed only for *C. glauca* inoculated with *G. fasciculatum*. This variation in response to fungus inoculation could be linked to the symbiosis efficiency under saline condition. Given that AMF improves plant growth by enhancing nutrients uptake (Sharifi et al., 2007), the difference in growth response could be related to a difference in nutrient acquisition particularly P ions which precipitate with Ca^{2+}, Mg^{2+} and Zn^{2+} ions in salt stressed soils and become unavailable to plants (Evelin et al., 2009, Park et al., 2009). The higher growth of plants inoculated with AMF in saline conditions could be linked to the increase of P uptake (Navarro et al., 2013) and to the reduction of NaCl toxicity by decreasing sodium and chloride uptake by fungi (Talaat and Shawkry, 2011). Furthermore, it has been demonstrated that any change in hyphae morphological properties under salt stress conditions can affect nutrients uptake (Wu et al., 2010). In this study, the suppressed growth of plants inoculated with *R. irregularis* could be related to hyphae morphological modification under high salt concentration which leads to the decrease of total P uptake by this fungi strain. Given that

Fig. 6. Chlorophyll content of *C. glauca* inoculated with *R. irregularis, G. fasciculatum* and *G. aggregatum* under different salt concentrations.



salt stress affects nutrient availability and competitive uptake, inoculation with efficient and suitable AMF will allow plants to have a better water status and lower the osmotic stress (Jahromi et al., 2008). As a consequence the selection of the suitable AMF is a key for the improvement of mycorrhizal effectiveness in salt stress condition.

For *C. equisetifolia*, at 150 mM of NaCl, the best growth obtained with *G. aggregatum* is also correlated to a higher total biomass. This response may be related to the compatibility between the fungus and plant. In our study, *G. aggregatum* and *G. fasciculatum* are more efficient in Casuarina biomass enhancement at high salt concentration than *R. irregularis*. *R. irregularis* has been reported to increase *Acacia auriculiformis* shoot biomass at 100 mM of NaCl (Diouf et al., 2005). Similar results were observed by Prodjinoto et al. (Unpublished), who showed that *R. irregularis* enhanced *C. equisetifolia* growth at 100 Mm of NaCl. These results suggest that *R. irregularis* is tolerant to salt but less tolerant than *G. aggregatum* and *G. fasciculatum*. In our study, *G. fasciculatum* was the most efficient AMF in increasing *C. glauca* root and shoot biomass under saline conditions. Similar positive results were reported in pepper plants inoculated with *G. fasciculatum* (Beltrona et al., 2013). In Gmelina, inoculation with *G. fasciculatum* increases shoot, root and total plant biomass in saline conditions (Dudhane et al., 2011).

Inoculation with appropriate AMF increases the total chlorophyll content in mycorrhizal plants under saline condition (Elahi et al., 2012). Similar results were obtained by Diaz and Garza (2006) with a higher chlorophyll level in inoculated plants. These data suggest that AMF have the capacity to counterbalance salt stress by reducing Na uptake and toxicity and thus help in maintaining chlorophyll content of the plant (Kaya et al., 2009). By secreting substances such as cytokinin that increase chlrorophyll development, AMF can enhance the chlorophyll synthesis (Thanaa and Nawar, 1994). However, increasing salt stress reduces the total chlorophyll (Elahi et al., 2012). This reduction in total chlorophyll could be due to the degradation of chlorophyll and chlorophyll protein complexes by salinity (Sheng et al., 2008) or to the suppression of enzymes involved in the synthesis of photosynthetic pigments (Murkute et al., 2009). It could also be due to an antagonistic effect of NaCl and N absorption, which is an important component of the structure of chrorophyll molecule (Grattan and Grieve, 1994) or to the negative effect of Na on Mg absorption, another important element for plant chlorophyll synthesis (Giri and Mukerji, 2004). However, AMF mitigates this negative effect by increasing Mg uptake (Giri and Mukerji, 2004). Navarro et al. (2013) found a higher concentration of Mg in *Alemow citrus* plants inoculated with AMF compared to the uninoculated plants.

5 Conclusion

Our results show that inoculation with AMF increases growth of *C. equisetifolia* and *C. glauca* plants in saline condition. *G. aggregatum* is more effective for *C. equisetifolia* and *G. fasciculatum* for *C. glauca*. These results suggest that, inoculation with a right AMF is a key to increase plant performance for the reforestation of lands affected by salt.

Acknowledgments

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Casuarina Leaf Litter: A Review

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Key words: branchlet, decomposition, nutrient accumulation, soil quality

Abstract

Casuarinas are extensively grown in forestry systems, particularly in China and India. They produce large quantities of litter. Needle-like branchlets are the major litter components (>95%). *Casuarina equisetifolia* produces up to 30 tonnes of litter per ha in a year. Litter covers the soil beneath Casuarinas forming a thick layer up to 12 cm deep. Nutrients present in the litter are released during its decomposition which are then recycled back into the ecosystem. Litter branchlets contain about 2% nitrogen depending on the species. More than 90% of the N released during the litter decomposition is from the branchlets. Nutrient accumulation increases with age of the litter as it decomposes. Up to 1567 kg ha^{-1} N accumulation in the litter was recorded in 34 year old *C. equisetifolia* stands, with an average addition of 45 kg ha^{-1} annually. Inorganic nutrient salts like phosphorus, potassium, calcium and magnesium are present in appreciable levels in the litter and are subsequently released. High C:N, N:P, lignin:N, phenolics:N, phenolics:lignin:N ratios in the litter affect its decomposition rates and determine the quality of the litter. Soil qualities (especially N content) improve during litter decomposition (along with roots and nodules), making the habitat more friendly towards the accession of other plant species in hostile habitats. Thus Casuarinas that inhabit these habitats as pioneer species can create a new ecosystem. Recalcitrant lignin and phenolic compounds present in the litter negatively affect its degradation rates. Phenolics released during litter decomposition can exert an allelopathic effect on the biota. Gathering litter for fuel in developing countries deprives the soil of additional nutrients from litter and potentially causes a reduction in the yield in subsequent generations. The chemical composition and physical structure of the litter has both beneficial and deleterious effects on the ecosystem which need further investigation.

1 Introduction

Many Casuarinas are native to Australia. They are among the most popular N_2 fixing multipurpose trees grown extensively in forestry systems in the world, more so in countries like China and India. They are able to grow in hostile habitats and have developed adaptations to overcome the harsh conditions. *Casuarina equisetifolia*, often referred as Australian pine, is studied more extensively. N-rich litter is produced in large quantities by Casuarinas comprising almost 10% of their above ground biomass (Lugo et al., 1990). The amount of litter produced increases with age. Appreciable amount of the available N is released into the ecosystem during litter decomposition along with other constituents. The released N plays an important role in changing soil chemistry (Hata et al., 2012).The phenolic compounds released during litter decomposition exhibit allelopathic effects on certain biota and considerable amount of litter accumulated under these trees interferes with the seed germination and seedling growth of certain plants (Barritt and Facelli, 2001; Patil and Hunshal, 2004; Hata et al., 2010). The physical structure of the litter may be either beneficial (Chiba, 2010) or deleterious to the native biota (Wheeler et al., 2011). More work is necessary to fully understand the impact of casuarina litter on the ecosystem. This paper describes the constituents of Casuarina litter and since branchlets constitute a major portion of Casuarina litter and regulate the litter decomposition, only the significant components present in them are described. The pattern of litter fall, litter decomposition, release and recycling of nutrients, improvement of soil properties and the allelopathic effects of the litter are also briefly dealt with.

2 Casuarina Leaf Litter

Since branchlets take over the function of leaves and are a predominant portion of the litter, Casuarina litter is generally referred to as leaf litter. Typical litter of Casuarinas is depicted in Fig. 1.

2.1 Constituents of Casuarina litter

More than 95% of the litter is composed of dead branchlets (Srivastava and Ambasht, 1996; Misra and Nisanka, 1997). The remaining portion of the litter contains occasional dead branches and seasonal dry inflorescences and fruits.

Table 1, Variation in major nutrients of Casuarina leaf litter (% dry weight)

Sample type	Casuarina species	Sample preprocessing	C	N	C:N ratio	P	K	Ca	Mg	Region of litter collection	Reference
Branchlet litter collected at monthly intervals from differently aged stands	*C. equisetifolia*	Dried at 80°C	Year 1: 32.552 Year 2: 36.230 Year 3: 39.707	Year 1: 1.577 Year 2: 1.617 Year 3: 1.693		Year 1: 0.182 Year 2: 0.191 Year 3: 0.183	Year 1: 0.381 Year 2: 0.365 Year 3: 0.364	Year 1: 1.842 Year 2: 1.925 Year 3: 1.911	Year 1: 0.308 Year 2: 0.327 Year 3: 0.347	Semiarid region of south India (alkaline soil with a pH of 8.1)	Uma et al. 2014
Freshly senesced freely falling leaf litter collected after shaking the plant	*C. glauca*	Dried at 60°C		1.90	25.1					North-central Florida	Bray *et al.* 2012
Senescent branchlets from trees of different ages collected from them	*C. equisetifolia*			Year 5: 0.767 Year 21: 0.912 Year 38: 1.165 Year 21: 0.016 Year 38: 0.016		Year 5: 0.022				Fujian Province China (subtropical monsoonal climate)	Ye et al.. 2012
Humic litter layer (top 2-3 cm)	*C. equisetifolia*	Air dried			34.2					Coastal forest region of northwest Taiwan	
Branchlet litter	*C. equisetifolia*	Air dried		0.85		0.16	0.42	0.51	0.30	Sodic soils of north India (subtropical region)	Singh et al.. 2011
Leaf litter	*C. equisetifolia* L.	Air dried	46.12	1.18						South India	Sugumaran and Seshadri, 2009
Senescent branchlets collected freshly from the tree	*C. equisetifolia*			Site 1: 1.254 Site 2: 1.495 Site 3: 1.729 Site 4: 0.912 Site 5: 1.077 Site 6: 0.969		Site 1: 0.043 Site 2: 0.029 Site 3: 0.034 Site 4: 0.007 Site 5: 0.011 Site 6: 0.017				Across a coastal gradient of Fujian Province, China. (subtropical– monsoonal climate)	Zhang et al., 2008
Litter collected from under the tree in dry season	*C. equisetifolia*	Air dried	47.19	1.36	34.8					Sub-Saharan region of Senegal	Diallo et al., 2006
Freshly fallen litter collected during dry season	*C. equisetifolia* Forst.	Air dried	47.2	1.36	34.8					Senegal (Sahelian-Sudanese climatic conditions).	Diallo et al., 2005
Branchlet litter picked from trees (collected during dry season).	*C. equisetifolia*	Air dried	47.18	1.3568	34.77					Senegal	Sall et al., 2003
Litter from forest floor (slightly decomposed litter)	*C. equisetifolia*	Dried at 65°C	Slightly decomposed: 53.13	Slightly decomposed: 1.203	Slightly decomposed: 44.4	Slightly decomposed: 0.016	Slightly decomposed: 0.136	Slightly decomposed: 1.453	Slightly decomposed: 0.172	North Puerto Rico coastal region (wet sub tropical forest).	Warren and Zou 2002
Branchlet litter collected from litter traps	*C. equisetifolia*	Dried at 80°C		1.25		0.071	0: 0.48	0: 1.35		Opencast coal mine spoils, central India (Tropical mon soonal climate).	Dutta and Agrawal, 2001
Freshly fallen branchlets collected from litter traps	*C. equisetifolia*	Air dried		1.59						South-eastern India	Jamaludheen and Kumar, 1999
Litter (branchelets- 75-96%)	*C. equisetifolia*							0.17	1.77	Sandy coast of Orissa, north India	Misra et al., 1997
Intact senescent branchlets collected from under trees	*C. equisetifolia*	Dry mass		0.86		0.03				Hawaii (Tropical climate)	Constantnide and Fowne, 1994
Freshly fallen litter	*C decussata* (Renamed as *Allocasuarina decussata)*	Air dried		0.44		0.005	0.19	1.19	0.28	South-western Australia (Medite rranean climate)	O'conell, 1988
Mean values			43.7	1.24	34.7	0.061	0.254	1.342	0.289		

Fig. 1. Leaf Litter of Casuarina

2.2 Composition of litter

Composition of the litter varies considerably depending on the species (Jamaludeen and Kumar, 1999), environment (Sayed et al., 2002) and geographic location (Zhang et al., 2008). The principal components of litter are the organic C (simple and complex carbohydrates, lignin, lipids and phenolics) and N compounds (proteins and nucleic acids) and the inorganic salts of P, K, Ca and Mg. The nutrient released into the soil is determined by the composition of these constituents in the litter (Diallo et al., 2005). N, C and significant inorganic salts of Casuarina litter estimated by various workers are summarized in Table 1. Up to 2% N is present in the fresh litter depending on the species and most of the N (up to 90%) released from the litter of Casuarinas has been found to be from branchlets (Ambasht and Srivastava, 1994). Aging litter has a high C:N ratio, although fresh branchlets are likely to have more N. Appreciable quantities of inorganic nutrients like P, K, Ca and Mg are also found in it. If Casuarinas grow on soils containing toxic inorganic mineral salts then their litter also contains these toxic substances (Sayed et al., 2002)

2.3 Pattern of litter fall in Casuarinas

The deciduous needle-like branchlets of Casuarinas are continually replaced by new growth. The variation in quantity of litter fall is dependent on the species, climatic conditions and geographical location (Clark and Allaway, 1996; Misra and Nisanka, 1997; Uma et al., 2014). Air temperature was determined to be the best predictor of leaf litter fall (Srivastava and Ambasht, 1996). Litter fall increases with age. Up to 30 tonnes ha^{-1} $year^{-1}$ of litter fall was reported in a 13 year old stand of *C. equisetifolia* in India (Misra and Nisanka, 1997). Accumulation of about 120 tonnes ha^{-1} of litter in a 34 year old stand of *C. equisetifolia* was reported in Western Africa (Mailly and Margolis, 1992).

Fig. 2. Flow chart for decomposition of Casuarina litter

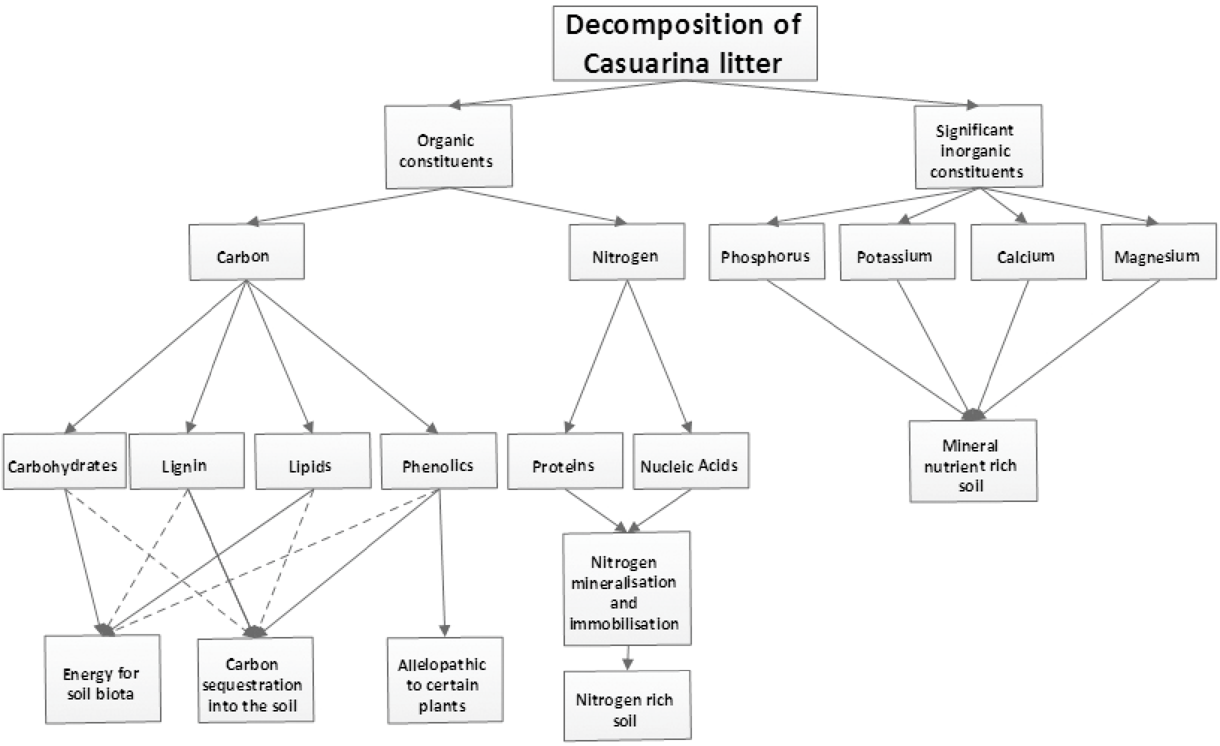

Table 2. Beneficial effects of Casuarina litter on the environment

Natural fertilizer	Enriches soil with organic matter and nutrients, especially with available N Aids Casuarinas in becoming pioneering species in degraded and waste lands
Improvement of soil properties:	
Improves soil pH	Useful for lowering soil pH of sodic soils
Conserves soil moisture	Used as a mulching agent (Sayed et al., 2011) Hinders flow of rain water and aids in controlling its surface runoff
Substrate for growing plants	Preferred substrate for growing Australian native orchids
As a fire retardant	Aids in controlling the spreading of wild fires (Scarrf and Westoby, 2006)
Provides food and/or shelter	In native habitats of Casuarinas In certain introduced habitats of Casuarinas (Chiba, 2010)
Fuel	Used as fuel in developing countries

2.4 Decomposition of the litter of Casuarinas

The initial rate of litter decomposition in Casuarinas is slow, mainly due to its components, and takes more than a year for complete decomposition. Thus, a large quantity of litter accumulates under the trees. Up to 12 cm of litter layer has been reported in *C. equisetifolia* plantations (Nagaraja et al., 2011). Litter decomposition changes according to the stage of decomposition in different layers of the litter. Favourable seasons for the maximum activity of litter decomposing organisms and for maximum litter fall may not coincide (Srivastava and Ambasht, 1996), which also leads to accumulation of litter beneath these plants.

Initially, easily accessible constituents of the litter are utilised by the litter decomposing organisms followed by a period of slower degradation when the more complex constituents of the litter are utilized.

A representative diagram of the pattern of Casuarina litter decomposition is depicted in Figure 2. Litter decomposing organisms utilize the C present in the litter for their metabolic activity. Simple carbon compounds are utilized rapidly while the more complex compounds are utilized at a much slower rate. Only a small fraction of lignin and phenolics are degradable. The residual fractions are sequestered into the soil thus increasing the soil C content. Lignin and phenolics may be complex with proteins and pectin making them unavailable for degradation (Sall et al., 2003). Most of the organic N compounds are converted into available forms of N that are readily utilized by the plants. High C:N ratios, N:P ratios (Mailly and Margolis, 1992), high N content (Bray et al., 2012), recalcitrant lignin and phenolic compounds and their ratios in relation to N determine the rate of litter degradation. The pattern of litter decomposition is determined by the amount of N, lignin, phenolics and C:N, N:P, lignin:N, phenolics:N, phenolics:lignin:N ratios present in it. Determination of these ratios may help in the prediction of the quality of the litter (Zhang et al., 2010). Appreciable quantities of inorganic nutrient salts are released into the soil during litter decomposition.

2.4.1 Recycling of the nutrients during decomposition of Casuarina litter

A major portion of nutrients released from the litter during decomposition is from the branchlet litter which are accumulated as the stand age increases and subsequently recycled back into the ecosystem via litter decomposing organisms. Concentration of the nutrients in the litter increased with stand age (Mailly and Margolis, 1992; Uma et al., 2014).Very old Casuarina stands have been reported to have accumulated large amounts of N in the forest floor, although it is likely that N is readily leached from the litter and its low residues become a limiting factor in litter decomposition. The forest floor of a 34 year old *C. equisetifolia* plantation contained 1567 kg ha^{-1} of N (Mailly

Table 3. Deleterious effects of Casuarina litter

Physical structure	Interferes with the nesting habit of native animals in certain regions (Wheeler et al., 2011). Exert negative effect on the germination of seeds of native plants (Hata et al., 2010; Craig et al., 1978).
Chemical components	Lignin: Affects the rate of decomposition. Phenolics and toxic minerals: Affect the rate of decomposition and exert allelopathic (toxic) effect on certain biota leading to the interference of the food chain in ecosystems in certain regions.
Litter removal	Depletion of nutrients may affect the yield of future generations. Decrease in the amount of development of fine roots may affect the yield of Casuarina stand (EnHua and Quiang, 2013). Increases in the incidence of bacterial wilt disease (Cao and Xu, 1990).

Table 4. Biota in Casuarina leaf litter

Organism	Reference
Bacteria	
Gram negative heterotrophs and Nitrifyers	Chen et al., 1999
Actinomycetes	Sayed et al., 2002 Chang et al., 2011
Fungi	
Hydrolytic and Lignin degraders	Uma et al., 2014
Lichens	Eldridge and Leys, 1999
Invertebrates	
Annelids	Warren and Zou, 2002 Ganihar, 2003
Arthropods	Warren and Zou, 2002 Rix, 2008; Uma et al., 2014
Mollusca	Chiba 2010 Uma et al., 2014

and Margolis, 1992). An average increase of 45 kg ha^{-1} $year^{-1}$ of N was recorded in this plantation.

C. equisetifolia litter released more N during its decomposition compared to the native species studied in the Oceanic Islands (Hata et al., 2012). The organic N compounds are mineralized by the litter decomposing organisms and are made available for other plants. In *C. glauca* litter, N mineralization occurred during the first month of decomposition and again during 4 to 8 months of litter decomposition in north-central Florida, USA (Bray et al., 2012). Highest N content and net mineralization was observed in soil samples amended with *C. equisetifolia* litter amongst various plant species studied (Diallo et al., 2005). N mineralization can be correlated to N content of the litter depending on the presence of other ingredients in the litter. Leaching of nutrients from the litter could also be one of the reasons for the variations found in the amount of N in litter by different authors. N content in the soil of mixed plantation increased when Casuarinas were grown with leguminous plants. Total N flux in the litter fall under mixed plantations with *Leucaena leucocephala* increased (about 56% over a 7 year period) in South America and in mixed plantations with *Eucalyptus robusta* or *Leucaena leucocephala* the C stores in the soil slightly reduced while the nitrogen stored in the soil increased by approximately 25% in 4-year-old plantations (Parrotta, 1999). The N and C of the litter immobilized in the bodies of the litter decomposing organisms are finally released into the soil after their death.

Concentrations of P vary in different constituents of Casuarina litter (Rathod and Devar, 2004). Appreciable quantities of P were found in the organic layer of 34 year old stand of *C. equisetifolia* as it accumulated slowly over the years (Mailly and Margolis, 1992). The K released during litter decomposition readily leaches after rainfall. Comparatively low levels of P and K found in sandy soils under Casuarinas in Bahamas (Buehler and Rodgers, 2012) could be due to their rapid rates of assimilation/leaching. Total annual return of Ca was comparatively high in *C. equisetifolia* litter (Rathod and Devar, 2004). Mg also accumulated beneath Casuarina litter. Total annual return of Mg is appreciable in *C. equisetifolia* litter and the nutrients returned followed the descending order Ca, N, K, Mg, P (Rathod and Devar, 2004). Mixed plantations of Casuarina with other plants showed better nutrient recycling. In mixed plantations, there was a significant increase in the inorganic salts of P, Ca, Mg in the soil but the K stores were not significantly different compared to monocultures (Parrotta, 1999).

2.4.2 Litter constituents interfering with nutrient cycling

Presence of high C:N, P:N, lignin:N, phenolics:N, phenolics:lignin:N (Jamaludeen and Kumar, 1999) ratios affect the rate of litter decomposition thus affecting nutrient recycling. Recalcitrant lignin and phenolics also interfere with nutrient cycling by binding to the nutrients and making them unavailable for degradation.

3 Allelopathic Effect of Casuarina Litter

Litter may have direct (by releasing allelopathic chemicals during decomposition) or indirect (by reducing nutrient availability by the formation of complexes with nutrients and allelopathic compounds) effect on litter decomposing organisms. Phenolics are also detrimental to the germination and growth of seedlings of certain plants (Patil and Hunshal, 2004; Lin et al., 2005) thus causing allelopathy. Only those plants that tolerate these compounds are able to grow under Casuarinas (Batish et al., 2001).

4 Influence of Casuarina Leaf Litter on Ecosystem

Casuarina leaf litter exerts both beneficial and harmful effects on its ecosystem. As large quantities of litter are produced by these plants, its effects on the ecosystem always merit consideration.

4.1 Beneficial effects of litter of Casuarina

Notable beneficial effects of Casuarina litter to mankind and other biota are summarised in Table 2.

4.2 Harmful effects of Casuarina litter

Deleterious effects of Casuarina litter on biota are summarised in Table 3. Together with its physical structure and its chemical constituent Casuarina litter is capable of changing the ecosystem in certain regions.

5. Litter Biota

Different organisms are found in the leaf litter of Casuarina (Table 4). The presence of adequate moisture and organic matter along with a protecting litter layer of Casuarinas created a mesic environment for fungal growth in Bahamas (Gochenuar, 1975). Profuse growth of mushrooms below Casuarina trees has been observed by the authors.

6 Future Plan of Work

Mushroom cultivation on *C. equisetifolia* wood chips yielded the highest amount of fruit bodies (Tisdale, 2004). Feasibility of mushroom cultivation on the Casuarina litter needs further study as the Casuarina litter contains substantial amounts of lignin which is utilised by mushrooms

7 Conclusion

Large quantities of N rich litter accumulated in Casuarina plantations is an important resource both in terms of economic utilization and nutrient balance. It needs to be effectively managed based on the agroforestry system and intended end use for which Casuarina is grown. Since fast and efficient decomposition of litter leads to release of nutrients in the soil, the specific role of many of the litter decomposing organisms needs to be studied further. Practices like gathering Casuarina litter for fuel should be discouraged as it imparts negative effects on the ecosystem.

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Effect of Bio-inoculants on Growth, Biomass and Nutrient Content of *Casuarina equisetifolia* Grown in Decomposed Coir Pith in Nursery Condition

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Key words: *Casuarina equisetifolia*, decomposed coir pith, root trainers, *Pseudomonas*, *Azospirillum*, *Trichoderma*, *Frankia*

Abstract

A nursery experiment was conducted to assess the effect of bio-inoculants (*Azospirillum, Trichoderma* and *Pseudomonas* applied both individually and in combinations) on seedling growth of *C. equisetifolia* grown in decomposed coir pith as substrate in root trainers. After six months, the plants were harvested and root and shoot length, collar diameter, root and shoot weight, nodule number and nodule weight were recorded. The total biomass was highest in *Azospirillum + Trichoderma + Pseudomonas* inoculated seedlings (54.8% increase over the control), followed by *Azospirillum + Pseudomonas* inoculated seedlings (49.2% increase over the control) and *Azospirillum* inoculated seedlings (47.3% increase over the control). Among the inoculated seedlings, *Azospirillum + Trichoderma + Pseudomonas* combination performed the best for biomass production and nutrient accumulation followed by *Azospirillum + Pseudomonas* combination and *Azospirillum*.

1. Introduction

Casuarina equisetifolia has been successfully introduced and cultivated in India and its cultivation area is steadily increasing as farm forestry plantations. It yields 120 -150 t ha^{-1} in short rotations of 2 to 4 years when inoculated with biofertilizers (Rajendran and Devaraj, 2004). The wood is highly suitable for firewood, charcoal production and as pulp wood for paper making. The high energy from the charcoal (3450 kJ kg^{-1}) makes it a good fire wood species (Orwa et al., 2009). It is also used in shelterbelts and windbreaks and commonly called as bio-shield plant. Due to these advantages, it is extensively planted in farm forestry, agroforestry and plantation forestry in a wide range of soil and climatic conditions.

Azospirillum is considered the most important rhizobacterial genus involved in improvement of plant growth or crop yield worldwide (Bashan et al., 2004). Through different mechanisms such as nitrogen fixation, phytohormone production, nitrate reduction and phosphate solubilization and mobilization it promotes plant growth after inoculation (Bashan and de-Bashan, 2010). Casuarina responds well to *Azospirillum* inoculation (Rajendran et al., 2003; Rajendran and Devaraj, 2004; Saravanan et al., 2013). *Trichoderma* spp. are free-living fungi that are common in soil and root ecosystems. It colonizes the rhizosphere rapidly and controls pathogenic and competitive microflora, improves plant health and stimulates root growth (Harman et al., 2004). They also solubilize a number of poorly soluble nutrients (Altomarne et al., 1991; Harman, 2000). *Pseudomonas* stimulates the plant growth through different mechanisms like antibiotic synthesis, plant hormone production, increasing P absorbance by plant and N stabling (Abdul-Jaleel et al., 2007).

Soil-less or soil-free growing media are commonly used in root trainers to improve general hygienic conditions in nurseries and to minimize the occurrence of soil-borne diseases (Mohanan, 2000a, b). Potting medium plays an important role in determining the growth of healthy fibrous root system. It physically supports the growing seedlings and both stores and supplies the nutrients, water and air to the root system and thereby promoting seedling growth. In recent times root trainers are increasingly being used to produce high quality seedlings with a well-developed root system. These containers are with open bottoms and vertical ribs, which help to avoid any kind of root coiling and the free flow of air constantly sloughing up the drainage hole resulting in proliferation of lateral roots. Decomposed coir pith can also substitute soil or sand in conventional nursery mixture for raising seedlings. Coir wastes after biodegradation can be effectively used as manure for increasing yield of crops (Ramasamy, 1986). Coir pith used as potting medium showed substantial increase in water holding capacity when tomato plants were grown on coir pith based potting mixture (Baskaran and Saravanan, 1997).

The decomposed coir pith is used as substrate for the production of planting stock in root trainer in the forest nurseries but plants grown in such media were found to have difficulty in early establishment under field conditions. This problem can be overcome by treating the plants with suitable biofertilizers. The objective of the present study was to assess the efficiency of individual and combined inoculation of bioinoculants on the growth and

Table 1. Growth of *Casuarina equisetifolia* seedlings inoculated with bioinoculants and grown in decomposed coir pith compost for 180 days after inoculation

Treatments	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Number of nodules	Collar diameter (mm)
T_1	29.66_{cd}	21.85_{cd}	51.52_{de}	4.41_{bc}	1.73_{bc}
T_2	23.60_{b}	19.07_{ab}	42.67_{b}	2.83_{ab}	1.47_{ab}
T_3	24.95_{b}	20.94_{bc}	45.89_{bc}	3.25_{ab}	1.47_{ab}
T_4	29.48_{cd}	21.40_{bcd}	50.88_{de}	4.08_{bc}	1.69_{bc}
T_5	30.50_{de}	23.85_{d}	54.35_{e}	5.08_{c}	1.85_{c}
T_6	26.55_{bc}	21.50_{bcd}	48.05_{cd}	4.00_{bc}	1.64_{bc}
T_7	33.59_{e}	26.73_{e}	60.32_{f}	7.00_{d}	2.41_{d}
T_8	19.35_{a}	17.05_{a}	36.40_{a}	2.25_{a}	1.30_{a}

Note: Means followed by a common letter(s) in the same column are not significantly different at the 5% level by DMRT (Duncan's Multiple Range Test); Treatments : T_1 – *Azospirillum* ; T_2 – *Trichoderma;* T_3 –*Pseudomonas* ; T_4 – *Azospirillum + Trichoderma* ; T_5 – *Azospirillum + Pseudomonas* ; T_6 – *Trichoderma + Pseudomonas*; T_7 - *Azospirillum + Trichoderma + Pseudomonas* ; T_8 – Control.

quality Casuarina seedlings grown in root trainer with decomposed coir pith used as potting medium.

2 Materials and Methods

2.1 Study area

The nursery experiment was conducted at the Plantation Division, Tamil Nadu Newsprint and Papers Limited (TNPL), Kagithapuram, Karur, Tamil Nadu, located at 11° 03' N latitude and 77° 59' E longitude at an elevation of 150m above mean sea level (msl). The annual maximum and minimum temperatures were 34 °C and 17 °C, respectively. The mean annual rainfall was about 725 mm and relative humidity ranged from 37.9 to 69.3%.

2.2 Seeds, potting media and experimental design

Casuarina equisetifolia fruits were collected from a plantation in the semi arid region of Cuddalore district of Tamil Nadu, India. Seeds were separated and graded and uniform size was used for raising seedlings. Seedlings were raised (about 5 cm height) in unsterilized sand in nursery mother bed and transplanted in 300cc root trainer with a potting mixture of unsterilized decomposed coir pith. The experiment was set up in a Completely Randomized Block Design (CRBD) with 8 treatments and 40 replicates. All the plants were kept under identical nursery condition up to 180 days.

2.3 Application of bioinoculants

Ten days after transplanting in root trainers, 15 ml each of *Azospirillum* (3.2x 10^9 cfu/ml), *Trichoderma* (3.0 x 10^7 cfu/ml) and *Pseudomonas* (2.5 to 3.0 x10^8 cfu/ml) were applied for single inoculation; for dual and triple inoculations, 7.5 ml and 5 ml each of bioinoculant were applied respectively. The inoculants were applied as per the following treatment schedule:

T_1 – *Azospirillum* (*Azospirillum brasilense*)
T_2 – *Trichoderma* (*Trichoderma viride*)
T_3 – *Pseudomonas* (*Pseudomonas fluorescens*)
T_4 – *Azospirillum + Trichoderma*
T_5 – *Azospirillum + Pseudomonas*
T_6 – *Trichoderma + Pseudomonas*
T_7 – *Azospirillum + Trichoderma + Pseudomonas*
T_8 – Control

The inoculants were applied to root trainers at 5 cm depth near the root zone. Uninoculated seedlings served as control. The seedlings were watered daily to maintain 80% field capacity and kept in nursery conditions for 180 days.

2.4 Assessing morphometric characters and nutrients

Twenty four randomly selected seedlings per treatment were harvested 180 days after transplanting and their shoot and root length, collar diameter, nodule number and fresh weight were recorded. They were cut at collar region and the shoot and root parts were dried separately in hot air oven at 70 °C until the weight became constant for biomass estimation (root and shoot dry weight). Total Nitrogen, Phosphorous, Potassium, Calcium and Magnesium content were determined by the standard methods. The data were statistically analyzed by conducting ANOVA with SPSS and treatment means were separated using Duncan's Multiple Range Test ($P< 0.05$) (Duncan, 1955).

3 Results

3.1 Morphometric observations

Seedling growth

All the seedlings survived till completion of the experiment. Seedlings with triple inoculation, T_7 showed the maximum length followed by dual inoculated plants T_5 and single

Table 2. Biomass (g plant^{-1}) of *Casuarina equisetifolia* seedlings inoculated with bioinoculants and grown in decomposed coir pith compost for 180 days after inoculation

Treatments	Needle dry weight	Stem dry weight	Total AGB	Root dry weight	Nodule dry weight	Total BGB	Total Biomass
T_1	2.22_{c}	1.35_{cde}	3.57_{c}	1.48_{bcd}	0.22_{bc}	1.71_{cde}	5.28_{de}
T_2	1.77_{b}	0.90_{ab}	2.67_{b}	1.11_{ab}	0.14_{ab}	1.26_{ab}	3.93_{b}
T_3	2.01_{bc}	0.94_{abc}	2.95_{b}	1.30_{bc}	0.14_{ab}	1.45_{bcd}	4.40_{bc}
T_4	1.96_{bc}	$1.21b_{cde}$	3.17_{bc}	1.36_{bcd}	0.15_{abc}	1.51_{bcd}	4.68_{cd}
T_5	2.27_{c}	1.36_{de}	3.64_{c}	1.59_{cd}	0.23_{c}	1.83_{de}	5.47_{e}
T_6	2.12_{bc}	1.14_{bcd}	3.26_{bc}	1.23_{abc}	0.16_{bc}	1.39_{bc}	4.66_{cd}
T_7	2.90_{d}	1.60_{e}	4.50_{d}	1.78_{d}	0.32_{d}	2.11_{e}	6.16_{f}
T_8	1.29_{a}	0.56_{a}	1.85_{a}	0.85_{a}	0.07_{a}	0.92_{a}	2.78_{a}

inoculated plants T_1 recording 39.6%, 33% and 29.3% increase over control respectively (Table 1). Within the dual inoculants, T_6 combination performed poorly with only 24.2% increase over the control. Similarly, among the single inoculated plants, T_2 inoculated plants showed poor seedling growth (14.7% increase over the control). Inoculation with T_7 induced maximum collar diameter (2.41mm), followed by dual inoculation with T_5 and single inoculation with T_1 (1.85 and 1.73 mm respectively). In contrast, collar diameter was the lowest in T_2 and T_3 treatments (1.47mm) (Table 1).

Number of nodules

Nodules were found in all the seedlings without inoculation of *Frankia*. However, higher nodule number was found with T_7 inoculation, compared to dual or single inoculation or control. Within the dual inoculants, T_5 combination resulted in higher nodule number. Among the single inoculated plants, T_1 treated seedlings had higher nodule number (Table 1).

Biomass estimation

The data pertaining to dry matter accumulation of needle, stem, root, nodule and total biomass are presented in Table 2. T_7 inoculation resulted in substantial increase in above ground biomass over the uninoculated control or dual or single inoculation. Within the dual inoculations, T_5 inoculation performed better in above ground biomass production. However, T_5 inoculation induced above ground biomass which was statistically on par with single inoculation with T_1 (Table 2).

The results on below ground biomass (root and nodule biomass) showed a significant difference among the treatments. Inoculation with *Azospirillum* alone and in combination with other inoculants viz., *Trichoderma* or *Pseudomonas* induced a significant increase in root and nodule biomass compared to other treatments (T_2, T_3 and T_6) (Table 2). The maximum total biomass was found in T_7 inoculated plants and it was 54.8% increase over the control. Seedlings inoculated with T_5 recorded 49.2% increase over the control. In the case of single inoculants, T_1 induced 47.3% increase over the control (Table 2).

3.2 Nutrient content

Seedlings treated with bioinoculants had significantly higher nutrient content in their tissues as compared to the control. Changes in total nitrogen, phosphorus, potassium, calcium, magnesium content of seedlings inoculated with bioinoculants and control are presented in Table 3**.** The nitrogen phosphorus, potassium, calcium, magnesium content reached the maximum in T_7 treatment. Nutrient content in tissues was in the order of Nitrogen>Calcium>Potassium>Magnesium>Phosphorus.

4 Discussion

The increase in the growth of seedlings inoculated with bioinoculants may be attributed to high accumulation of nutrient in the plant tissue. In the present study, 300cc sized root trainers were used which produced maximum height, collar diameter and biomass in six month old seedlings. The above results corroborate with earlier studies on three month old seedlings of *Casuarina equisetifolia* (Rathore et al., 2004).

Azospirillum inoculated seedlings showed better growth and root biomass when compared to the control. Growth may be attributed due to increased root biomass and accumulation of nitrogen (Wong and Sternberg, 1979; Rajendran et al., 2003) and the production of gibberellins and cytokinin like substances (Tien et al., 1979) which promote the growth of the seedlings. The above results corroborate with earlier studies on quality seedling production of *C. equisetifolia* by Rodriguez-Barrueco et al. (1991) and Rajendran et al. (2003), Casuarina trees treated with bioinoculants in farm forestry by Rajendran and Devaraj (2004) and *Moringa oleifera* by Rengamani et al. (2006). In the present study *Pseudomonas* inoculated seedlings produced better plant height, stem girth and total biomass. It may be due to inoculation of *Pseudomonas* which has shown stable and consistent capacity to

Table 3. Nutrient content (g plant^{-1}) of *Casuarina equisetifolia* seedlings inoculated with bioinoculants and grown in decomposed coir pith compost for 180 days after inoculation.

Treatments	N	P	K	Ca	Mg
T_1	0.11_e	0.007_e	0.07_c	0.10_e	0.025_d
T_2	0.06_b	0.004_b	0.05_b	0.06_b	0.013_b
T_3	0.08_c	0.005_{cd}	0.06_b	0.06_b	0.018_c
T_4	0.10_d	0.006_d	0.06_b	0.09_d	0.019_c
T_5	0.12_e	0.007_e	0.08_d	0.11_f	0.024_d
T_6	0.08_c	0.004_{bc}	0.07_c	0.07_c	0.019_c
T_7	0.15_f	0.008_f	0.10_e	0.12_g	0.030_e
T_8	0.04_a	0.002_a	0.04_a	0.03_a	0.008_a

solubilise insoluble phosphorus and thus making it available to plants.

Trichoderma are present in substantial quantity in nearly all agricultural soils and in other substrates such as decaying wood and their use is only now being recognized world over as an alternative in plant disease control (Harman et al., 2004). Among all the treatments, combined inoculations of *Azospirillum* + *Pseudomonas* + *Trichoderma* produced excellent growth, biomass and tissue nutrient concentration. Combination of bioinoculants is a major cause for success of both the plant establishment and the sustainability of bioinoculants and confirms the beneficial effects of microbial consortium over conventional method of single inoculant application (Raja et al., 2006). The greater height, diameter and dry matter of the *C. equisetifolia* seedlings due to co-inoculation of all the biofertilizers might strongly improve accumulation of nitrogen due to *Azospirillum* (Rajendran, 2003), more phosphorus uptake by Phosphobacterium (Kuccy, 1987).

Nutrient management has been well recognized since early times, which has become highly relevant with the advent of commercial forestry, where there is always a thrust to increase the production and biomass removal besides maintaining the site fertility. Estimation of the essential mineral elements in plants is an important aspect in the study of plant growth and ecosystem structure. Growth in the case of fast growing species, it becomes more essential to study the geochemical cycle of the essential elements in support of their survival in future. In the present estimation nutrient uptake was higher in seedlings treated with bio inoculation**.** Nutrient accumulation of seedling is in the order of nitrogen > calcium > potassium > magnesium > phosphorus.

Artificial application of *Frankia* as nodule suspension increased dry matter yield of Casuarina. Similarly, Rajendran et al. (2003) obtained increased overall growth, nodulation, shoot and root dry weight and nitrogen content by inoculation of *Frankia* in *C. equisetifolia*. However in the present assessment of seedlings grown in decomposed coir pith, nodules were found in all the seedlings without inoculation of *Frankia*. Nodulation was higher in seedlings treated with phosphate solubilizing microorganisms individually and in combinations with other biofertilizers. It shows that coir pith compost support to *Frankia* infection in seedlings naturally and phosphate solubilizing microorganisms help to improve the nodule number and nodular biomass in the seedlings.

5 Conclusion

Bioinoculants act as perpetually renewable inputs helping in better tree crop nutrient management and maintenance of soil health, better soil and water management leading to improved forestry practices. It is inferred that under appropriate technology, the use of efficient microbial inoculants yield increased growth and biomass of *C. equisetifolia* seedlings. The present study clearly shows that the application of *Azospirillum* along with *Trichoderma* and *Pseudomonas* plays a significant role in increasing the growth response of *C. equisetifolia* seedlings in a stipulated period, thereby producing good quality planting stock. These treated seedlings may perform better in nutrient impoverished soil too.

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Growth Response Based on Sex in *Casuarina equisetifolia* Inoculated with *Frankia*

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Key words: *Casuarina equisetifolia*, *Frankia*, growth, biomass, nodulation, symbiotic effectiveness, nitrogenase activity.

Abstract

Casuarina equisetifolia is predominantly dioecious in India and thus offers scope for gender-based selection for growth and other traits of economic importance. Being an actinorhizal plant benefitting from biological nitrogen fixation through symbiotic relationship with the actinomycete, *Frankia,* it is possible to further improve the selections through inoculation with the most effective source of the symbiont. A study was undertaken to assess the variation in growth, nodulation and nitrogenase activity among different sexes in three contrasting locations (coastal, riverine and inland) and determine the effect of *Frankia* inoculation on male and female plants. A higher frequency of male trees (57 to 60%) was found compared to female (38 to 40%) and monoecious trees (1 to 3%). Male plants also showed better growth than other two sexes in all three regions. Among the three regions, trees in riverine area showed the highest nodulation frequency, nodule biomass, nitrogen content and nitrogenase activity. Increase in growth and biomass due to *Frankia* inoculation under pot culture was more for male plants than female plants compared to the uninoculated control plants. Nodule extract from riverine region was the most effective in improving growth and biomass. Male plants showed better response to *Frankia* inoculation than female plants in terms of plant growth and biomass accumulation under pot culture up to 9 months age. The present study indicates the scope available for sex-based selection for growth, biomass and symbiotic efficiency through more studies involving a broad genetic base of host and symbiont and field tests covering the economic rotation age of Casuarina.

1 Introduction

Casuarina plays an important role in the economy and environment in India. It has the capacity to flourish under stressed environment that include soil acidity and salinity, water stress (both flooding and drought) high temperature and other adverse conditions. The outstanding ability of Casuarina to grow in such conditions is due to its symbiotic association with *Frankia* that fixes atmospheric nitrogen (Bond, 1983). Among many actinorhizal plant species, the potential of Casuarina as a multipurpose tree crop is recognized worldwide. It is also widely used for sand dune stabilization, shelterbelts, and windbreak in agroforestry systems.

Casuarina equisetifolia was introduced in India during the late 19th century. In Tamil Nadu State*, C. equisetifolia* cultivation has been accepted as an alternative land use pattern for improving nutrient status of soil, contributed by the root nodule and litter fall. Many studies suggest increasing productivity of casuarina by selection of both fast growing host tree genotypes and by inoculating highly effective compatible strain of *Frankia*. In India, *C. equisetifolia* is a predominantly a dioecious species with male and female flowers borne on separate individuals. However a small proportion of monoecious trees also occur. It has been reported that female trees in general showing lower girth and lesser biomass despite their slight edge over the others in respect to height (Kondas et al., 1985). Not many reports exist on the relationship between sex of the tree and symbiotic association with *Frankia* on the growth and other economically important traits in *C. equisetifolia*.

Keeping in view of the above facts, the present study was undertaken with the following objectives: to determine relationship between growth and sex in coastal, riverine and inland regions, to quantify nodulation of Casuarina in different regions as influenced by nitrogen fixing ability, to test the response of vegetatively propagated plants of different sexes to inoculation with *Frankia* and to study the effect of best host-endophyte interaction with respect to growth performance, physiological and biochemical characters.

2 Materials and Methods

2.1 Sex expression and growth in Casuarina plantations

Casuarina plantation at coastal (at the sea shore), riverine (at the river bank) and inland (interior cultivated land) areas near Annamalainagar, Cuddalore District, Tamil Nadu were surveyed for studying proportion of different sexes and their growth. The three regions were selected on the basis of differences in soil texture namely coastal sandy, sandy loam and sandy clay loam and hard laterite soil. In

Table 1. Screening of growth performance with respect to dbh (cm) and height (m) in male, female and general *C. equisetifolia* plantation at different regions

Sex	Male		Female		Monoecious	
Region	dbh	height	dbh	height	dbh	height
Coastal	4.34bd ±0.14	9.89d ±0.17	4.20d ±0.17	9.66d ±0.11	4.27d±0.11	9.77d±0.13
Riverine	4.14b ±0.13	8.65 ±0.20	3.48 ±0.21	7.83 ±0.32	3.814±0.12	8.23±0.18
Inland	2.73 ±0.11	7.33d ±0.12	2.32 ±0.12	6.94d ±0.16	2.525±0.10	7.136±0.10

Note: In a column, the means followed by the same letters are not significant at p = 0.05 using DMRT; ± = standard deviation.

each region, two- three year old plantations located at least a kilometer away from each other were selected. In each plantation, 100 trees were selected randomly in a criss-cross manner to cover the entire plantation. Tree height and diameter at the breast height (DBH) were measured and sex of the tree was noted during the flowering season.

In each plantation, the 50 trees were selected randomly in a criss-cross manner (Zhang and Torrey, 1985). Fresh young nodules were collected from each plantation of different soil texture to study their nitrogen fixing capacity – nitrogenase activity (acetylene reduction assay; Hardy et al., 1973), amino nitrogen **(**Stuart, 1935) and total Nitrogen (Nelson and Sommers, 1973).

2.2 Pot culture experiments

The pot culture experiments were conducted with vegetatively propagated male and female trees grown in pots.

2.2.1 Collection of nodules and preparation of inoculum

Fresh young nodules were collected from all three regions and surface sterilized. As it is rather difficult to culture *Frankia*, in the laboratory for the purpose of plant inoculation the fresh nodule suspension was prepared from nodule sources collected from 3 different regions (Callaham et al., 1979). These nodule preparations contain active and infective *Frankia*, confirmed by SEM of nodule section for the presence of endophyte.

2.2.2 Raising potted plants

Shoot cuttings taken from the selected male and female “plus” trees were propagated using rooting hormone IBA at 5000 µg L^{-1} inside mistless poly tunnels. Three months old rooted cuttings were treated with respective nodule suspension for 20 minutes and transferred to 45 cm diameter earthern pots filled with a mixer of unsterilized 3:1 ratio of river sand and red soil and were watered regularly. The experimental pots were arranged in a randomized block design (RBD) with 12 plants for each treatment as replicates. Experiments were carried out in the Botanical garden of Annamalai University. The uninoculated rooted cuttings were kept as control for both sexes.

2.3 Data collection and statistical analysis

Three months after inoculation, six plants were removed along with the entire root system from the pots and assessed for shoot and root length, dry weight, symbiotic effectiveness, root nodulation to select the best host-endophyte combination. These studies were continued with the remaining six plants at the age of six months only for the best host-endophyte combinations. Experimental results were statistically analyzed by DMRT and ANOVA.

3 Results

3.1 Sex expression in different regions

The distribution of male, female, monoecious trees of *C. equisetifolia* plantations in different regions are presented in Fig.1. In all the three regions, proportion of male trees (57, 63 and 60.5% in coastal, riverine and inland regions respectively) was higher than that of female trees (40, 38 and 38% in coastal, riverine and inland regions respectively). Monoecious trees constituted only a fraction in all three regions (3, 1, 1.5% in coastal, riverine and inland regions respectively).

Table 2. Nitrogenase activity and nitrogen content in nodules of *Casuarina equisetifolia* collected from different regions.

Region	Amino nitrogen (mg g^{-1})	Nitrogenase activity (µg g^{-1})	Total nitrogen (%)
Coastal	2.484^{b}±0.019	15.914^{a}±1.560	0.233^{b}±0.035
Riverine	3.087^{a}±0.099	18.568^{a}±0.881	1.530^{a}±0.296
Inland	0.821^{c}±0.012	3.951^{b}±0.265	0.135^{b}±0.019

In a column, the means followed by the same letters are not significant at p < 0.05 using DMRT; ± = standard deviation;

Fig. 1. Percent sex distribution of *Casuarina equisetifolia* at different regions

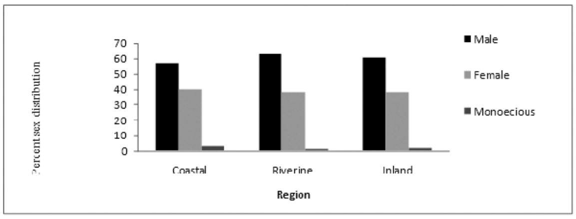


Fig.2. Effect of *Frankia* inoculation on the growth of *C. equisetifolia* at 3 months' age

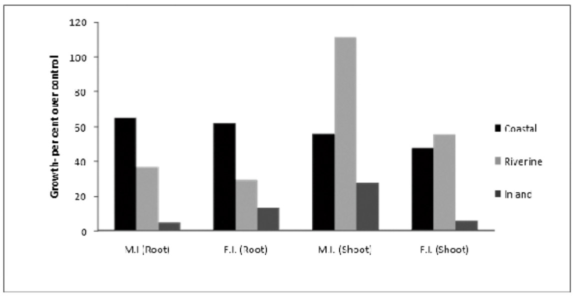
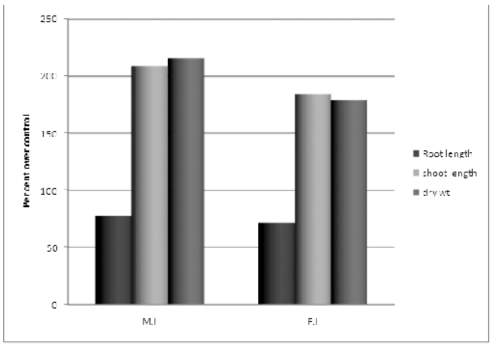


Fig. 3. Effect of *Frankia* inoculation on growth and biomass of *C. equisetifolia* at nine months'age



M.I- Male inoculated   F.I - Female inoculated

3.2 Growth of plantations in different regions

Tree growth (height and DBH) was found to be significantly different among the coastal, riverine and inland regions. In general, trees in the coastal region showed better growth than the other two regions. There was also significant growth difference among the three sexes. Male trees recorded better growth than female and monoecious trees (Table 1).

3.3 Nitrogenase activity and nitrogen content in nodules

The highest nitrogenase activity of 18.56 n moles of ethylene g^{-1} fresh weight h^{-1} was observed in nodules of riverine region. This region also showed the highest amino (3.08 mg g^{-1}) and total nitrogen (1.53%) in the nodules. Nodules of inland region recorded the lowest nitrogenase activity 3.95 n moles of ethylene g^{-1} fresh weight h^{-1}) and amino (0.82mg g^{-1}) and total nitrogen content (0.13%; Table 2).

3.4 Effect of *Frankia* inoculation

3.4.1 Nodulation

Male plant inoculated with nodule suspension from riverine region showed the highest (83.3) nodulation frequency, number of nodules per plant (10) and dry weight (1.18 g) followed by female plants inoculated with same nodule source (Table 3). The lowest nodulation frequency was observed in male plants inoculated with nodule suspension from coastal region followed by female plants treated with coastal and inland nodule sources. However these values were still found to be better when compared to uninoculated control (8.3%). Between the two sexes, male plants in general showed better values for these characters than female plants (Table 3).

3.4.2 Growth and biomass

All inoculated plants showed better growth than uninoculated controls (Fig. 2). Among the three months old inoculated plants, male plants showed the maximum root length of 64.8% over control with the nodule source from coastal region and maximum shoot length of 111.5% over control was observed with nodule source from riverine region. Female plants inoculated with nodule source from coastal region showed 61.5% more root length than control and 55.2% more shoot length than control with nodule source from riverine region. Male plants inoculated with nodule source from riverine region recorded maximum dry weight (569 g) over control and the minimum total dry weight (458 g) over control was observed with nodule source from inland region (Table 4). At nine months age, male plants showed 77.8% and 208.9% length of root and shoot over control respectively whereas the female plants showed 71.2% and 184% over control respectively (Fig. 3). Dry weight of the sampled plants, the total nitrogen content in root, shoot and nodules also showed similar trend.

3.4.3. Symbiotic effectiveness

The per cent symbiotic effectiveness in terms of dry weight was found maximum (669%) in male plants inoculated with nodule suspension from riverine region in three months old plants. In female plants too the maximum (360%) was observed in plants inoculated with nodule suspension from riverine region (Table 4). The same trend was observed in nine months old plants as well. At the age of nine months, inoculation with *Frankia* enhanced plant growth, stem girth, fresh weight and dry weight of both male and female plants. However, male plants showed superior growth characters at different age of the plants than the female plants. Male plants also showed higher number of nodules and nitrogenase activity (data not shown).

Table 3. Effect of inoculation on the nodulation of male and female *C. equisetifolia*

Region	Nodulation frequency (%)		Nodules plant^{-1}		Nodule dry weight (g plant^{-1})	
	Male	Female	Male	Female	Male	Female
Control	8.3	8.3	1^{Dd}	1^{Dd}	0.021^{D}g	0.03^{D}g
Coastal	33.3	50	5^{C}c ±0.71	4^{C}c±0.471	0.53^{C}e±0.022	0.28^{C}f±0.018
Riverine	83.3	66.6	10^{A}a ±0.89	7^{A}a±0.745	1.18^{A}a±0.012	0.71^{A}b±0.011
Inland	66.6	50	6^{B}b±0.87	5^{B}b±0.48	0.71^{B}c±0.005	0.59^{B}d±0.024

± = standard deviation
In a column, means (treatments) followed by different letters (capital letters) are significantly different (p=0.05), and in a row means (sex) followed by different letters (small letters) are significantly different (p=0.05) for root and shoot separately using DMRT.

4. Discussion

Variation in sex expression among the three regions (coastal, riverine and inland) may be due to interactions between genetic factors and environment considering the seed source of the plantations surveyed are not different. Sex expression has been reported to be varying among different provenances of *C. equisetifolia* (Luechanimitchit, 2002). The occurrence of higher proportion of male trees than female and monoecious trees is similar to observations reported from Thailand (Luechanimitchit and Luangviriyasaeng, 1996). The present study concurs with Dorairaj and Wilson (1981) who found male and monoecious trees showing better growth than female trees in coastal plantations. But the same authors found growth of female trees the best in riverine plantations while Kondas et al*.,* (1985) reported monoecious trees showing more vigorous growth than other sexes. No strong relationship between sex and growth was found in plantations of *C. equisetifolia* in Thailand (Luechanimitchit and Luangviriyasaeng, 1996). More studies involving different provenances and clones tested in contrasting locations are needed to determine whether sex of the individual influences on the growth of Casuarina.

The variation observed in the nitrogenase activity of nodules across the three study areas may be due to different strains of *Frankia* involved in nodulation in these areas (Baker and Torrey, 1990). In general, nodules showed the highest nitrogenase activity in the coastal region compared to the inland region. Casuarina has been grown in the coastal areas of India for more than a century and hence it is possible that most effective strain evolved in these areas over a period of time. Casuarina is being cultivated in the inland areas only recently and hence the potential inoculum may be limited in this region. Nodules originated from different soil nutrient status can also influence the variation in nitrogenase activity as reported by Sheppard et al. (1988). The variation in amino nitrogen content of nodules can be related to the level of nitrogeanse activity which influences nitrogen accumulation due to fixation and also seasonal changes (Wheeler et al., 1983).

The substantial improvement in growth and biomass of both male and female plants inoculated with *Frankia* shows the potential of biological nitrogen fixation through symbiosis for improving productivity in Casuarina. The nodule suspension employed as inoculum might have consisted more than one strain of *Frankia* resulting in infection and yield improvement in all treatments. The general of trend of male plants showing higher nodulation frequency, number of nodules, nodule weight, growth and biomass than female plants can be exploited for selecting such plants for clonal forestry with adequate field testing to confirm the superiority. Female plants with high symbiotic efficiency and growth will be useful as maternal parents in breeding programmes. Studies with more diverse genetic base and *Frankia* strains in future will be useful for a better understanding of gender-based symbiotic efficiency in Casuarina.

Table 4. Effect of Frankia inoculation on biomass and symbiotic effectiveness over control in male and female plants of *C. equisetifolia*

	Male inoculated		Female inoculated	
Region	total dry weight (g)	symbiotic effectiveness (%)	total dry weight (g)	symbiotic effectiveness (%)
Coastal	503.6	603.6 ±6.516	185.9	285.9±3.504
Riverine	569.1	669.1 ±7.189	260	360.1 ± 2.525
Inland	458.5	558.6±6.931	78.8	178.4 ± 4.667

5. Conclusion

Casuarina plants show different levels of nodulation and nitrogenase activity and sex expression in coastal, riverine and inland locations. Nodules collected from these three locations differed in their symbiotic efficiency with male and female plants. Male plants showed better growth in the plantations and also responded better than female plants to *Frankia* inoculation in pot culture experiments. More studies are needed to confirm these trends with a broad genetic base and host and the symbiont for improving the productivity in Casuarina.

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Age-related Changes in Tannin and Nutrient Concentrations of *Casuarina equisetifolia* Branchlets and Fine Roots

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Key words: stand age, total phenolics, condensed tannin, protein precipitation capacity

Abstract

Tannin and nutrient levels of *Casuarina equisetifolia* at different developmental phases (juvenile, mature and senescent phases) were examined to discuss the different functions between fine roots and mature branchlets. Results showed that branchlets contained relatively higher contents of total phenolics (TP), extractable condensed tannin (ECT), protein-bound condensed tannins (PBCT), total condensed tannin (TCT) and nutrients compared to fine roots. On the contrary, fiber-bound condensed tannins (FBCT) contents were higher in fine roots than in mature branchlets. Protein precipitation capacity (PPC) was higher for juvenile and mature plantations in mature branchlets than in fine roots, and lower for senescent plantations, while N:P ratio showed an opposite trend. Significant relationships were found between branchlets and fine roots for phosphorus, TP and TCT. In addition, the relationship between PPC and TP or TCT in branchlets, as well as PPC and TCT in fine roots was also significant. These results indicated that tannin level and nutrient concentration were affected by stand age, and *C. equisetifolia* could adjust its secondary metabolism and nutrient level between above- and below-ground with stand development.

1. Introduction

Plant chemistry often changes through the process of maturation (Donaldson et al., 2006). Plant developmental changes can occur rapidly, for example, as leaf matures and senesces through a growing season, or slowly, as long-lived perennial plants reach reproductive maturity and senescence. These changes can have effects on ecological interactions and ecosystem processes (Kraus et al., 2003; Donaldson et al., 2006). At the ecosystem level, variation in tannin content can influence litter decomposition and nutrient cycling rates (Hattenschwiler and Vitousek, 2000; Kraus et al., 2003). Whereas relatively little is known about the effects of stand age on tannin content of plants, many previous studies showed that nutrient availability, especially N and P, limits plant growth in most terrestrial ecosystems (Knecht and Goransson, 2004). Nitrogen to phosphorus (N:P) ratios have been applied to identify thresholds of nutrient limitation (Rejmankova, 2005).

Casuarina equisetifolia is a nitrogen-fixing tree of social, economic and environmental importance in tropical/subtropical littoral zones of Asia, the Pacific and Africa. There are more than 300,000 ha of *C. equisetifolia* plantations in the coastline of southern China (Zhong et al., 2010). Despite the widespread planting and known ecological and physiological properties of *C. equisetifolia*, there is scant information about the tannin and nutrient dynamics of *C. equisetifolia* forest with stand development. Since plant ecophysological traits are associated with ageing, we hypothesise that tannin and nutrient contents of branchlets and fine roots change with stand development. To evaluate this hypothesis, a field investigation of *C. equisetifolia* stand covering juvenile, mature and senescent phases was conducted at Chihu Forestry Center of Huian County, Fujian Province, China.

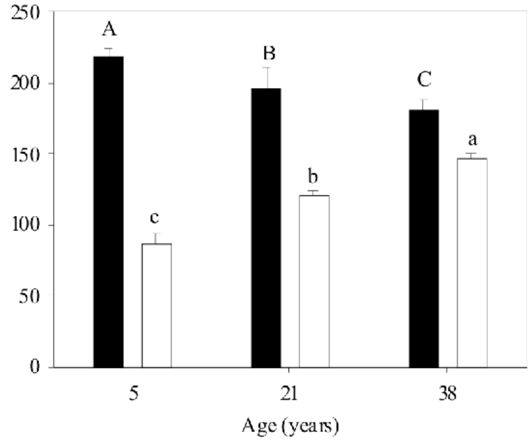
2 Materials and Methods

2.1 Materials

The study was carried out at Chihu Forestry Center of Huian County (23°45′N, 118°55′E), Fujian Province, China. The climate of the region belongs to southern subtropical maritime monsoon climate, with annual temperature ranging from 2.2 °C to 37 °C. Mean annual precipitation and evaporation are 1029 mm and 2000 mm, respectively. The rainy season is from March to October, and the dry season is from November to February.

In September 2012, we selected *C. equisetifolia* plantations, which were planted in 2004 (juvenile phase), 1988 (mature phase) and 1971 (senescent phase), respectively, at Chihu

Fig. 1. Content of total phenolics (TP) as a purified tannin standard in branchlets and fine roots of *Casuarina equisetifolia*; black bars are for branchlets and white bars for fine roots; for branchlets and fine roots, means with different capital and small letters are significantly different at the $P < 0.05$ level, respectively.



Forestry Center of Huian County, Fujian Province, China. Mature branchlets and fine roots were collected. All samples were taken to the laboratory immediately after sampling and cleaned with distilled water. The washed branchlets and fine roots were immediately freeze-dried for 48 h and then ground to pass through the 40-mesh sieve. The samples were stored at –20 °C prior to the chemical analyses.

2.2 Chemical analyses

Procedures described by Lin et al. (2006) were used to determine total phenolics (TP), extractable condensed tannins (ECT), protein bound condensed tannins (PBCT), and fibre bound condensed tannins (FBCT) and protein precipitation capacity (PPC). Total condensed tannin (TCT) content was calculated by adding the respective quantities of ECT, PBCT and FBCT (Terrill et al., 1992). A radial diffusion assay was used to determine the protein precipitation capacity (PPC) (Hagerman, 2011).

Plant samples were digested with sulfuric acid and hydrogen peroxide. The N concentrations of plant samples were determined by the micro Kjeldahl method (Yoshida et al., 1976), while the P concentrations were determined by ascorbic acid-antimony reducing phosphate colorimetric method (Institute of Soil Science, 1978).

2.3 Statistical analysis

All measurements were replicated five times. A one-way analysis of variance (ANOVA) was performed with stand age as the treatment factor. The Student–Newman–Keuls multiple comparison method was used to test the significance of differences between any two stand ages. All analyses were performed by SPSS15.0 for Windows.

3 Results

3.1 Changes in total phenolic (TP) content

TP contents of mature branchlets decreased from 218.5 ±6.06 mg g^{-1} to 180.4 ±7.70 mg g^{-1} during stand development. On the contrary, TP content of fine roots was in the order of senescent stand > mature stand > juvenile stand (Fig.1). TP contents in branchlets were higher compared to fine roots in all stands ($P < 0.05$).

3.2 Changes in condensed tannin content

The ECT content in branchlets of *C. equisetifolia* was in the order of juvenile stand > senescent stand > mature stand. It was the lowest in roots of mature stand, but there was no significant difference between juvenile and senescent stand (Fig. 2a).

The PBCT contents of branchlets and fine roots were both lower in mature stand than in juvenile and senescent stands (Fig.2b). The FBCT contents in branchlets were in the order of juvenile stand > senescent stand > mature stand. While the FBCT contents of fine roots increased during stand development (Fig. 2c).

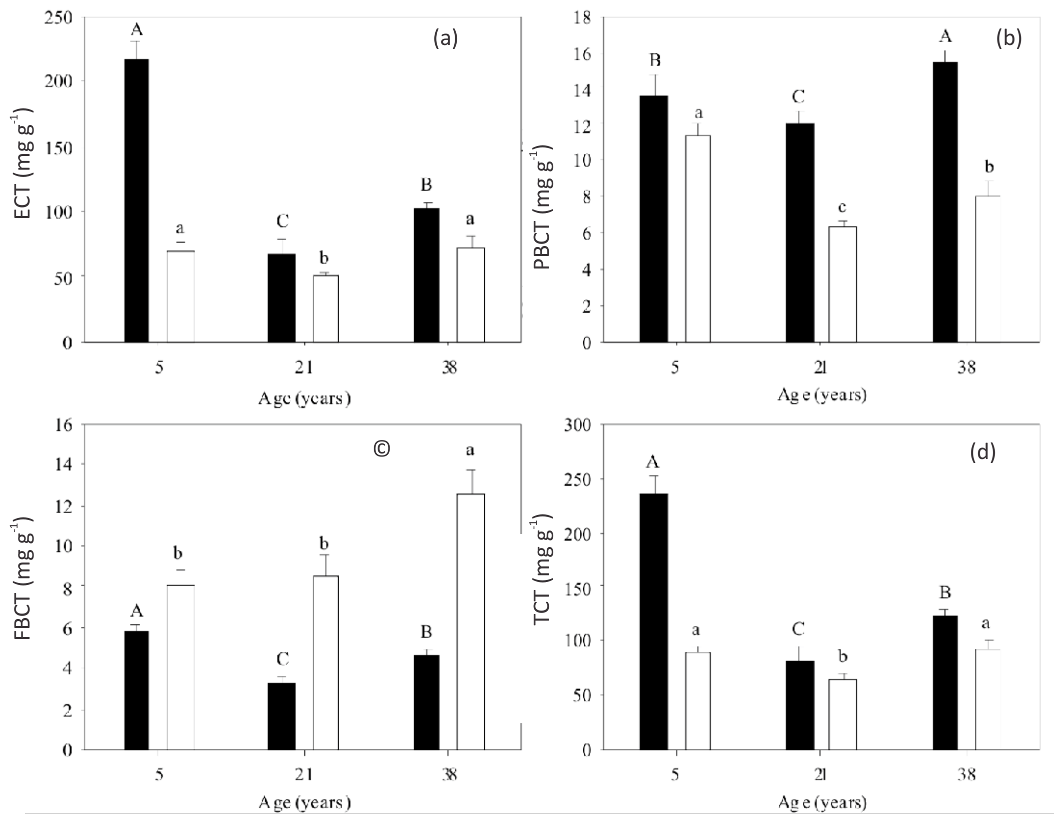
The TCT followed the pattern similar to ECT during stand development (Fig. 2d). The condensed tannins of branchlets were higher than fine roots except for FBCT.

Table 1. Changes in nitrogen (N), phosphorus (P) concentrations in branchlets and fine roots of *Casuarina equisetifolia* with stand development

Nutrient		Stand age (years)		
		5a	21a	38a
N ($mg·g^{-1}$)	Branchlet	15.16 (0.95)$_C$	18.81 (0.65)$_B$	20.07 (0.81)$_A$
	Fine root	12.10 (0.48)$_B$	11.27 (0.42)$_C$	14.70 (0.38)$_A$
P ($mg·g^{-1}$)	Branchlet	0.76 (0.05)$_A$	0.72 (0.05)$_A$	0.59 (0.04)$_B$
	Fine root	0.32 (0.02)$_C$	0.38 (0.04)$_B$	0.51 (0.05)$_A$
N:P	Branchlet	20.01 (1.13)$_C$	26.06 (1.54)$_B$	34.08 (3.01)$_A$
	Fine root	37.74 (2.44)$_A$	29.59 (2.76)$_B$	28.85 (2.78)$_B$

Means with different letters are significantly different at the $P < 0.05$ level. Values in parenthesis are standard errors.

Fig 2. Changes in extractable condensed tannins (ECT), protein-bound condensed tannins (PBCT), fibre-bound condensed tannins (FBCT) and total condensed tannin (TCT) contents in branchlets and fine roots of *Casuarina equisetifolia*; black bars are for branchlets and white bars for fine roots; for branchlets and fine roots, means with different capital and small letters are significantly different at $P < 0.05$ level, respectively



3.3 Changes in protein precipitation capacity (PPC)

Similar to TP contents, PPC in branchlets and fine roots decreased and increased with stand development, respectively (Fig. 3). PPC in branchlets were higher than fine roots in juvenile and mature stands, but no significant difference was found in senescent stand.

3.4 Changes in N and P concentrations and N:P ratios

N and P concentrations in branchlets and fine roots obviously increased during stand development except for P in branchlets, which decreased with stand development (Table 1). N:P ratio of branchlets increased and decreased in fine roots during stand development, and were all above 20.

4 Discussion

TP contents in branchlets decreased with stand development, indicating that stand age has an impact on TP contents. *C. equisetifolia* in juvenile stand possessed a greater selective pressure for defense than mature and senescent trees. Phenolics confer protection against herbivores is a likely explanation for such patterns.

Both ECT and TCT contents were also the highest in juvenile stand. But ECT and TCT of branchlets and fine roots were the lowest in mature stand. *C. equisetifolia* trees grow rapidly in mature stand. According to the protein competition model hypothesis, protein demand should be highest when the plants grow rapidly, and allocation to those phenolics that are derived from phenylalanine should simultaneously decrease (Jones and Hartley, 1999), as phenylalanine is the common precursor of either protein or condensed tannins synthesis (Hattenschwiler and Vitousek, 2000). In addition, the fact that branchlets contain much higher contents of TP and ECT compared to fine roots may indicate that branchlets are experiencing more intense selective pressure than fine roots.

PPC had a positive linear correlation with TP and TCT (Table 2), which was consistent with that reported in a previous study (Zhang et al., 2008). These observations suggest that both hydrolysable and condensed tannins contribute to the protein precipitation capacity of the branchlets.

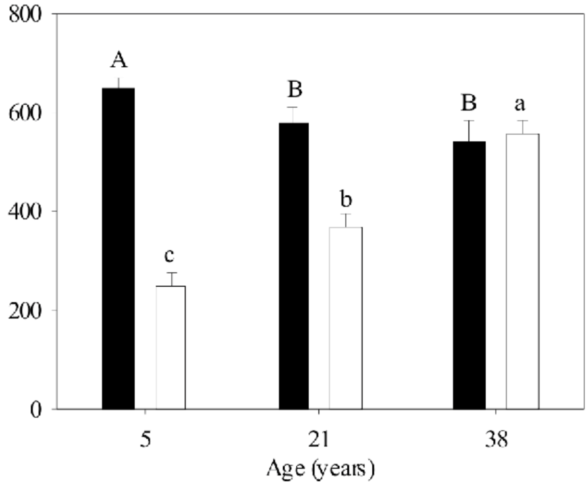
There were significant shifts in nutrient concentrations of *C. equisetifolia* branchlets along the stand age

Table 2. Correlative coefficient of selected branchlets and fine roots of *Casuarina equisetifolia*

Variable	R^2	F	P
N_B-N_R	0.224	3.756	0.075
P_B-P_R	0.556	16.944	<0.001
TP_B-TP_R	0.810	55.483	<0.001
ECT_B-ECT_R	0.257	4.506	0.054
$PBCT_B$-$PBCT_R$	0.048	0.657	0.432
$FBCT_B$-$FBCT_R$	0.001	0.007	0.934
TCT_B-TCT_R	0.278	4.997	<0.05
TP_B-PPC_B	0.547	15.693	<0.01
TP_R-PPC_R	0.913	137.081	<0.001
TCT_B-PPC_B	0.472	11.617	<0.01
TCT_R-PPC_R	0.036	0.491	0.496

N_B, N_R = nitrogen concentrations in branchlets and fine roots, respectively; P_B, P_R = phosphorus concentrations in branchlets and fine roots, respectively; TP_B, TP_R = total phenolic concentrations in branchlets and fine roots, respectively; ECT_B, ECT_R = extractable condensed tannin concentrations in branchlets and fine roots, respectively; $PBCT_B$, $PBCT_R$ = protein-bound condensed tannin concentrations in branchlets and fine roots, respectively; $FBCT_B$, $FBCT_R$ = fibre-bound condensed tannin concentrations in branchlets and fine roots, respectively; TCT_B, TCT_R = total condensed tannin concentrations in branchlets and fine roots, respectively; PPC_B, PPC_R = protein precipitation capacity in branchlets and fine roots, respectively.

Fig. 3. Changes in protein precipitation capacity (PPC) in branchlets and fine roots of *Casuarina equisetifolia*; black bars are for branchlets and white bars for fine roots; for branchlets and fine roots, means with different capital and small letters are significantly different at the $P < 0.05$ level, respectively



chronosequence (Table 1). N concentrations in branchlets increased with stand development, which was in accordance with the observation of Yuan and Chen (2010). The changes in green-leaf N with stand development might be related to the dilution effects because the greater leaf biomass production rates in younger stands could result in reduced N concentrations even if absolute uptake rates are high (Yuan and Chen, 2010).

Nitrogen to phosphorus (N:P) ratio has been applied to identify thresholds of nutrient limitation (Güsewell and Koerselman, 2002; Rejmankova, 2005). Thresholds of foliar N: P ratios were found to be <14 for nitrogen limitation and >16 for phosphorus limitation (Güsewell and Koerselman, 2002). In the present study, the N:P ratios of branchlets and fine roots were all above 20, indicating that three stands were P-limited.

5 Conclusions

Junvenile stands of *C. equisetifolia* contained relatively higher total phenolics, extractable condensed tannin, total condensed tannin contents, and protein precipitation capacity in branchlets compared to those in mature and senescent stands. N concentrations in branchlets increased with stand development, while P concentrations showed a decreasing trend. N:P ratios in mature branchlets were above 20, and increased with stand development. The tannin level and nutrient concentration were affected by stand age.

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Response of *Casuarina equisetifolia* and *C. junghuhniana* to *Frankia* Under Field Conditions

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Key words: *Casuarina equisetifolia*, *Casuarina junghuhniana, Frankia,* nitrogen, survival, growth

Abstract

Casuarina equisetifolia and *C. junghuhniana* are being cultivated in southern India mainly for pulp and paper production and scaffolding for construction. The roots of *C. equisetifolia* and *C. junghuhniana* normally produce root nodules where atmospheric nitrogen (N) is fixed through the symbiotic relationship with *Frankia.* Therefore, it is necessary to introduce *Frankia* in casuarinas at the seedling stage for better growth and nutrient improvement. In this study, the effect of inoculation of cultured *Frankia* on the seedling growth of *C. equisetifolia* and *C. junghuhniana* was assessed. The inoculated seedlings showed better growth and biomass production than uninoculated seedlings. The N accumulation was also higher (2.7 to 2.9 mg g^{-1}) in *Frankia* inoculated seedlings than control (0.8 to 0.83 mg g^{-1}). These seedlings were planted in a farmland at Karaikal, Puducherry, India, to test the effect of *Frankia* inoculation on the survival and growth of trees under field conditions. At 2 years age, growth of inoculated plants was significantly increased compared to uninoculated control without any additional organic or inorganic fertilizers. The soil nutrient status was also found improved due to *Frankia*. The survival of the *Frankia* inoculated seedlings was 94.6% in the field conditions and this study confirms that application of *Frankia* for growth improvement of casuarinas is an alternate to chemical fertilizers.

1 Introduction

Casuarina equisetifolia L. and *C. junghuhniana* Miq. are nitrogen fixing trees cultivated in around 500,000 ha in India mainly in the States of Tamil Nadu, Andhra Pradesh, Odisha and Pondicherry. They are multipurpose trees, used as fuel wood, pulpwood, poles and providing services like shelterbelts, windbreaks (Duke, 1983), rehabilitating mine spoils and nutrient poor areas. Its importance in protecting coastal environment has been realized after the occurrence of Tsunami in 2004 and it became a major component in many coastal afforestation programmes. The annual planting target requires production of a large number of high quality seedlings to maximize survival and growth in plantations. While genetic improvement through selection and breeding has resulted in substantial genetic gain for growth, it can further be improved through promoting the symbiotic relationship between Casuarina and *Frankia* leading to increased biological nitrogen fixation. Nitrogen-fixation is the biological process responsible for reduction of molecular nitrogen into ammonia carried out by *Frankia* through interaction with Casuarina in the root nodules (Benson and Silvester, 1993). Nitrogen is generally considered one of the major nutrients for plant growth. As part of the symbiotic relationship with *Frankia*, Casuarina trees convert the 'fixed' ammonium ion to nitrogen oxides and amino acids to form proteins and other molecules (e.g., alkaloids). In return for the 'fixed' nitrogen, the trees secrete sugars to the symbiotic bacteria (Santi et al., 2013). It has been estimated that *Frankia* fixes atmospheric N up to 362 kg ha^{-1} $year^{-1}$ (Shantharam and Mattoo, 1997).

Generally, Casuarina seedlings are inoculated with *Frankia* by treating with crushed root nodules collected from mature trees. This practice may not be successful if the root nodules contain dead or inactive *Frankia* leading to poor or absence of nodule formation. Application of inorganic nitrogen fertilizer like Diammonium phosphate fertilizer increases cultivation cost (Nicodemus, 2009). The objective of the present study was to develop a cost effective and easy to adopt inoculation procedure for *Frankia* and to test its effect on survival and growth of plants both in nursery and field conditions. The use of such inoculation has the potential to reduce the use of chemical fertilizers and planting cost.

2 Materials and Methods

2.1 Seedling production

Seeds of *C. equisetifolia* and *C. junghuhniana* were obtained from the seed bank of Seed Technology Division, Institute of Forest Genetics and Tree Breeding (IFGTB). They were sown in the nursery beds containing pure sand with sufficient water spray. After germination, 10 days old seedlings with uniform height (about 5 cm) were transplanted to polythene bags (10 x 14 cm) containing sterilized sand and soil (1:1 v/v).

Table 1. Growth and biomass of *Casuarina equisetifolia* and *C. junghuhniana* seedlings to *Frankia* inoculation at 90 days under nursery conditions.

	C. equisetifolia		*C. junghuhniana*	
Parameters	Inoculated	Control	Inoculated	Control
Collar Diameter (cm)	$1.58_b \pm 0.21$	$0.80_a \pm 0.04$	$1.88_b \pm 0.16$	$0.73_a \pm 0.02$
Shoot length (cm)	$126.3_b \pm 1.80$	$61.12_a \pm 0.18$	$159.4_b \pm 1.42$	$72.32_a \pm 0.12$
Root length (cm)	$24.40_b \pm 0.52$	$12.10_a \pm 0.26$	$52.4_b \pm 0.58$	$13.22_a \pm 0.31$
No. of lateral roots	$12.80_b \pm 0.44$	$4.23_b \pm 0.19$	$21.8_b \pm 0.34$	$6.42_a \pm 0.16$
Shoot dry weight (g $plant^{-1}$)	$6.33_b \pm 0.13$	$2.10_a \pm 0.16$	$9.1_b \pm 0.11$	$4.30_a \pm 0.13$
Root dry weight (g $plant^{-1}$)	$3.56_b \pm 0.11$	$1.65_a \pm 0.09$	$5.8_b \pm 0.06$	$3.85_a \pm 0.02$
R/S ratio	$0.56_b \pm 0.04$	$0.78_a \pm 0.03$	$0.63_b \pm 0.07$	$0.89_a \pm 0.04$
Nodulation time (days)	21	–	25	–
Number of nodules	11.6 ± 0.13	–	24.3 ± 0.11	–
Nodule biomass (mg $plant^{-1}$)	45.3 ± 1.10	–	22.35 ± 0.16	–

Mean followed by same letters are not significantly at $p < 0.05$ of DMRT, same as following Tables

2.2 Inoculation of *Frankia* strains in the seedlings

The root nodules of *C. equisetifolia* and *C. junghuhniana* were collected from the field grown trees in coastal plantations on sandy clay loam soil. Two strains of *Frankia* were isolated from them and named as CeCb1 from *C. equisetifolia* and CjCb2 from *C. junghuhniana*.

The *Frankia* strains CeCb1 and CjCb2 cultured in P media broth were inoculated in the root zone of the seedlings at the rate of 10 ml per seedling. Seventy five seedlings each of *C. equisetifolia* and *C. junghuhniana* were treated with the *Frankia* strains CeCb1 and CjCb2 respectively. An equal number of uninoculated seedlings were kept as control. The seedlings were arranged in a randomized block design consisting 15 replications with 5 seedlings of each treatment in a replication. The seedlings were placed in the shade house of IFGTB's research nursery and watered regularly for 3 months.

2.3 Evaluation of growth, biomass and tissue Nitrogen (N) content

Twenty five seedlings of each treatment (5 replications) were utilized to assess Collar Diameter, shoot length, root length, number of lateral roots, dry weights of shoot and root, nodulation time, number of nodules and nodule biomass. The total N content was estimated in root and shoot sample using KELPLUS auto analyzer (Jackson, 1973).

2.4 Field planting

The remaining 50 seedlings of each treatment were planted in a field test in a coastal farm at Karaikal, Puducherry, India by adopting a randomized block design with 10 replications each containing 5 seedlings of a treatment planted at a spacing of 1.5 x 1.5 metres. Height and diameter growth of trees were assessed two years after field planting.

2.5 Analysis of soil nutrients

The major soil nutrients (N, P and K), soil pH and electrical conductivity of the soil were analyzed according Jackson (1973) before and two years after planting of *Frankia* inoculated field trial.

2.6 Statistical analysis

Each measured variable in the nursery and field experiments were statistically analyzed using Duncan's multiple range test (SPSS ver. 17).

Fig.1. Tissue N content in *C. equisetifolia* (1) and *C. junghuhniana (2)*

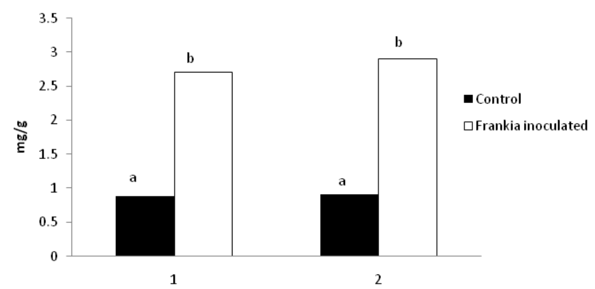

Fig.2. Survival rate of *Frankia* inoculated seedlings of *C. equisetifolia* (1) and *C. junghuhniana* (2) after two years

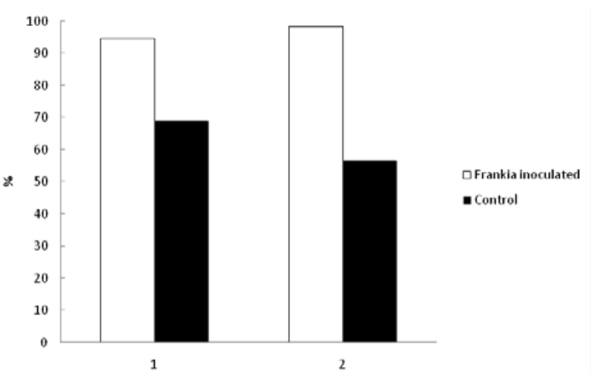

Table 2. Field performance of *Casuarina equisetifolia* and *C. junghuhniana* seedlings inoculated with *Frankia* after two years (mean of 10 replicates)

Treatment	*C. equisetifolia*		*C. junghuhniana*	
	Height (m)	GBH (cm)	Height (m)	GBH (cm)
Frankia	8.6_b ±0.18	11.8_b ±0.15	9.8_b ±0.19	10.25_b ±0.14
Control	3.2_a ±0.12	5.6_a ±0.17	4.6_a ±0.13	4.8_a ±0.11.

3 Results

3.1 Seedling growth and biomass

Nodulation was observed 21 days after inoculation in *C. equisetifolia* and 25 days after in *C. junghuhniana*. The initial infection has showed as clubbed roots in the seedlings and the nodule development occurred after 21 to 25 days. Seedlings inoculated with *Frankia* showed significantly increased shoot length, root length, collar diameter and bio mass compared to uninoculated control seedlings (Table 1). Between the two species, *C. equisetifolia* showed higher nodule biomass than *C. junghuhniana* although the later had more number of nodules than the former. *C. equisetifolia* had 11.6 nodules per seedling weighing 45.3 mg whereas there were 24.3 nodules in *C. junghuhniana* weighing 22.35 mg. The R/S ratio was significantly lower for *Frankia* inoculated seedlings than the uninoculated control.

3.2 Tissue N content

Significant difference in total nitrogen content was observed between uninoculated and inoculated seedlings of both the species. The total N content was 2.7 to 2.9 mg g^{-1} dry weight in the inoculated seedlings whereas it was 0.89 mg g^{-1} in the uninoculated control seedlings (Fig. 1).

3.3 Field performance

The *Frankia* inoculated seedlings showed better survival of 94.6% in *C. equisetifolia* and 98.3 % in *C. junghuhniana* compared to 70% and 58% survival for control of respective species in the field test. *Frankia* inoculated seedlings showed significantly ($p<0.05$) higher growth in terms of height (8 to 10 m) and stem girth (10 to 12 cm) compared to the uninoculated controls (Table 2). Three fold increase of growth was observed due to inoculation of *Frankia* in the seedlings under field conditions than control.

3.4 Soil nutrients

The soil nutrients particularly N was significantly increased after planting of *C. equisetifolia* and *C. junghuhniana* seedlings inoculated with *Frankia*. The electrical conductivity and soil pH have also changed after planting (Table 3).

4 Discussion

The results showed that *Frankia* inoculation could increase the growth and biomass in Casuarinas under nursery conditions and also help in successful establishment and fast growth under field conditions without additional fertilizer input. Earlier studies reported that the increase in growth and biomass of Casuarina due to inoculation of *Frankia* might be strongly correlated with improved accumulation of nitrogen (Karthikeyan et al., 2011; 2013). This study further supports the positive response of *C. equisetifolia* and *C. junghuhniana* in the nursery to *Frankia* application confirms *Frankia* dependency of Casuarina in field conditions. Similar results were reported for *Frankia* (nodule suspension) inoculation employed in *C. equisetifolia* seedlings (Muthukumar and Udaiyan, 2010). In some of the studies the effect of *Frankia* on growth promotion in actinorhizal plants has been demonstrated in sterile soil substrates. Hence, inoculation of *Frankia* in nursery conditions is beneficial for *C. equisetifolia* and *C. junghuhniana* which produce more root nodules in nursery and field conditions.

In this study nodulation occurred from 21 to 25 days in the seedlings of Casuarina but Vergnaud et al. (1985) obtained axenic nodulation in *Alunus glutinosa* within 10 days. This also showed that there is a difference in nodulation behavior among actinorhizal plants. It was reported that nodulation helps for N transfer to *C. cunninghamiana* seedlings by *Frankia* inoculation (He et al., 2005). High nodule number and biomass in the seedlings of *C. equisetifolia* and *C. junghuhniana* inoculated with *Frankia* reflects the symbiotic nitrogen fixation that helps in host photosynthesis (Arnone and Gordon, 1990) where the ATP

Table 3. Soil nutrient status before and after planting with *Frankia* inoculated Casuarina

Treatment	pH	EC (mS)	N (mg kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})
Before planting	6.00 ±0.14	0.08 ±0.02	1.30 ±0.02	1.98 ±0.13	4.00 ±0.06
Two years after planting	6.8 ±0.12	0.19 ±0.01	3.63 ±0.03	2.40 ±0.16	5.30 ±0.08

supplemented to *Frankia*. The increased biomass in the seedlings of both the species of *Casuarina* could be the result of increased nutrient inflow through *Franki*a. The higher tissue N content of *Frankia* inoculated seedlings than the uninoculated control plants shows the influence of *Frankia* in N fixation (Franche et al., 2009). The increased growth of *Frankia* inoculated seedlings under field conditions may be due to increased uptake of N through *Frankia*. Earlier studies also showed that the growth of *Alnus cordata* inoculated with *Frankia* increased the growth and survival in field conditions due to the uptake of N (Lumini et al., 1994). Soil nutrients were improved due to the activities of *Frankia*. Therefore, the results from this study support the inoculation of cultured *Frankia* to the seedlings of *C. equisetifolia* and *C. junghuhniana* for enhancement of growth, biomass and nutrient uptake. This method of inoculation of *Frankia* in the seedlings of *C. equisetifolia* and *C. junghuhniana* will be beneficial for early establishment in the field without the addition of chemical fertilizers. The fresh root nodules produced through inoculation of *Frankia* in Casuarina under field conditions also indicate the health of the trees.

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Genetic Improvement of Casuarinas: Past Achievements and Priorities for Future

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Abstract

Compared to commercially important tree genera such as *Acacia*, *Eucalyptus* and *Pinus*, investment in genetic improvement in Casuarinas is recent. In the past, Casuarinas were principally grown for environmental protection and ecological restoration, and the main use of wood was in the form of firewood and charcoal not as pulpwood or sawn timber. Until mid 1990s, most of the work related to genetic improvement focused on species and provenance trials. Four species, *Casuarina cunninghamiana*, *C. equisetifolia*, *C. glauca* and *C. junghuhniana* have been identified as most suitable for large scale planting. Of these, *C. equisetifolia* is the most extensively planted Casuarina with probably close to a million hectares under cultivation in China, India and Vietnam. The first large-scale progeny trials of *C. equisetifolia* were established in India in the late 1990s and those of *C. junghuhniana* were established in Thailand in the early 2000s. The first cycle of breeding has been completed for both species with significant genetic gain in the productivity. Advances in the understanding of genetic structure and reproductive biology have been integrated into operational breeding and deployment programs. As these programs have now moved to advanced generation there is a pressing need for infusion of genetic material to maintain a broad genetic base. There is an increasing interest in interspecific hybrids of the four species. This calls for more effective controlled pollination techniques as well as effective vegetative propagation techniques for deployment of the hybrid clones.

1. Introduction

Casuarinas are a group of 96 species of trees and shrubs belonging to family Casuarinaceae (Turnbull, 1990). The natural distribution extends from Southeast Asia to Australia and the Pacific. Until 1980 all species were included in a single genus *Casuarina*. Currently four genera: *Allocasuarina* (Johnson 1982), *Casuarina*, *Ceuthostoma* (Johnson 1988) and *Gymnostoma* (Johnson, 1980) are recognized based on evidence of anatomy, biogeography, cytology and morphology. Species of the Casuarinaceae family are well known for their environmental adaptability. They thrive in deserts and coastal sand dunes, mine dumps and other places where nutrients and water are scarce, and where salt and wind are excessive. The most extensively planted species belong to the genus *Casuarina* mainly *C. equisetifolia, C. cunninghamiana, C. glauca* and *C. junghuhniana* with probably close to a million hectares of *C. equisetifolia* under cultivation in China, India and Vietnam.

Research interest in Casuarinaceae species began in the late 1950s and early1960s when the biological nitrogen fixation ability and association with *Frankia* were realized. Research on genetic improvement and breeding of Casuarinas, however, is more recent compared to other commercially important species of *Eucalyptus* and *Pinus*. Those early activities were concerned mainly with provenance test in the 1980s with most of the efforts focusing on *C. equisetifolia, C. cunninghamiana, C. glauca* and *C. junghuhniana*.

2. Genetic Resources Research

2.1 Early investigation in provenance variation

Provenance seed collection in Australia by CSIRO Australian Tree Seed Centre (ATSC) in the early 1980s made it possible to study provenance variation in *C. cunninghamiana* and *C. glauca*. The first of such trials were established in Egypt in 1982 and 7-year growth results reported by El-Lakany (1990) (Table 1). More provenances of *C. cunninghamiana* and *C. glauca* were collected and field trials planted on several locations in California in 1987; two-year results were reported by Merwin (1990) (Table 2). These early trials revealed considerable variation among provenances of both Casuarina species and encouraged more investigation in genetic variation of other Casuarina species.

Casuarinas are ecologically and economically important in the coastal areas of southern China. Systematic introduction of Casuarina species including *C. cunninghamiana* and *C. glauca* was carried out in Hainan province in 1987. One-year results confirmed early rapid growth of these two species (Table 3). Another provenance trial with a wider range of seedlots of both species was planted in Guangdong province in 2002. This trial revealed

Table 1. Results of *C. cunninghamiana* and *C. glauca* provenance trials at age 7 years in Egypt (source: El-Lakany, 1990)

CSIRO Seedlot No.	Provenance	Survival (%)	Height (m)	Diameter (cm)
C. cunninghamiana				
13125	Bathurst, NSW	48	3.48	2.91
13127	Singleton, NSW	58	4.90	4.31
13129	Tenterfield, NSW	79	4.91	4.75
13134	Gympie, Qld	67	5.67	5.15
13148	Cobargo, NSW	66	3.65	3.95
13149	Uriarra Crossing ACT	57	3.48	3.05
13508	Augathella, Qld	100	6.98	7.32
13510	Banana Ck, Qld	68	5.35	5.30
13511	South Dululu, Qld	89	7.38	7.33
13512	Fletcher Ck, Qld	88	6.55	6.05
13513	Oasis, Qld	91	7.16	6.83
13514	Petford, Qld	80	5.88	5.09
13515	Mareeba, Qld	92	7.13	7.62
13516	Lakeland Downs, Qld	79	5.57	5.50
13517	Helenvale, Qld	82	5.00	4.83
13518	Mt Molloy, Qld	93	5.96	6.05
13519	Kinduro, Qld	95	6.17	6.11
13520	Marlborough, Qld	74	5.85	5.65
	LSD 1%	9.2	1.82	1.47
C. glauca				
13128	Singleton, NSW	65	4.22	3.34
13135	Caloundra, Qld	70	4.64	4.95
13137	Ballina, NSW	77	5.70	4.77
13139	Woolgoolga, NSW	59	4.49	3.74
13141	Coffs Harbour, NSW	67	4.77	4.23
13142	Taree, NSW	75	5.82	5.57
13143	Mangrive Ck, NSW	77	4.59	4.25
13144	Ulladulla, NSW	71	5.87	5.64
13146	Buladelah, NSW	80	5.26	4.81
	LSD 1%	7.7	1.97	1.63

considerable provenance variation similar to that found in Egypt and California (Table 4).

2.2 International provenance seed collection and evaluation of *C. equisetifolia*

Following a recommendation of the Second International Casuarina Workshop in Cairo, Egypt in 1990, the ATSC coordinated an international seed collection of *C. equisetifolia* during 1990-1992. In total 67 provenances were collected in 21 countries both within and outside the species' natural distribution range. Details of this collection was described in Pinyopusarerk et al. (2004).

The origins of these seedlots can be categorized into 5 broad regions

(1) Natural distribution in Australia and the Pacific

(2) Natural distribution in Southeast Asia

(3) Locations of introductions in Asia

(4) Location of introductions in Africa

(5) Location of introductions in Central America

Thirty eight provenance trials were established in 20 countries during 1992-1994 and involved 60 provenances and land races. Results from 25 trials were synthesized in a

Table 2. Two-year height (m) of *C. cunninghamiana* and *C. glauca* provenance trials in California, USA. (Source: Merwin, 1990)

CSIRO Seedlot No	Provenance	Anderson	Woodland	Davis	Oregon House	Palermo
C. cunninghamiana						
14919	Uriarra Crossing, ACT	2.63	3.03	4.49	4.26	4.39
14996	Wagga Wagga, NSW	2.70	3.09	4.68		4.45
14997	Cowra, NSW	2.67	2.97	4.55		4.38
14998	Bathurst, NSW	2.83	3.15	4.72	3.68	4.57
14999	Hartley, NSW	2.57	2.96	4.32	3.10	4.22
15000	Dubbo. NSW	2.75	3.08	4.69		4.34
15001	Coonabarabran, NSW	2.69	3.05	4.64	3.47	4.44
15002	Grafton, NSW	2.62	3.03	4.46		4.22
15003	Wauchope, NSW	2.73	2.99	4.70	3.46	4.33
15004	Singleton. NSW	2.85	3.31	4.80		4.87
15006	Bega, NSW	2.69	3.08	4.58		4.51
15007	Kangaroo Valley, NSW	2.55	2.99	4.64		4.68
15601	Glen Innes, NSW	2.69	2.86	4.53	3.51	4.28
Local	Davis, California				3.22	3.97
	LSD 1%	0.19	0.18	0.18	0.35	0.5
C. glauca		Rockeford	Five Point			
15930	St Georges Basin, NSW	1.89	1.78			
15932	Bundeena, NSW	1.78	1.68			
15934	Buladelah, NSW	1.80	1.75			
15935	Port MacQuarie, NSW	1.89	1.81			
15938	Grafton, NSW	1.91	1.82			
15939	Ballina, NSW	1.87	1.77			
16361	Narooma, NSW	1.93	1.79			
16362	Singleton, NSW	1.80	1.69			
16363	Penrith, NSW	1.74	1.68			
17200	Ipswich, NSW	1.75	1.61			
	LSD 1%	0.23	0.08			

technical report by the Australian Centre for International Agricultural Research (Pinyopusarerk et al., 2004). There was a considerable amount of genetic variation among natural provenances and land races. There was also evidence of provenance x site interaction which would be expected from a large and diverse number of seed sources and trial locations. Natural provenances from Southeast Asia and land races from Asia were generally more vigorous, while natural provenances from Australia-Pacific region grew slowest. Most seed sources had good bole length but planted-stand seed sources showed better stem straightness.

2.3 International provenance trials of *C. junghuhniana*

ATSC arranged a range-wide seed collection of *C. junghuhniana* from across the natural range in Indonesia in 1993 which was followed by establishment of provenance trials in Asia and Africa during 1995-1997. The natural distribution of *C. junghuhniana* extends from near sea level to 3000 m above sea level (asl). Results showed that low-altitude provenances outperformed high-altitude provenances in India and Thailand where the trial was located at 45-500 m asl while high-altitude provenances grew faster than low-altitude provenances in Tanzania where the trial was planted at 1500 m asl (Table 5).

2.4 Casuarina breeding programmes

Records indicate that only three countries, China, India and Thailand, have carried out systematic breeding programs for Casuarina species.

Table 3. One-year growth results of species and provenance trials of Casuarinas in China (Source Zhong, 1990)

CSIRO Seedlot No	Provenance	Survival (%)	Height (m)	D0 (cm)	D1.3 (cm)
C. cunninghamiana					
13515	Mareeba, Qld	100	2.58	3.61	1.19
13520	Marlborough, Qld	89	2.21	3.44	0.98
15003	Wauchope, NSW	92	2.53	3.81	1.38
Local	Ledong, Hainan	78	1.82	2.49	0.61
Local	Qionghai, Hainan	89	2.94	3.45	1.48
C. glauca					
13141	Coffs Harbour, NSW	97	2.25	3.29	1.03
13146	Bodalla, NSW	86	2.21	3.04	0.91
15218	Caloundra, Qld	97	2.08	2.59	0.78
Local	Zhanjiang, Guangdong	86	2.50	3.46	1.34
C. equisetifolia					
14196	Wangetti Beach, Qld	81	2.11	2.79	0.68
14492	Karumba, Qld	86	2.20	2.63	0.68
14505	Murgonella, NT	83	2.44	2.40	0.92
Local	Wenchang, Hainan	89	2.90	3.88	1.50
C. cristata					
14593	Bourke, NSW	71	1.31	1.70	0.08
14843	Gilgandra, NSW	94	1.43	1.89	0.20
15240	Goondiwindi, Qld	94	1.17	1.81	0.06
C. torulosa					
13377	Mt Lewis, Qld	46	1.11	1.82	-
13992	Stradbroke Island, Qld	42	1.23	1.26	-
C. junghuhniana					
13950	Tanzania	92	3.12	4.85	2.04
14006	Mugaga, Kenya	80	2.58	3.95	1.37
Allocasuarina littoralis					
13876	Gordon and Chill Cks, Qld	47	1.22	2.84	-
13986	Woolgoolga, NSW	89	2.20	3.33	0.76

India commenced implementation of a breeding plan of *C. equisetifolia* in 1997 setting up large breeding populations in three States (Nicodemus et al., 2011). One generation of breeding has yielded 13-28% gain in wood production. Clones of outstanding families have already been tested and deployed. The newly introduced *C. junghuhniana* was found to be faster growing and more drought and disease tolerant than *C. equisetifolia*, and tested superior clones are also now available. Intra- and interspecific hybrid families of the two species were produced through control pollination. Superior hybrid individuals have been identified through field test and will soon be released to growers. The breeding program in India has progressed to second generation which consists of genetic material from the first generation.

In China, first generation breeding populations of *C. equisetifolia*, *C. junghuhniana*, *C. cunninghamiana* and *C. glauca* were planted in 2008. Outstanding individuals were selected for clonal test. Grafted plants of the selected trees were propagated for intra- and interspecific hybridization among the four species. Preparation is currently underway for establishment of new breeding populations of *C. equisetifolia* and *C. junghuhniana*.

In Thailand, the program focuses on *C. junghuhniana* only and the first breeding cycle begun in 2004 was completed,

Table 4. Growth performance of *C. cunninghamiana* and *C. glauca* provenances at age 6 years at Raoping, Guangdong, China. Stem form was scored as 1 (very crooked) to 4 (very straight) (Source: Xu, 2011)

CSIRO Seedlot No.	Provenance	Survival (%)	Height (m)	Diameter (cm)	Stem form
C. cunninghamiana					
13508	Augathela, Qld	64	4.15	5.24	1.7
13516	West Normanby River, Qld	93	6.13	7.15	3.1
13520	Marlborough, Qld	86	5.91	6.96	2.6
13521	North Queensland, Qld	95	6.55	8.43	3.3
15002	Grafton, NSW	76	5.03	6.25	2.3
15004	Singleton, NSW	62	5.89	6.49	2.1
15574	Clairview, Qld	81	7.44	8.99	3.3
15601	Glen Innes, NSW	64	3.46	4.17	1.6
C. glauca					
13128	Singleton, NSW	53	4.08	4.16	2.2
13142	Taree, NSW	64	4.03	4.60	2.2
13143	Mangrove Ck, NSW	60	3.83	3.97	1.9
13146	Bodalla, NSW	76	3.36	3.03	1.7
13987	Coffs Harbour, NSW	52	3.85	4.03	1.6
15218	Caloundra, Qld	76	4.20	4.59	2.0
15930	Jervis Bay, NSW	55	3.5	3.65	1.8
15938	Yuragir National Park, NSW	69	5.42	6.37	2.0
15941	Burrum Heads, Qld	70	4.66	5.13	2.0

and superior clones already available for deployment. Three second-generation breeding populations were planted in 2013 involving selected individuals and families from the first generation and infusion of new material collected in Timor.

2.5 Genetic diversity studies

Genetic diversity among populations of *C. cunninghamiana* from the large geographic range was determined using allozyme electrophoresis techniques in the mid 1980s (Moran et al., 1989). High correlation with latitude was found. Results supported the taxonomic revision into two subspecies. More studies on genetic diversity in *Casuarina* and *Allocasuarina* species were conducted in the 2000s using AFLP, ISSR and RAPD markers (Elavazhagan et. al., 2011; Ndoye et al., 2011; Ramakrishnan et al., 2011; Kamalakannan et al., 2006; Yasodha et al., 2004; Ho et al., 2002). These studies revealed differentiation between the two genera and high genetic distance between *C. equisetifolia* and *C. junghuhniana*. There was also evidence of introgression involving *C. equisetifolia* and *C. junghuhniana*, *C. equisetifolia* and *C. glauca*, and perhaps *C. cunninghamiana* under cultivation.

3. Priorities for future

Genetic improvement of Casuarinas has progressed to second- or third-generation breeding cycles. The current breeding populations constitute genetic material selected in the first generation. There is an urgent need to obtain infusion material to maintain or broaden the genetic base. As interspecific hybridization is of particular interest, there is a need to better understand the reproductive biology of the species concerned in order to achieve more effective controlled pollination.

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Table 5. Height and diameter (DBH) of *C. junghuhniana* provenances in Thailand (4 years) and Tanzania (2 years) (sources: Pinyopusarerk et al., 2005, Mwihomeke et al., 2002)

				Thailand		Tanzania	
CSIRO seedlot	Seed source	Origin of mother trees	Altitude (m)	Height (m)	DBH (cm)	Height (m)	DBH (cm)
17844	Wetar, Indonesia	Old Uhak	5	10.44	9.58	5.35	4.35
17877	Timor, Indonesia	25 km SW Soe	550	9.59	8.76	4.93	4.50
17878		Noelmina River	170	10.63	8.92	5.18	5.03
19489		Kapan, Kupang	600	10.08	8.52	5.23	4.63
19490		Camplong	600	9.82	8.23	5.38	4.68
19491		Soe, Buat	800	9.75	8.38	4.90	4.18
	Mean			9.92	8.51	5.12	4.60
18845	Bali, Indonesia	Mt Pohen	2000	-	-	7.35	6.58
18846		Mt Pengalongan	1500	-	-	7.83	7.35
18848		Bukit Abang	1500	8.01	7.10	7.13	5.78
18849		Kintamani	1500	6.63	6.05	7.38	6.18
	Mean			7.36	6.61	7.31	6.47
18948	Java, Indonesia	Mt. Kawi	2000	6.66	5.87	7.14	6.45
18949		Mt. Argopuro	1500	6.35	5.70	6.20	5.68
18950		Mt. Bromo	1600	7.04	6.38	7.70	7.33
18951		Mt. Arjuno West	1350	6.99	6.41	6.96	6.22
18952		Mt. Willis	1500	6.36	5.54	7.03	6.48
18953		Mt. Arjuno East	1350	7.04	6.37	-	-
18954		Mt. Bromo	2500	6.51	5.65	7.23	6.68
	Mean			*6.73*	*6.01*	*7.04*	*6.47*
19237	Kenya	Meru	1750	5.90	5.54	-	-
19238		KEFRI HQ	2080	7.14	6.18	7.05	6.83
19239		KARI-Muguga	2060	6.50	5.87	7.03	7.10
19240		Muka Mukuu		7.18	6.56	5.95	5.88
	Mean			6.66	6.03	6.67	6.60

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Clone Trial of *Casuarina junghuhniana* in Thailand

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Abstract

Twenty-nine outstanding individuals of *Casuarina junghuhniana* were selected from a provenance/progeny trial planted in Thailand for clonal test. These individuals consisted of 23 from Indonesia (Bali, Java, Timor and Wetar) and 6 from Kenya. The local commercial hybrid *C. junghuhniana* x *C. equisetifolia* was included as control. Eleven of these trees were male and 17 were female, 2 had no sex expression. The selected trees were firstly propagated by marcottage and subsequently multiplied by rooted cuttings for use as planting material. The experiment was laid out in a randomized complete block design with 6 replicates, 6 (2x3) trees per plot, and 3 x 2m spacing. The trial, planted in August 2008, was located at Saiyoke, Kanchanaburi province (latitude 14° 22' N, longitude 98° 56' E, altitude 370 m). The area receives a mean annual rainfall of 1500 mm and a mean annual temperature of 25.7°C. The soil is clay loam, pH 5-6. Height (Ht) and diameter at breast height (DBH) have been assessed annually. Stem form (axis persistence and stem straightness), branching habit (density, angle and thickness) and flowering were assessed by scoring when trees were 4 years old. Growth data assessed at age 4 years were subjected to an analysis of variance. There were highly significant differences (P<0.001) in all the growth parameters assessed. Eight clones (1 Bali, 1 Timor, 4 Wetar and 2 Kenya) showed high survival (83-100%) and rapid growth (Ht 12.9-15.1 m, DBH 10.4-14.6 cm) with good stem form comparable to that of the local commercial hybrid. No correlations between sex of the clones and general growth performance were observed. Based on the growth and stem form, these 8 clones are considered suitable for plantation establishment.

1. Introduction

Casuarina junghuhniana Miq. (syn. *C. montana*) of the Casuarinaceae family is a deciduous tree, 25-35 m tall and 50-80 cm in diameter. The natural distribution is restricted to the eastern part of the Indonesian Archipelago where it occurs on Java, Bali, Lombok, Sumbawa, Flores, Alor, Sumba, Wetar and Timor Islands (Pinyopusarerk and House ,1993; Mile, 1996). It grows in various habitats such as hard stony ground, steep slopes of water-ways, valley sides and mountains, and in crater valleys. It is a principal species on the slopes of volcanoes at altitudes of 1500-3100 m but also at lower altitudes in Wetar and Timor, where it occurs from near sea level to 1500 m altitude. It is long-lived and well adapted to a monsoonal climate with a distinct dry season. Rainfall in the natural distribution ranges from 700 mm to 1500 mm, and up to 2500 mm in highland places in Java (Mile, 1996). Temperature varies widely with altitudes with mean annual minimum of 13 °C and mean annual maximum of 31°C. The species tolerates a variety of soil conditions, from compact clays to coastal volcanic soils and alkaline shales on Timor. When trees reach a few metres in height they are fire tolerant and sprout readily after being damaged by fire.

Casuarina junghuhniana has been introduced successfully to some countries in Africa including Kenya, Uganda and Tanzania and, is increasingly being planted as an agroforestry tree (Okorio et al., 1994; Mwihomeke et al., 2002). It is an important plantation species in China (Zhong and Bai 1996; Zhong et al., 2011) and India (Varghese et al., 2011; Rawat et al., 2011). The species was introduced to Thailand for provenance trials in 1997 (Pinyopusarerk et al., 2005). This was followed by a more detailed assessment of genetic variation in progeny trials in 2004 (Luechanimitchit et al., 2011). Outstanding individuals in the progeny trial were subsequently selected and propagated by marcottage. Rooted cuttings were then produced for clonal test. This paper reports results of a clone trial at 4 years of age.

2. Materials and Methods

2.1 Plant material

The trial involved 30 clones of which 29 were original trees selected from a progeny trial and one local commercial hybrid clone *C. junghuhniana* x *C. equisetifolia* (Table 1). Of the 29 clones 23 were from Indonesia Bali (2), Java (5), Timor (9) and Wetar (7), and 6 from Kenya. Eleven of the clones were male, 17 were female, and 2 had no sex expression.

2.2 Planting site

The planting site belongs to the Thai Royal Forest Department's Experimental Station at Saiyoke,

Table 1. Detail of *Casuarina junghuhniana* clones included in the trial

Clone No.	Original source tree	Country	Sex
1.	Kintamani, Bali	Indonesia	M
2.	Mt Abang, Bali	Indonesia	F
3.	Mt Argopuro, East Java	Indonesia	M
4.	Mt Arjuno, East Java	Indonesia	F
5.	Mt Arjuno, East Java	Indonesia	F
6.	Mt Arjuno, East Java	Indonesia	F
7.	Mt Kawi, East Java	Indonesia	M
8.	Buat, Soe, Timor	Indonesia	F
9.	Buat, Soe, Timor	Indonesia	M
10.	SW Soe, Timor	Indonesia	F
11.	Camplong, Timor	Indonesia	F
12.	Camplong, Timor	Indonesia	F
13.	Kupang, Timor	Indonesia	M
14.	Kupang, Timor	Indonesia	F
15.	Kupang, Timor	Indonesia	M
16.	Noelmina, Timor	Indonesia	M
17.	Old Uhak, Wetar	Indonesia	M
18.	Old Uhak, Wetar	Indonesia	N/A
19.	Old Uhak, Wetar	Indonesia	F
20.	Old Uhak, Wetar	Indonesia	M
21.	Old Uhak, Wetar	Indonesia	F
22.	Old Uhak, Wetar	Indonesia	F
23.	Old Uhak, Wetar	Indonesia	F
24.	Kari-Muguga	Kenya	M
25.	KEFRI HQ, Nairobi	Kenya	F
26.	KEFRI HQ, Nairobi	Kenya	F
27.	Meru	Kenya	N/A
28.	Muka, Mukuu	Kenya	F
29.	Muka, Mukuu	Kenya	F
30.	Local hybrid	Thailand	M

Kanchanaburi province (latitude 14° 22’ N, longitude 98° 56’ E, altitude 370 m). Mean annual temperature is 25.7 °C and mean annual rainfall is 1500 mm with 4-5 months of dry season. The soil is clay loam, pH 5-6.

2.3 Experimental design

The experiment was laid out in a randomised complete block design with 6 replicates, 6 (2x3) trees per plot, and 3m x 2m spacing. Two guard rows of the same species were planted surrounding the entire experiment. The experimental area was ploughed prior to the planting in August 2008.

2.4 Assessment

At 4 years of age, all trees were measured for height (Ht) and diameter at breast height (Dbh). Plot mean survival was calculated by counting the number of trees presented in the height data. In addition, each tree was scored subjectively for eight morphological characteristics: axis persistence; stem straightness; density, thickness and angle of permanent branches; length and thickness of branchlets; and flowering. Details of the scoring procedures are given in Table 2. This set of morphological characteristics summarises the morphological attributes of individual trees.

2.5 Data analysis

Each characteristic was analysed to determine differences between clones. Measurements on the trees within each plot were summarised to plot means, variances and counts using the data pre-processing package DataPlus 3.0 (Williams et al., 2000). Normality checks were conducted on the plot means of the data, especially the categorical measurements. Analysis of variance (ANOVA) was performed on the plot mean using the statistical package GenStat Discovery Edition 3 (Buysee et al., 2004).

3. Results and Discussion

Highly significant differences ($P<0.01–0.001$) were observed for all growth parameters among the clones assessed at 4 years of age (Table 3).

3.1 Survival

Most of the clones had good to very good survival rate (>80%). Only 5 clones survived less than 80%; these were from Timor (Clone No. 8, 10, 13) and Kenya (Clone No. 25, 27). It is not known whether the low survival was caused by poor rooting quality or poor adaptability, but it is noted that these low surviving clones also had poorer height and diameter growth.

3.2 Height and diameter growth

There was a considerable differentiation in height and Dbh among clones (Table 3). The tallest clone (15.1 m) was from Wetar (No. 20) while the largest diameter (13.7 cm) was also from Wetar (No. 19). The other Wetar clones also grew well and were among the top rankings. Wetar provenances were among the fastest growing in the previous provenance and progeny trials in Thailand (Pinyopusarerk et al. 2005; Luechanimitchit et al. 2011). The slowest growing clone was from Kenya (No. 27) with a mean height of 9.1 m and Dbh of 7.3 cm, which accounted for 40 and 46% respectively smaller than the most vigorous clones. However, there were two clones from Kenya (No. 24 and No. 29), and one each from Bali (No. 2) and Timor (No. 12) which also performed very well in height and Dbh. The poor growth results shown by many Timor clones were not expected as the original materials from this Indonesian island performed very well in provenance and progeny trials.

Table 2. Characteristics assessed in the *C. junghuhniana* clone trial at Kanchanaburi, Thailand

Characteristic	Unit	Explanation
Quantitative traits		
Height (Ht)	m	Height of the tallest stem
Diameter (Dbh)	cm	Measured at 1.3 m above ground
Qualitative traits		
Axis persistence *(Axpst)*	1-6	Ability of tree to retain its primary stem axis 1 = Multiple stems from ground level 2 = Forking in 1st (lowest) quarter of stem 3 = Forking in 2nd quarter 4 = Forking in 3rd quarter 5 = Forking in 4th quarter 6 = No forking
Stem straightness *(Strst)*	1-4	1 = Very crooked, > 2 serious bends 2 = Slightly crooked, >2 small bends or <2 serious bends 3 = Almost straight, 1-2 small bends 4 = Completely straight
Branching habit Permanent branches Density *(Denpb)*	1-4	First order branches originating from the main stem 1 = Very high, regularly branched, internode mainly 15 cm 2 = High, irregularly branched, internode around 15 cm 3 = Low, irregularly branched, internode around 30 cm 4 = Very low, sparsely branched, internode >30 cm
Thickness (Thkpb)	1-4	1 = Very heavy, >3 branches diameter >⅓ of adjacent stem 2 = Heavy, 1-3 branches diameter > ⅓of adjacent stem 3 = Light, branch diameter ≤⅓ of adjacent stem 4 = Very light, branch diameter ≤¼ of adjacent stem
Angle *(Angpb)*	1-2	1 = Upright, <60° 2 = Horizontal, >60°
Branchlets Length *(Lenbl)*	1-2	Deciduous branchlets (needles) 1 = Short, <15 cm 2 = Long, >15 cm
Thickness *(Thkbl)*	1-2	1 = Fine 2 = Coarse
Flowering *(Flowr)*	1-2	1 = No flowering 2 = Yes (regardless of sex)

The widely planted, local commercial hybrid clone *C. junghuhniana* x *C. equisetifolia* was clearly outperformed by many pure *C. junghuhniana* clones. Based on height and diameter growth, many new clones have proved far more superior. These clones are Bali (No. 2), Timor (No. 12), Wetar (No. 17, 19, 20, 22), and Kenya (No. 24, 29).

3.3 Stem form (Axis persistence and stem straightness)

Clones showed marked variation in axis persistence and stem straightness despite the vigorous emphasis on these traits in plus tree selection. For example, clone No. 10 from Timor, No. 27 from Kenya and No. 18 and 21 from Wetar had very crooked stem (mean score 1.8-2.7), while clone No. 1 and 2 from Bali, No. 9, 13 from Wetar, No. 28, 29 from Kenya, and the local hybrid No. 30 had near perfect to perfect score (3.8-4.0). The results indicated that many phenotypes did not perform well as clones relative to the performance of the original source trees. Inconsistent results where selected superior phenotypes have not performed well as expected in clonal tests have been reported (Kageyama and Kikuti, 1988). These results underpin the importance of a rigorous testing program in clonal forestry.

3.4 Branching characteristics

Most of the clones had low branching density, i.e. internode length around 30 cm. The local commercial hybrid (No. 30) and Kitamani, Bali clones (No. 1) had highest density among the clones tested. One particular clone from Soe, Timor (No. 10) had very low density, mean score 3.8.

Branch thickness varied significantly between clones although the majority of clones were found to have thick

Table 3. Mean values for survival, growth and morphological characteristics at 4 years old of a *C. junghuhniana* clone trial in Thailand

No.	Original source tree	Surv	Ht	Dbh	Axpst	Strst	Denpb	Thkpb	Angpl	Lenbl	Thkbl	Flowr
1	Kintamani, Bali	96.8	13.1	9.0	5.8	4.0	2.2	2.4	1.0	1.0	2.0	1.6
2	Mt Abang, Bali	97.6	14.6	11.7	6.0	3.8	3.0	2.0	1.7	1.0	2.0	2.0
3	Mt Argopuro, East Java	91.9	10.5	8.2	3.9	3.5	2.5	2.0	1.3	1.0	1.8	1.6
4	Mt Arjuno, East Java	94.2	14.0	10.1	5.4	3.1	2.9	2.1	1.8	1.0	2.0	2.0
5	Mt Arjuno, East Java	85.9	12.8	10.1	5.7	3.3	3.0	1.9	1.7	1.0	1.8	1.8
6	Mt Arjuno, East Java	97.1	11.2	8.3	5.7	2.9	3.0	2.1	1.8	1.0	1.5	1.6
7	Mt Kawi, East Java	97.7	12.7	9.5	5.5	3.2	2.9	2.0	1.3	1.0	1.7	1.9
8	Buat, Soe, Timor	64.4	11.2	8.6	5.9	2.3	2.9	2.0	2.0	1.0	1.0	1.9
9	Buat, Soe, Timor	89.8	12.1	10.6	5.5	3.8	2.8	1.9	1.7	1.1	2.0	2.0
10	SW Soe, Timor	71.9	14.0	11.6	5.7	1.8	3.7	1.7	2.0	1.0	2.0	1.7
11	Camplong, Timor	88.4	11.9	9.4	5.8	3.5	3.0	2.1	1.3	1.4	2.0	1.2
12	Camplong, Timor	97.5	13.2	11.6	5.8	3.4	2.8	1.8	1.7	1.0	2.0	1.5
13	Kupang, Timor	78.5	10.6	8.1	5.8	3.8	2.9	2.3	1.3	1.0	1.5	1.6
14	Kupang, Timor	86.0	11.2	8.5	5.8	3.7	2.9	2.2	1.3	1.0	1.3	1.3
15	Kupang, Timor	80.2	10.9	9.2	3.5	3.1	2.9	1.9	1.5	1.0	1.8	1.6
16	Noelmina, Timor	92.3	13.2	10.7	5.3	2.9	2.9	1.9	1.2	1.0	1.9	2.0
17	Old Uhak, Wetar	100.0	12.9	11.5	5.7	3.2	2.6	1.9	1.5	1.1	2.0	1.8
18	Old Uhak, Wetar	88.5	14.5	11.2	5.6	2.7	3.1	1.7	1.7	2.0	2.0	1.0
19	Old Uhak, Wetar	94.5	14.6	13.7	5.5	3.1	2.7	1.6	1.9	1.1	2.0	1.0
20	Old Uhak, Wetar	88.3	15.1	13.1	5.8	3.5	3.0	1.6	2.0	1.1	2.0	1.7
21	Old Uhak, Wetar	85.3	13.9	11.1	5.7	2.7	2.9	1.7	1.7	1.2	1.7	2.0
22	Old Uhak, Wetar	83.4	14.4	12.6	5.5	3.1	2.7	1.7	1.5	1.0	2.0	2.0
23	Old Uhak, Wetar	94.8	12.9	12.7	5.1	3.3	2.8	1.6	1.7	1.0	2.0	2.0
24	Kari-Muguga	97.9	14.3	12.2	5.3	3.6	2.9	2.0	1.8	1.0	2.0	1.5
25	KEFRI HQ, Nairobi	66.4	11.0	8.4	5.6	3.1	2.8	2.0	2.0	1.0	1.8	1.8
26	KEFRI HQ, Nairobi	94.5	11.4	9.9	5.6	2.9	2.5	1.5	1.5	1.0	2.0	1.7
27	Meru	59.7	9.1	7.3	5.3	2.5	2.7	2.0	2.0	1.9	2.0	1.0
28	Muka, Mukuu	88.6	11.8	8.0	5.7	3.8	2.6	2.8	1.0	1.1	1.8	1.5
29	Muka, Mukuu	91.7	14.3	10.4	5.6	4.0	2.8	2.0	1.2	1.0	1.8	1.9
30	Local hybrid	86.6	12.8	8.6	6.0	3.9	2.3	2.7	1.3	2.0	1.8	1.0
	Overall mean	87.7	12.7	10.2	5.5	3.2	2.8	2.0	1.6	1.1	1.8	1.6
	F probability	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	L.S.D. (5%)	19.6	1.2	1.2	0.48	0.37	0.39	0.39	0.44	0.2	0.33	0.36

branches, i.e. diameter greater than 1/3 of the adjacent main stem. The local hybrid clone clearly had the smallest branches of all, diameter ≤1/3 of the adjacent main stem.

Branch angle was also found to differ considerably among clones with about half the clones having upright angle and the other half horizontal angle. Most of the clones had short but coarse branchlets. Very few clones developed long branchlets and these were the local hybrid, and one each from Wetar (No. 18) and Kenya (No. 27).

C. junghuhniana is often planted for windbreaks and as such branching characteristics are important issues as they influence the stability of trees in windstorms. Dense and fine branches with a more horizontal insertion are considered suitable structure for windbreaks. In this regard, many clones are considered to meet such criteria. These include clone No. 2 from Bali, clone No. 12 from Timor, clone No. 19 and 20 from Wetar, and clone No. 24 and 29 from Kenya.

3.5 Flowering

With a few exceptions, most clones have flowered at 4 years of age. Only three clones including the local male hybrid and No. 18 from Wetar and No. 27 from Kenya which did not flower. With regard to the latter two clones, we have continued to monitor the original trees in the progeny trial which was planted in 2004, and after 10 years none of these trees have yet to attain first flowering.

The representation of both male and female clones in the trial confirms that *C. junghuhniana* is a dioecious species. Based on the growth data presented above, however, there was no evidence to indicate differentiation between the two sexes in terms of growth vigour.

Conclusions

This study clearly demonstrates considerable variation among clones of *C. junghuhniana* and confirms the importance of a rigorous field testing program to evaluate the relative merits of the clones. Based on height and diameter growth and stem form traits, eight new clones are considered suitable for plantation establishment for wood production. These clones are trees originally selected from Bali, Timor, Wetar and Kenya.

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Controlled pollination of *Casuarina equisetifolia*

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Key words: *Casuarina equisetifolia,* controlled pollination, fruit set, seed germination rate, pollination chambers.

Abstract

Current breeding strategy of casuarinas in China emphasizes controlled pollination option for developing intra- and interspecific hybrids among key species. The conventional controlled pollination method by bagging yielded poor success rates with low fruit set and poor seed germination. Using *Casuarina equisetifolia* as a test species, controlled pollination was conducted in closed chambers instead of pollination bags. Results showed that fruit set and seed germination rate increased significantly from 7 % to 90 %, and 8 % to 52 % respectively compared with the conventional bagging method. It is proposed to study this new method with other key casuarina species such as *C. cunninghamiana* and *C. glauca*.

Introduction

Casuarina equisetifolia L. Johnson (Casuarinaceae) is a nitrogen-fixing tree native to Southeast Asia, Australia and Pacific islands (Pinyopusarerk and House, 1993). It is of considerable social, economic and environmental importance in China, representing more than 90% of the estimated 300,000 hectares of total casuarina planted areas (Zhong et al., 2011). Genetic improvement program for *C. equisetifolia* is actively underway in China.

Hybridization in plant can provide a rapid means for increasing genetic variation and producing new gene combinations which can potentially enhance vigor in growth or resistance. The importance of intra- and inter-specific hybridization for genetic improvement of forest trees has been evident for at least 50 years (Zobel and Talbert, 2003). Controlled crossing has been an increasingly important method to concentrate the best alleles from a range of selected trees and obtain genetic gain for tree breeding (Harbard et al., 1999).

Controlled pollination of forest tree is generally conducted manually in the field. Bagging of the flowers is used to exclude undesired pollens. Scaffoldings , which are erected using steel pipes and expensive, are required when pollinating flowers in the tall trees. In casuarinas, very poor fruit set (< 1 %) and low seed germination rate obtained from the conventional method has been reported in India (Nicodemus et al., 2011). Thus, the use of conventional method for controlled pollination of casuarinas is inefficient and costly.

Grafting is used in agriculture and forestry to induce dwarfing of tree to facilitate pollination practice, and to accelerate the onset of flowering, which in turn shortens the breeding cycle (Perez et al., 2007; Darikova et al., 2011). Our experiences with *C. equisetifolia*, *C. cunninghamiana* and *C. glauca* indicate that grafted plants with physiologically mature scions flower in the following season, which offers a precondition for developing a new method for controlled pollination in casuarinas (Zhang, 2013).

The objective of this study was to develop a more efficient method for controlled pollination in casuarinas using grafted plants of *C. equisetifolia*.

1. Materials and methods

The experiment was conducted in Huian county, Fujian province of China. The nursery site was surrounded by mature *C. equisetifolia* plantations from which scions and male pollen were collected for this experiment.

1.1 Controlled pollination methods

In March 2009, scions of male and female trees from a 20-year-old *C. equisetifolia* seedling plantation were grafted on stock plants grown in containers. The grafted plants were about 100 cm tall when the controlled pollination experiment commenced in April 2010. Three pollination methods were carried out in three replicates of four grafted female plants each as follows.

Method 1 – conventional bagging: Twelve grafted female plants with visible flower buds were laid out on an open ground. They were arranged into three groups of four (2 x 2) spaced at 1 m apart. On each plant, a branch was selected and tagged. All female flower buds except five to be pollinated were removed. The flower bud branches were bagged in glassine paper bags (25 x 15 cm) before anthesis. When male and female flowers were in full bloom, the bags were opened, and fresh pollen collected from a nearby plantation was manually pollinated on the female flowers using brushes at 8:00-10:00 am. Immediately thereafter, the pollinated flowers were rebagged to exclude undesired pollen. The manual pollination was repeated two days after. The bags were removed 15 days later after confirmation of drying and withering of the female flowers.

Method 2 – pollination chambers: Three clear plastic sheet chambers 1.5 m, 1.5 m and 2 m in length, width and height

Table 1. Mean fruit set, number of seeds per cone and seed germination rate of three pollination methods in *C. equisetifolia*

Pollination method	Fruit set (%)	Number of seeds per cone	Seed germination (%)
1. Conventional bagging	7.0±6.2 [c]	68.3±13.3 [b]	8.1 ±5.9 [c]
2. Pollination chambers	89.9±12.0 [a]	81.8±16.1[a]	51.8±10.1 [a]
3. Open pollination	69.5±13.8 [b]	74.5±15.2 [ab]	28.3±9.9 [b]

Note: Data were mean ± SD. Data followed by the same superscript letter were not significantly different by Duncan's multiple range test (P < 0.05).

respectively were placed inside a glasshouse. These chambers functioned as pollination bags. Each chamber had a separate entrance through overlapping of two plastic sheets. Twelve grafted female plants with visible flower buds were chosen. On each plant a branch with visible flower buds was tagged. All flower buds except five to be pollinated were removed. Four grafted female plants were then placed inside each of the three chambers (as 3 replicates). When female flowers were in receptive stage, one male plant with the inflorescences shedding pollen was placed in the centre of the four female plants inside each chamber. Male inflorescences were shaken by hand to disperse the pollen at 8:00-10:00 am. This shaking was repeated for five days. When all female flowers had withered about 15 days later, the grafted plants were moved to open area outside the glasshouse.

Method 3 – open pollination (control): The same twelve female plants in Method 1 were used. A branch with visible flowers on each plant was tagged. All flowers apart from five to be pollinated were removed. Male trees in the adjacent plantation provided a pollen source for the open pollination. In addition, ten grafted male trees were placed around the female plants to provide supplementary pollen.

1.2 Assessment and data analysis

In November 2010 approximately five months after pollination treatments, the number of the fruits (cones) on the tagged branches was counted. The cones were air-dried individually in the sun to extract the seed, after which the number of seeds in each cone was counted. All the seeds were then sown to determine the germination rate.

Data were analysed using SAS statistical package (reference) on the number of cones (fruit set), number of seeds per cone, and seed germination rate. Arcsin transformations were performed for percentage data. Duncan's Multiple Range Test at 5 % level was used to compare the differences among treatment means.

2. Results

There were significant differences ($P < 0.05$) between pollination methods in fruit set, number of seeds per cone and seed germination rate (Table 1).

The highest fruit set (89.9 %) was obtained by pollination in chambers, followed by open pollination (69.5 %) and conventional bagging (7 %).

Pollination in chambers also yielded the highest number of seeds per cone, average 81.8 seeds. There was no difference in the number of seeds per cone between conventional bagging (Method 1) and open pollination (Method 3). The mean values recorded for these two methods were 68.3 and 74.5 seeds respectively.

Germination was very poor (8.1 %) in the seeds obtained by the conventional bagging method. The best result (51.8 %) was displayed by controlled pollination in the chambers. The seeds obtained from open pollination had 28.3 % germination rate.

3. Discussion

Normally, pollination and fertilization in casuarinas can be accomplished without difficulty under outdoor conditions, however, environmental factors such as winds, rain, temperature and humidity can affect the outcome of seed production. Low pollen density in the air may also reduce the chance of viable pollen landing on receptive stigma, leading to pollination limitation (insufficient pollen transfer by vectors) in female flowers (Jennifer et al., 2001). In contrast, in the small and closed space of pollination chambers, higher density of pollen shed from male inflorescences enhances more viable pollen grains to stick to receptive stigmas. This indicates the higher fruit set by pollination chambers method than that of open pollination method.

The majority of casuarina species are dioecious, wind-pollinated and primarily obligate outbreeders (Barlow, 1981) that is an essential prerequisite for developing this controlled pollination method using pollination chambers. Compared with conventional controlled hand pollination method, this method can eliminate adverse effects of outdoor environments, and reduce cost of materials and labor used in conventional controlled pollination. Apart from casuarina species, some other wind-pollinated tree species with obligate outcrossing characteristic, such as dioecious species, monoecious but self-incompatible species may also be undertaken using the new method. The present results warrant further study with other casuarinas such as *C. cunninghamiana* and *C. glauca* as interspecific hybridization among these casuarinas are a priority of the casuarina breeding program in China.

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Diversity for Pulp and Paper Production Ability in *Casuarina equisetifolia*

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Key words: *Casuarina equisetifolia*, pulping, anatomy, lignin, charcoal, breeding objective

Abstract

The wood anatomical and pulping parameters of 15 plus trees of *Casuarina equisetifolia* were analyzed for selecting candidates with desirable pulp qualities for further genetic improvement. Clones CPCE890108, CPCE890110 and CPCE890301 had high basic density (737-787 kg m^{-3}) and CPCE890110 contained maximum ash content of 2.48 %. The fibre length and diameter for tested samples were found to be in normal range of hard woods for pulp and paper production. Pulp yield of a few clones was more than 50 % which was greater than that of threshold level of 47 %. Clones CHCE890201, CHCE890905, CHCE892604, CPCE890108 and CPCE893702 were found to possess desirable pulp traits and suitable to grow as raw materials for paper manufacturing. Clone CPCE890110, with the highest ash content with low screen yield and fibre strength factor though may not be suitable for paper production, could *per se* be propagated and cultivated for production of quality charcoal and poles for scaffolding. The existing variability and genetic diversity from end use perspective need to be exploited by including wood properties as a breeding objective in Casuarina improvement programmes.

1 Introduction

Casuarinas were introduced into the Indian sub-continent during the 1860s to power steam locomotives, when Indian railway was formed. Though Casuarinas are a group of 96 species, three species *viz. Casuarina equisetifolia, C. stricta* and *C. suberosa* were introduced at the same time in the Nilgiris of the Madras Presidency. *C. equisetifolia* was found to be the most suitable for large scale plantations. The species thrives well in comparison to many indigenous and exotic species in barren, saline and alkaline soils and tolerates high pH. It is also a good soil reformer as organic matter returned to the soil in the form of foliage in relatively higher amounts and also fixes atmospheric nitrogen with the help of actinorhizal *Frankia.* The amount of nitrogen fixed is around 84 g $tree^{-1}$ at the age of two years which is more than 170 kg ha^{-1} of nitrogen with the plant density of 2500 trees ha^{-1} (Dommergues et al., 1990). Thus Casuarinas have become pioneer species to establish on nutrient-poor sites and in stressed environments.

C. equisetifolia is an excellent fuelwood with a specific gravity of 0.8 and calorific value of about 5000 k cal kg^{-1} of dry wood (Anon, 1984). Casuarina wood is increasingly preferred as raw material for paper / rayon industry, and also finds use as poles and scaffolding. Thus, the genetic studies with a good combination of plant physiology and biotechnological approaches will help a great deal to exploit the potential of this species in degraded and wasteland afforestation programme. The demand for Casuarina wood for paper and pulp production is increasing substantially (Hegde, 1993) necessitating use of superior planting material for increasing yield per unit area. High-yielding Casuarina plantations will benefit both farmers and wood based-industries apart from contributing towards environmental and soil amelioration.

2 Materials and Methods

The wood analysis was carried out for 15 Candidate Plus Trees (CPTs) selected from two geographical locations on the coastal areas of Tamil Nadu viz. Chengelpet and Chidambaram. The purpose of the study was to find out suitable genotypes for various end uses including pulp and paper making. Samples for wood analysis were taken from 100 cm above the ground level and analysis was carried out at the Paper and Pulp Research Institute, Jaykaypur, Odisha. Physical and chemical wood traits were studied and analyzed.

2.1 Physical properties

2.1.1 Wood density

Wood density, defined as "ratio of the mass of a quantity of the wood to its volume and expressed in terms of weight per unit volume", was determined as per the Technical Association for Paper and Pulp Industries, New York (TAPPI) Method Number 258 Om-85. This method describes the measurement of the basic density of pulp wood in the form of disks by taking cross section of logs. The green volume in ml was determined by water displacement method and then the disks were dried in an oven at 105 ± 3 C to constant weight.

Wood density (kg m^{-3}) = (Oven dry weight (g) / Green volume (cc)) x 1000

2.1.2 Fibre morphology

The fibrous elements of the CPTs which are commonly encountered in paper making were identified on the basis of their morphology. Fibre length (mm) and diameter (micron) were measured microscopically. The unbleached pulp was partially delignified with an acidified sodium chlorite solution at 60 °C for 5 minutes. Then the pulp was washed with distilled water. A small portion of the pulp was thoroughly dispensed and was taken for slide preparation. The prepared slides were examined for the measurements microscopically.

2.1.3 Slenderness ratio

The slenderness ratio is a derivative property based on fibre dimensions. The short and thin walled fibres exhibit plastic formation to offer more surface contract and fibre bonding. The slenderness ratio was calculated as per below.

Slenderness ratio = Fibre length / Fibre diameter

2.2 Proximate chemical analysis

Wood logs were chipped and the chips were ground to a fine particle size in Wiley grinder. The powdered material was passed through 40 mesh screen and the screened material was taken for proximate chemical analysis following TAPPI standard method (TAPPI, 1954).

2.2.1 Ash

Ash is an approximate measure of the mineral salts and other inorganic matter in wood. Ash was determined according to TAPPI method number T211 : Om – 85 by keeping wood dust in an electric Muffle furnace at a temperature of 575 °C till complete burning. Complete ignition was indicated by the absence of black particles.

Ash (%) = (Weight of the ash (g) / Weight of moisture free test specimen (g)) x 100

2.2.2 Alcohol-Benzene extractive

Alcohol-Benzene extractive (A-B extractive) describes the procedure for determining the amount of solvent-soluble and non-volatile material in wood. Alcohol-benzene extractive was determined according to TAPPI method number T204 : Om - 88. In this method, the extractive was determined in an extraction apparatus consisting of Soxhlet extraction flask, extraction tube and Allihn type condenser. The air dried 5 gm wood dust was taken in an extraction thimble, placed in the soxhlet apparatus. The extraction flask was filled with 150 ml of alcohol-benzene mixture. The flask was fitted to the apparatus, and the solvent was heated up at least 6 times per hour till boiling. The achieved extract was dried in the oven for 1 h at 105 ± 3 °C and weighed. A-B extractive was calculated using the following formula.

A-B extractive (%) = (Oven dry weight of extract (g) / Oven dry weight of wood (g)) x 100

2.2.3 Lignin

Lignin represents incrusting material forming a part of the cell wall and middle lamella in wood. It is an aromatic and amorphous substance containing phenolic methoxyl, hydroxyl and other constituent groups. The wood contains about 20-30% lignin, removal of which is a main objective of pulping and bleaching processes.

Lignin was determined according to TAPPI method number 222: Om-88, a procedure for determining the acid-insoluble lignin in wood (TAPPI, 1943). In this method, 1 g of moisture free wood dust was taken in a 100 ml beaker to which 15 ml cold (15 °C) 72% sulphuric acid was added. Then the beaker was kept in a water bath at 20 °C for 2 h with frequent stirring. The material in the beaker was then transferred to a flask and total volume was adjusted to 575 ml with distilled water. This solution was boiled for 4 h by maintaining constant volume. The insoluble matter was allowed to settle. The liquor was then filtered through a crucible and washed finally with hot water till it becomes acid free. The crucible was dried in the oven at 105 ± 3 °C till constant weight. Lignin was calculated with following formula.

Lignin (%) = (Oven dry weight of lignin (g) / Oven dry weight of test specimen (g)) x 100

2.2.4 Hollocellulose

Hollocellulose was determined according to acid chlorite method (TAPPI, 1954). The extractive free wood dust was transferred to a conical flask. 160 ml distilled water, 0.5 ml glacial acetic acid and 1.5 g of sodium chlorite was added successively to the flask. This flask was placed with occasional shaking in a steam bath at a temperature of 80 °C for 1 h. Then, without cooling, additional 0.5 ml glacial acetic acid and 1.5 g of sodium chlorite were added. Acetic acid and sodium chlorite reactions were repeated four times. At the end, the flask was cooled in an ice bath below 10 °C. The Hollocellulose was filtered in a tared Gooch crucible and finally washed with ice water. The crucible and Hollocellulose were dried in the oven at 100 °C till constant weight.

Hollocellulose (%) =(Dry weight of Hollocellulose (g)/ Oven dry weight of the wood (g)) x 100

2.2.5 Pulp yield

The kraft pulping process was adopted for pulping. In this process, chemical used was white liquor which was containing NaOH and Na_2S. The wood chips were cooked in a 15 L capacity laboratory rotary digester. Following conditions were maintained during cooking to determine the pulp yield.

- Active alkali (Na_2S) : 17%
- Sulphadity : 18%
- Wood liquor ratio : 1 : 2.7
- Cooking temperature : 165 °C
- Time for cooking : 90 to 120 m
- H factor : 1380

Table 1. Criteria for awarding weightage to develop index value for various wood traits of *Casuarina equisetifolia*

Static	Wood density	Fibre length	Fibre diameter	Slenderness ratio	Ash content	A-B Extract	Lignin	Hollo-cellulose	Kappa Number	F S Factor
≥ 21 % of Mean	1.00	1.00	10.00	1.00	1.00	10.00	1.00	10.00	1.00	
> 10-20 % of Mean	2.50	2.50	7.50	2.50	2.50	7.50	2.50	7.50	2.50	7.50
Mean	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
≤ 1-10 % of Mean	7.50	7.50	2.50	7.50	7.50	2.50	7.50	2.50	7.50	2.50
≤ 11-20 % of Mean	10.00	10.00	1.00	10.00	10.00	1.00	10.00	1.00	10.00	1.00
≤ 21 % of Mean	0	0	0	0	0	0	0	0	0	0

Pulp yield (%) = (Oven dry weight of screened pulp / Oven dry weight of wood chips) x 100

2.2.6 Kappa number

Kappa number is defined as the volume (ml) of 0.1 N potassium permanganate solution consumed by one gram of moisture free pulp. Kappa number of pulp was measured as per TAPPI method number T 236: Cm-85. This method applies to determine the relative hardness, bleachability and degree of delignification of the pulp. 2.5 g moisture free pulp was dispersed in 500 ml distilled water with a stirrer. After complete dispersion the volume was made up to 800 ml. The beaker was kept in a water bath at 25 °C. Then 100 ml 4 N-sulphuric acid was added followed by 100 ml 0.1 N-potassium permanganate solution. After 10 minutes, the reaction was stopped by adding 40 ml of 10% potassium iodide solution. The solution was titrated against 0.1 N-sodium thiosulphate solution, a few drops of a starch indicator were added towards the end of the reaction. Similarly the blank reading was obtained using the same method as above but omitting the pulp.

Kappa number= ((b-a) x f) / w

Whereas a = amount of thiosulphate consumed by test specimen (ml), b = amount of thiosulphate consumed by blank (ml), w = oven dry weight of pulp, and f = factor for correction to a 50 per cent permanganate consumption.

2.2.7 Fibre strength factor

Fibre strength is an important parameter which determines the quality of pulp being produced. Evaluation of hand sheets was done using the trouble shooter, which provides a measure of the strength of the constituent fibres and also allows rapid characterization of basic pulp quality.

Unbleached pulp was partially delignified with an acidified sodium chlorite solution at 60 °C for 5 minutes. The washed pulp was taken for making hand sheets as per TAPPI method number T 205 : Om - 88. 60 g m^{-2} sheets were prepared. The test strips of 2.5 x 10 cm size were cut from these hand sheets. Fibre strength factor (F.S. factor) was determined by pulmac trouble shooter (Model - TS 100) by re-wetting the strips. From the readings, F.S. factor was calculated as follows.

F.S. Factor = (Mean wet zero span value x Std (60 g m^{-2}) basis wt.)) / Actual average basis weight

The index value was developed for each of the tree through individual scores for above mentioned individual traits and then by adding these individual index scores. The indexing was calculated by awarding a range of weightage (Table 1).

3 Results and Discussion

The wood analysis was carried out to understand the utility of *C. equisetifolia* in paper and pulp industry. The results for

Table 2. Mean, range and standard deviations for various anatomical and pulp properties in *Casuarina equisetifolia*

S. No.	Descriptive Properties	Mean	Maximum	Minimum	Standard deviation
1	Wood Density (kg m^{-3})	670.60	787.00	585.00	64.41
2	Fibre Length (mm)	0.95	1.07	0.86	0.05
3	Fibre Diameter (micron)	13.78	16.70	8.95	1.95
4	Slenderness Ratio	0.07	0.10	0.06	0.01
5	Ash (%)	0.88	2.48	0.52	0.48
6	A-B Extract (%)	1.78	2.71	1.08	0.46
7	Lignin (%)	24.25	27.03	22.95	1.37
8	Holocellulose (%)	71.79	75.17	67.00	2.39
9	Kappa Number	17.02	21.00	14.70	1.62
10	FS Factor	28.77	34.28	22.57	3.67
11	Pulp yield (%)	49.34	51.95	45.10	2.33

Table 3. Indexing for various wood traits in *Casuarina equisetifolia*

Clone	Measured value										Index value											
	WD	FL	FD	SR	Ash	ABE	LIG	HOC	KN	FSF	WD	FL	FD	SR	Ash	ABE	LIG	HOC	KN	FSF	Total	Rank
CHCE890905	705	0.94	13.50	0.07	0.92	2.22	23.30	75.17	15.95	32.76	7.50	10.00	1.00	10.00	7.50	7.50	10.00	2.50	10.00	5.00	71	1
CHCE890903	671	0.96	14.90	0.06	0.92	1.38	22.95	73.90	17.56	25.20	7.50	7.50	2.50	10.00	7.50	0.00	10.00	2.50	7.50	0.00	55	2
CHCE890201	633	0.93	14.40	0.06	0.57	2.22	23.67	67.00	14.85	31.00	10.00	10.00	2.50	10.00	0.00	7.50	10.00	1.00	0.00	2.50	54	3
CHCE892602	651	0.97	14.80	0.07	0.62	1.52	26.24	74.05	15.97	30.70	10.00	7.50	2.50	10.00	0.00	0.00	7.50	2.50	10.00	2.50	53	4
CHCE892604	616	0.96	14.60	0.07	0.66	1.08	24.70	73.00	16.70	31.00	10.00	7.50	2.50	10.00	0.00	0.00	7.50	2.50	10.00	2.50	53	5
CHCE893003	615	0.94	10.84	0.09	1.26	1.94	24.24	69.20	17.56	32.66	10.00	10.00	0.00	2.50	2.50	2.50	10.00	1.00	7.50	5.00	51	6
CHCE892003	596	0.95	14.10	0.07	0.97	1.09	23.64	68.10	15.38	27.29	0.00	10.00	2.50	10.00	5.00	0.00	10.00	1.00	10.00	1.00	50	7
CHCE891003	585	1.07	15.20	0.07	0.59	1.86	23.73	72.44	18.50	34.28	0.00	5.00	5.00	10.00	0.00	2.50	10.00	2.50	7.50	5.00	48	8
CHCE893004	594	0.96	11.47	0.08	0.87	1.92	23.82	73.30	16.67	25.42	0.00	7.50	0.00	5.00	10.00	2.50	10.00	2.50	10.00	0.00	48	9
CPCE893702	693	0.86	8.95	0.10	0.52	2.32	23.06	71.00	17.30	25.27	7.50	10.00	0.00	2.50	0.00	7.50	10.00	1.00	7.50	0.00	46	10
CPCE890108	737	0.97	13.60	0.07	0.78	1.52	23.09	71.01	16.91	28.00	7.50	7.50	1.00	7.50	0.00	0.00	10.00	1.00	10.00	1.00	46	11
CHCE890401	689	0.94	14.40	0.07	0.64	1.73	26.93	72.60	14.70	32.80	7.50	10.00	2.50	10.00	0.00	1.00	5.00	2.50	0.00	5.00	44	12
CPCE890110	764	1.01	14.50	0.07	2.48	1.77	23.34	74.50	21.00	24.38	5.00	7.50	2.50	10.00	2.50	1.00	10.00	2.50	2.50	0.00	44	13
CPCE891802	723	0.96	16.70	0.06	0.67	1.47	24.03	71.23	17.64	28.15	7.50	7.50	7.50	0.00	0.00	0.00	10.00	1.00	7.50	1.00	42	14
CPCE890301	787	0.90	14.80	0.06	0.68	2.71	27.03	70.30	18.58	22.57	5.00	10.00	2.50	0.00	0.00	7.50	5.00	1.00	7.50	0.00	39	15

WD-Wood density (kg m^{-3}); FL-Fibre length (mm); FD-Fibre diameter (μm); SR-Slenderness ratio; Ash – Ash content (%); ABE-Alcohol-Benzene extractive (%); LIG-Lignin (%); HOC-Hollocellulose (%); KN-Kappa number; FSF-Fibre strength factor

physical, proximate chemical analysis, pulping and indexing are presented in Tables 2 and 3. The maximum physical parameters viz. wood density, fibre length and fibre diameter were recorded for CPCE890301 (787 kg m^{-3}), CHCE891003 (1.07 mm) and CPCE891802 (16.7 micron), respectively. Proximate chemical analysis also varied significantly for various traits, similarly (Table 2).

The indexing developed for various parameters demonstrated that index value varied from 39 (CPCE890301) to 71 (CHCE890905) with an average value of 49 (Table 3). The indexing depicted that out of 15 tested genotypes, seven genotypes had index values more than mean index value, and therefore could well be used for pulp and paper manufacturing.

It is well documented that the quality of wood affects the quantity and quality of pulp production. The quality of wood varies within the species depending on the genetic characteristics, growth pattern and environment in which it is grown. Selection of specific genotype for specific end uses such as paper and pulp becomes important to meet the increasing demands. It may be appropriate to mention an example of Aracruz Florestal, Brazil had initially selected 5000 plus trees of eucalypts from an area of 36000 hectare population. These trees were tested for specific gravity and yield of bleached pulp and 625 trees were screened through preliminary standards. On the basis of wood characteristics, further screening was carried out to bring down to 25 most useful trees to be promoted for clonal propagation for large scale plantations. In this way, the pulp yield was improved by 23% and total productivity of pulp on per hectare basis was increased by 135% (Zobel, 1993).

The wood density of a tree species is one of the most influential factors controlling strength and several other characteristics of the paper sheet. It is a known factor that the amount of wood needed to produce one tonne of air dried pulp is calculated from a ratio of wood density and pulp yield (Storebraten, 1990). However, high wood density creates hindrance in digestion and therefore not preferred in pulping as it requires higher amount of chemicals.

The proximate chemical analysis gives an idea of potentiality of raw materials for paper making (Rao et al., 1999). All soluble material comes under category of extractives, and are totally undesirable in pulp and paper making. The A-B extract affects pulp yield, paper quality and drainage characteristics of paper machine.

Fibre slenderness ratio is an important factor related to paper conformability as short and thin walled fibres produce good slenderness ratio, which is related to paper sheet density and pulp digestibility (Ona et al., 2001) as well as, increase tearing resistance. This is partly because short and thin fibres are readily collapsed to double walled ribbons and produce good surface contact and fibre to fibre bonding (Ogbonnaya et al., 1997).

The fibre diameter governs flexibility and therefore thin walled fibres favourably affect bursting and tensile strengths and folding endurance of paper. A decrease in the variable, which measures flexibility and wet plasticity of fibres, results in higher degree of conformability gives rises a sheet of higher density or lower bulk. This ultimately leads to good physical strength with more opaque sheet and less porosity. Therefore, papers made from such fibres expected to have increased mechanical strength and thus be more suitable for writing, printing and packaging purposes (Neto et al., 1996).

4 Conclusion

The substantial clonal variation in pulp yield and other traits related to papermaking offers scope to select and breed trees suitable for pulpwood production.

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Clonal Evaluation of *Casuarina junghuhniana* for Growth and Stem Form in South India

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Abstract

A multilocation clonal testing conducted with 10 clones of *Casuarina junghuhniana,* two clones of *C. equisetifolia*, one seedlot of each species and a widely planted natural hybrid clone showed substantial variation for growth, stem straightness, wood density and pulp yield. The trials were conducted in three locations in Tamil Nadu State under contrasting soil and cultural conditions between 2009 and 2013. The design adopted was Randomized Complete Block Design with 4 to 5 ramets of each clone planted in 10 replications. Survival, growth (height and diameter) and stem straightness were assessed up to the age of four years. Clonal and seedling accessions differed significantly ($p<0.001$) for all these characters. Two clones of *C. junghuhniana* were found to be superior to all other entries in terms of growth. The two seedlots from seed orchards also showed better growth than many clones including the benchmark clone. The best clone of *C. junghuhniana* in terms of growth also possessed 2% more pulp content than the benchmark clone. Two clones that recorded the most outstanding growth in all locations were deployed for commercial planting.

1 Introduction

Within two decades of its systematic introduction in 1996, *Casuarina junghuhniana* proved to be a more preferred species than the widely cultivated *C. equisetifolia* for various end uses like pulp wood, fuel wood and poles in India. It is faster growing and better adapted to dry land cultivation than *C. equisetifolia*. No incidence of blister-bark disease which causes heavy mortality in *C. equisetifolia,* has been reported in *C. junghuhniana* under cultivation. This versatile species provides new opportunities for improving productivity of Casuarina plantations through selection and breeding. The excellent coppicing ability of this species makes it amenable for clonal forestry. Clonal plantations raised from branch cuttings of *C. equisetifolia* showed slow growth during early ages, plagiotrophism and precocious flowering leading to highly variable growth (Santhakumar et al., 2001; Warrier and Ganesan, 2010). Coppice shoots of *C. junghuhniana* readily root and quickly establish in plantations after planting.

Twenty one provenance and landrace seedlots from Indonesia, East Timor, Kenya and Tanzania received from the Australian Tree Seed Centre, CSIRO were tested in coastal and inland locations from 1996 and six of them from East Timor were found to be the most adaptable and fast-growing. These trials were converted into seed orchards by removing the inferior provenances and seeds supplied to tree growers. Cultivation of *C. junghuhniana* steadily increased during the last decade and is generally found to be faster growing than *C. equisetifolia*. It was well-suited for inland regions where Casuarina is not cultivated so far. Since Casuarina plantations are raised in short rotations of 3 to 5 years in India, clonal forestry will lead to rapid genetic improvement and increasing wood production. Considering these advantages, a clonal selection and testing programme was undertaken in Tamil Nadu to identify fast-growing clones with straight stems suitable for the principal end uses in India. This paper reports results from the clonal tests.

2 Materials and Methods

2.1 Clonal selection

Around 100 outstanding trees were selected based on their superiority for height, diameter and stem straightness traits among different provenances in trial plots located at Coimbatore, Panamaplli (Kerala) and Puducherry. Selected trees were felled at 15 cm from ground level and maintained with water and fertilizer application for coppice shoot production. Rooted cuttings developed from one-month old coppice shoots were assembled in a clone bank in Forest Campus, Coimbatore and used as the base material for producing requisite number of clonal plants for multilocation testing. Clones were deployed in field tests in batches and the present report is from the earliest clonal test involving 10 clones selected from six provenances (Table 1). A widely planted natural hybrid clone of *C. junghuhniana* and *C. equisetifolia*, two clones of *C. equisetifolia* and one seed orchard seedlot each of *C. equisetifolia* and C. *junghuhniana* were included as benchmark accessions.

2.2 Clonal Testing

Three trials were established in the year 2009 at Pugalur (Lat.: 11° 04'; Long.: 77° 99'; Alt.: 450 m; rainfall: 900 mm;

Table 1. Details of clonal and seedling accessions of Casuarina tested in Tamil Nadu, India

Clone Number	Test ID	Type	Species	Provenance	Selection Location
1	CJ9	Clone	*C. junghuhniana*	Noelmina River, Timor	Puducherry
2	CJ10	Clone	*C. junghuhniana*	Camplong, Timor	Puducherry
3	CJ11	Clone	*C. junghuhniana*	Kapan, Kupang, Timor	Puducherry
4	CJ12	Clone	*C. junghuhniana*	Old Uhak, NE Wetar	Puducherry
5	CJ13	Clone	*C. junghuhniana*	Kapan, Kupang, Timor	Puducherry
6	CJ15	Clone	*C. junghuhniana*	Sw Soe, Timor	Puducherry
7	CJ16	Clone	*C. junghuhniana*	Buat, Soe, Timor	Puducherry
8	CJ17	Clone	*C. junghuhniana*	Old Uhak, NE Wetar	Puducherry
9	CJ207	Clone	*C. junghuhniana*	Camplong, Timor	Coimbatore
10	CJ 208	Clone	*C. junghuhniana*	Camplong, Timor	Coimbatore
11	101	Clone	*C. junghuhniana x C. equisetifolia*	Thailand	Marakkanam
12	102	Seedling	*C. junghuhniana*	IFGTB Seed Orchard	Coimbatore
13	103	Seedling	*C. equisetifolia*	IFGTB Seed Orchard	Coimbatore
14	MTP 1	Clone	*C. equisetifolia*	Clone released by FCRI, MTP	Mettupalayam
15	MTP 2	Clone	*C. equisetifolia*	Clone released by FCRI, MTP	Mettupalayam

red soil), Pallipalaym (Lat.: 11° 34'; Long.: 77° 72'; Alt.: 600 m; rainfall: 850 mm; black soil) and Emapalli Lat.: 11° 38'; Long.: 77° 89'; Alt.: 600 m; rainfall: 850 mm; black soil) in Tamil Nadu State. The trials were planted in randomized complete block design with ten replications each having four (Pallipalayam and Emapalli) or five trees (Pugalur) of an accession. Trees were planted at a spacing of 1.5 m in pits containing a basal dose of farm yard manure (one kg) and NPK fertilizer (10 g). Trial plots were irrigated once in a week and maintained weed-free through periodic ploughing between the rows.

2.3 Assessment and data analysis

All the trees in the test plots were assessed for height, diameter at breast height (DBH) and stem straightness annually up to 4 years age. Conical volume was used as a derived character of height and DBH. Stem straightness was assessed in a scale of 1 to 4 (4 – straight stem; 3 – one minor bend; 2- 1 to 2 bends; 1- more than 2 bends). Wood density and pulping traits of the clone with the best growth and one of the benchmark clones were assessed. The data was subjected to analysis of variance as per Williams and Matheson (1994) using the statistical software Gestat (15th edition).

3 Results and Discussion

All accessions recorded high survival (90% and above) in the test plots. Highly significant variation (P<0.001) was found among the clonal and seedling accessions for growth and stem straightness (except in Emapalli) in all three locations. Clone number 1 (Test ID: CJ9) recorded the fastest growth and the straightest stem consistently in the three trials. At four years age, this clone had a height of 12.8 to 14.8 m, DBH 8.8 to 13 cm and volume 0.029 to 0.067 m^3 in different locations (Table 2). Compared to the most widely planted benchmark clone 11 (MKM – natural hybrid), it showed nearly 25% better height, 43% DBH and 142% volume growth across locations. It also possessed straight poles scoring the maximum of 4 points in two of the three locations which was nearly 9% better than the check clone. Many other clones of *C. junghuhniana* particularly clone 4 showed better growth than the benchmark clones but had a stem form inferior to the benchmark clone. Growth of the two clones of *C. equisetifolia* (14 and 15) was less than the trial mean and benchmark clone in all locations.

Since *C. junghuhniana* is in its early stages of domestication and breeding, reports on clonal variation for growth and other characters of economic importance are scarce for this species. Clones selected from a set of provenances similar to the present study showed highly significant variation for growth, stem form and branching traits in Thailand (Luechanimitchit et al., 2016). The best performing clones were from the Wetar provenances and a clone from Noelmina River recorded above-average growth similar to the results obtained in the present study. The best clone (1) belongs to the Noelmina River provenance and the second-ranking clone (4) was from the Wetar provenance. These provenances showed the best growth in provenance and progeny trials conducted earlier in India and Thailand (Pinyopusarerk et al., 2005; Nicodemus et al., 2005; Luechanimitchit et al., 2011; Nicodemus et al., 2015). Since the best clones are from the most adaptable provenances

Table 2. Growth and stem straightness of four year old clonal and seedling accessions of Casuarina tested in Tamil Nadu, India

		Pugalur				Pallipalayam				Emapalli			
Clone No.	Test ID	Height (m)	DBH (cm)	Volume (m^3)	Stem St'ness	Height (m)	DBH (cm)	Volume (m^3)	Stem St'ness	Height (m)	DBH (cm)	Volume (m^3)	Stem St'ness
1	CJ 9	14.79	10.29	0.0441	3.91	14.19	13.00	0.0667	4.00	12.83	8.82	0.0291	4.00
2	CJ 10	12.09	7.61	0.0223	3.04	9.20	6.48	0.0161	3.47	11.01	7.63	0.0209	3.83
3	CH 11	11.39	6.58	0.0154	3.41	11.98	9.66	0.0313	3.55	10.33	6.51	0.0136	3.69
4	CJ 12	13.49	9.95	0.0364	3.05	11.95	10.29	0.0381	3.67	9.66	6.26	0.0136	3.90
5	CJ 13	12.90	8.67	0.0274	3.23	11.11	8.34	0.0236	3.29	10.67	6.99	0.0182	3.66
6	CJ 15					10.35	7.09	0.0172	3.30	11.14	7.55	0.0182	3.83
7	CJ 16	11.99	7.42	0.0203	3.40	11.77	9.04	0.0287	3.83	10.90	7.07	0.0164	3.79
8	CJ 17					10.21	7.63	0.0192	3.82	10.02	6.67	0.0127	3.70
9	CJ 207					10.56	7.57	0.0190	3.03	10.45	6.46	0.0146	3.73
10	CJ 208					12.41	9.81	0.0348	2.83				
11	MKM	12.57	6.90	0.0173	3.12	10.70	7.78	0.0205	3.91	10.23	7.69	0.0200	3.92
12	CJS	13.78	8.89	0.0305	3.00	11.91	10.45	0.0410	3.03	11.00	7.70	0.0200	3.67
13	CES	13.38	8.16	0.0257	3.11	13.02	11.64	0.0504	3.50	11.45	7.95	0.0230	3.68
14	MTP1	11.19	5.53	0.0111	3.20	10.17	6.62	0.0127	3.76	10.05	6.30	0.0127	3.50
15	MTIP2									9.93	6.08	0.0110	3.63
	Mean	12.76	8.00	0.0250	3.25	11.39	8.96	0.0306	3.50	10.69	7.12	0.0174	3.75
	LSD	1.203	1.33	0.01045	0.4269	1.34	1.814	0.01365	0.4312	1.37	1.233	0.0080	NA
	F-prob	<0.001	<0.001	<.001	<0.001	<0.001	<0.001	<.001	<0.001	0.00	<0.001	<.001	NS

Table 3. Pulping characters of Casuarina clones at 4 years age

Parameter	Casuarina Clone		Eucalyptus	
	CJ 9	CJ MKM	Seedling	TNPL Clone
Bulk density (kg m^{-3})	247	270	204	203
Basic density (kg m^{-3})	556	484	520	457
Chemical addition (%)	17	17	17	17
Screened pulp yield (%)	51.71	50.62	45.63	52.01
Kappa Number	17.1	25	21.2	22.4

tested in similar environments, they have been deployed in commercial plantations to increase the productivity.

It is important to note that the two seedlots from seedling seed orchards (SSO) of *C. equisetifolia* and *C. junghuhniana* have shown excellent growth, in fact outperforming many of the clones including the benchmark accessions (Table 2). These SSOs were established with a broad genetic base comprising provenance and landrace seedlots from the entire distribution range of the two species. Inferior provenances and individuals were gradually thinned out to improve the quality of seed output. The genetically improved seedlots used in the present study constitute the second generation progeny possessing high level of adaptability and vigorous growth in different planting environments. Faster growth of seedlings compared to clonal plants has been reported for *C. equisetifolia* (Santhakumar et al., 2001; Warrier and Ganesan, 2010). This underscores the need for retaining both the seedling and clonal options as outputs from Casuarina breeding programmes and subjecting them to rigorous field testing. Seedling propagation of Casuarina is simple and cost-effective and hence affordable to smallholding farmers who raise the major portion of Casuarina plantations in India.

A preliminary testing of pulping traits of the fast growing clone 1 and the widely planted benchmark clone 11 showed differences between them (Table 3). Clone 1 had a basic density of 556 kg m^{-3} at four years age which is nearly 15% more than the benchmark clone. They require the same chemical addition of 17% but the pulp yield of clone 1 was 51.71% compared to 50.62% of the benchmark clone. Increase in pulp yield by more than 2% is likely to result in high conversion rate of paper from wood raw material for the industries. Screening more clones for pulp yield and other traits important for papermaking is necessary to select clones specific for pulpwood production.

4 Conclusion

Highly significant variation was observed for growth and stem straightness among clonal and seedling accessions of *Casuarina equisetifolia* and *C. junghuhniana* tested at three locations in Tamil Nadu, India. The best clone of *C. junghuhniana* showed superior growth and stem form than the benchmark clone and seedling accessions. It also possessed higher basic density and pulp content. The top ranking clones identified in the present study have the potential to increase plantation productivity in South India. Seeds from seed orchards also showed superiority over the benchmark clone providing a cost-effective means of providing genetically improved planting material to resource-poor farmers.

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Screening of *Casuarina equisetifolia* Clones for Tolerance to Salinity and Identification of a Biochemical Marker

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Abstract

A study was conducted to screen 20 clones of *Casuarina equisetifolia* for their salinity tolerance and also to identify a biochemical marker for tolerance. Screening was done in a glass house in sand culture in which the concentration of NaCl was increased at the rate of 0.2% every 5 days, from 0 to 2.4% over a period of 60 days. Growth in terms of dry weight and growth rate were estimated and the Relative Growth Rate (RGR) was calculated. The clones were ranked on the basis of RGR and Tolerance Index (TI) calculated as the product of RGR and survival percentage. They were also ranked based on visual scoring of salt damage symptoms which included succulence of the needles, necrosis of needle tips, colouration of the needles due to anthocyanin accumulation and drying of needles. RGR ranged from 7.39 % to 40.88 %, TI from 338 to 3666 and visual score for salt damage symptoms from 3 to 7.4 indicating a high degree of variation among the clones providing scope for selection for salinity tolerance. Seven clones viz., CP 3903, CH 3001, TNPP-2, TNRM-4, TNAM-2, TNPP-4 and TNVM-3 with above average RGR, TI and symptom score were grouped as salinity tolerant clones and the remaining as intolerant to salinity. The comparison of these clones in terms of the pattern of proline accumulation, at different levels of salinity ranging from 0 to 350 mM NaCl revealed that there was no consistency in the pattern. The "critical point" or the point of NaCl concentration at which there is a spurt in proline accumulation could not be located in many clones and even when located did not correlate with tolerance to salinity. Therefore, it is hypothesized that accumulation of proline may not be the only mechanism of tolerance operating in the species, and it may vary among sub-species and provenances, which will require an in-depth study.

1 Introduction

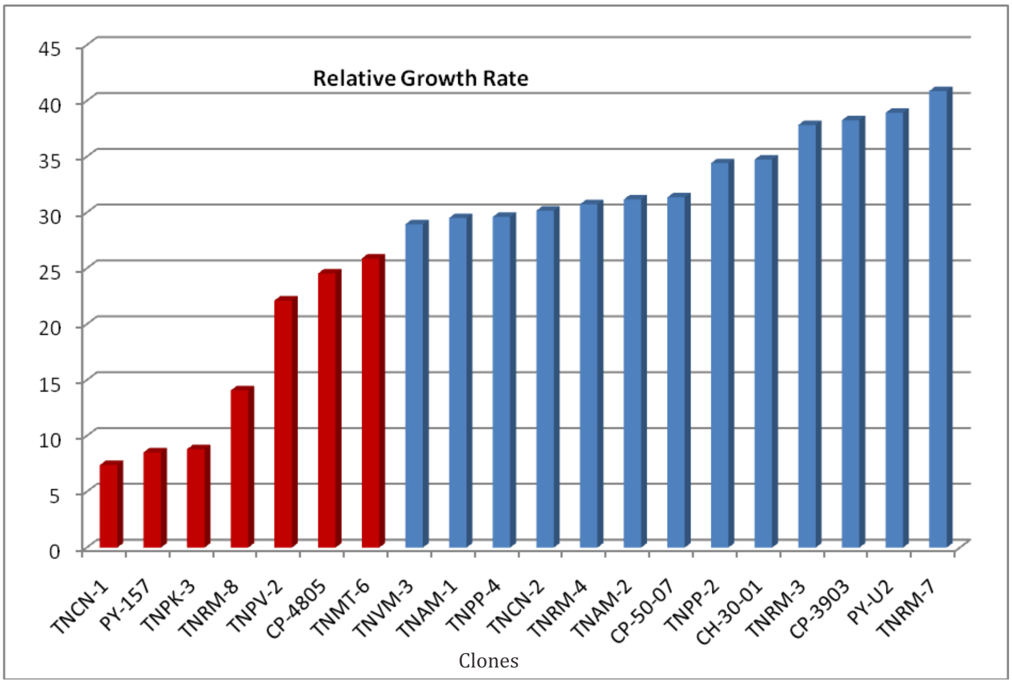
Genetic improvement of a species can be carried out for three purposes- firstly for use in productive lands under normal plantation practices, secondly for use in marginal lands, and thirdly for special uses like pulpwood, fuelwood and timber. Most of the genetic improvement programmes have been focussed only on the purpose first stated, so far. The second purpose is gaining importance due to emphasis on environmental amelioration and progress of forest plantations into degraded and marginal lands hitherto unused. Selection of *Casuarina equisetifolia* clones for the purpose of afforestation of problem soils and marginal lands was carried out at the Institute of Forest Genetics and Tree Breeding, Coimbatore, India, by Point Grading method, at a selection intensity of 1 in 10000, from 8- to 10-year-old plantations raised on stressed sites, under conditions of drought, salinity, disease and pests. After assessing 180 trees, 51 were selected. Of these only 45 could be rooted and these were assembled in a clone bank at the Institute (Jayaraj et al., 2001).

Studies have been made on the tolerance of Casuarinas to salinity and alkalinity besides other physico-chemical stresses. Several experiments have been conducted to rank the Casuarinas in terms of their tolerance to salinity, and a wide variation is reported among the species, in the following order of decreasing salinity tolerance : *C.obesa*> *C. glauca*>*C. equisetifolia*>*C. cunninghamiana* >*C. cristata* (Clemens et al., 1983). Significant provenance variation has also been reported within *C. glauca* (Girgis et al., 1992). It has been suggested that there is a high degree of genetic variation in salt tolerance in Casuarina and that it is possible to develop salt tolerant cultivars for reclaiming salt damaged forests or to establish plantations with low quality brackish water (Allen et al., 1994). With this background a study was conducted to screen 20 selected clones of *C. equisetifolia* for their tolerance to salinity and also to identify a biochemical marker for the tolerance.

2 Materials and Methods

The screening was done in a glass house in sand culture, using sterilized washed sand as the medium in which the concentration of NaCl was increased at the rate of 0.2% every 5 days. The concentration was increased from 0 to 2.4% over a period of 60 days. Growth in terms of dry weight was measured with 5 ramets maintained under treatment and 5 ramets as control. Growth rate was estimated using the average values by the formula

Fig. 1. Relative Growth Rate (RGR) of *Casuarina equisetifolia* clones subjected to salinity stress



[log_n(final weight)- log_n (initial weight)] /2 and the Relative Growth Rate was calculated using the formula RGR=(GRt/ GRc)x100, where GRt is the growth rate of the treatment and GRc is the growth rate of the control. Tolerance Index (TI) was calculated for each clone using the formula TI= RGR x Survival percentage. The clones were ranked on the basis of RGR as well as TI.

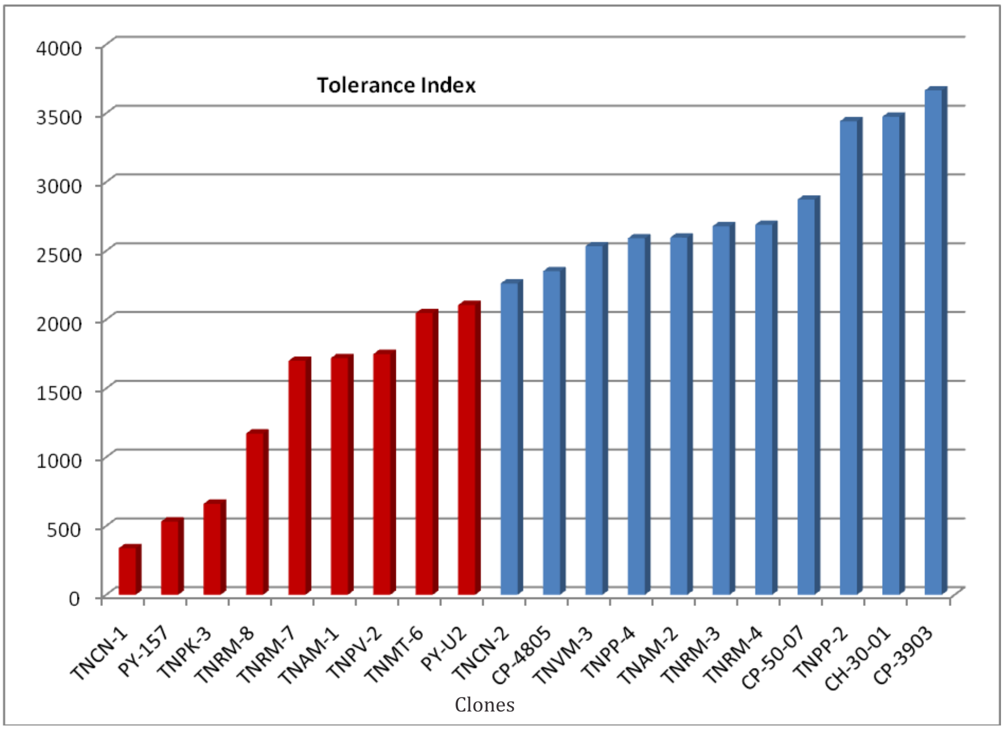
The symptoms of salt damage included succulence of the needles, necrosis of needle tips, colouration of the needles due to anthocyanin accumulation and drying of needles. Visual scoring of the symptoms was also done for the clones using the following scores: 1- no symptom; 3- symptoms visible on older leaves but plant is healthy; 5- symptoms visible on all leaves; 7- severe symptoms; and 9- plant is dead (Jayaram et al., 2000). The clones were ranked on the basis of the scores also.

In order to identify a biochemical marker for the salinity tolerance, the method used by Dreier (1983 a) was tried. The selected 20 clones were grown in water culture, and after rooting, they were subjected to different levels of NaCl stress using concentrations ranging from 0 to 350 mM NaCl. The concentration of proline was estimated in the needles and plotted against the concentration of NaCl, to determine the critical point, the concentration of NaCl at which proline started accumulating rapidly. The critical point determined biochemically was correlated with the salt tolerance determined in terms of RGR, Tolerance Index as well as the visual score, to determine the validity of the procedure of rapid screening for salinity tolerance in *C.equisetifolia*.

Table 1. Salinity Damage Score of clones of *Casuarina equisetifolia* clones subjected to salinity stress

Clone	Maximum Salinity Damage Score	Tolerance status
TNRM-7	7.4	Intolerant
TNAM-1	7	Intolerant
TNCN-1	7	Intolerant
PY-157	6.4	Intolerant
TNCN-2	6.25	Intolerant
PY-U2	6.25	Intolerant
TNRM-3	6	Intolerant
TNMT-6	5.9	Intolerant
TNPK-3	5.9	Intolerant
CP-50-07	5.4	Intolerant
TNRM-4	5	Tolerant
TNPV-2	5	Tolerant
TNRM-8	4.9	Tolerant
TNAM-2	4.75	Tolerant
TNPP-4	4.7	Tolerant
TNVM-3	3.8	Tolerant
CP-4805	3.75	Tolerant
CP-3903	3.25	Tolerant
TNPP-2	3	Tolerant
CH-30-01	3	Tolerant

Fig. 2. Tolerance Index of *Casuarina equisetifolia* clones subjected to salinity stress



3 Results

The visual score for salt damage symptoms ranged from 3 to 7.4 (Table 1). RGR of the 20 clones ranged from 7.39% to 40.88 % and Tolerance Index ranged from 338 to 3666 (Table 2; Fig. 1 and 2). A comparative evaluation of the three methods of screening showed that 11 clones could be classified as salinity tolerant by having Tolerance Index above average, while 13 could be identified having above average RGR and 10 having visual score above 5. Seven clones were common in the list of tolerant clones identified by all the three methods, viz., clones CP 3903, CH 3001, TNPP-2, TNRM-4, TNAM-2, TNPP-4 and TNVM-3. Clones TNCN-2, TNRM-3 and CP 5007 were found tolerant using Tolerance Index and RGR, but scored poorly in the visual scoring method. The clones that were found either below average in tolerance to salinity in terms of TI and RGR or scoring more than 5 in visual score were treated as intolerant to salinity and they are TNRM-8, TNRM-7, TNMT-6, TNAM-1, TNPV-2, TNPK-3, TNCN-1, TNCN-2, PY-157, PY-U2, CP-4805, CP-5007 and TNRM-3.

The comparison of these clones in terms of the pattern of proline accumulation, at different levels of salinity ranging from 0 to 350 mM NaCl revealed that there was no consistency in the pattern. The "critical point" or the point of NaCl concentration at which there is a spurt in proline accumulation could not be located in many clones and even when located did not correlate with tolerance to salinity.

4 Discussion

Both RGR and TI have been found reliable in identifying salinity tolerant clones through nursery studies, with TI being more rigorous than RGR alone. Combining this information with the Visual Scoring, can help in a more rigorous screening. Seven clones have been found salinity tolerant by all the three methods, indicating the reliability of all the methods.

It has been found in crop plants that under stress due to NaCl, there is a concentration of NaCl, above which the proline content strongly rises, called as the "critical point". In salt sensitive plants this "critical point" is low, and the determination of the critical point by measurement of proline concentration could serve as a basis for analysis of salt tolerance. This has been confirmed in 19 cultivars of crop plants (Dreier, 1983b), *Brassica juncea* (Jain et al., 1991) and egg plant (Jain et al., 1987). Rapid screening by detection of "critical point" in the present study, did not correlate well with the tolerance observed, possibly because of various biochemical factors that may be involved in salinity tolerance in Casuarina, other than accumulation of proline. The differences in the visual symptoms of damage, viz., drying of needle tips, succulence of needles, colouration of needles and dropping of needles which are mutually exclusive in the clones is itself an indication of differences in mechanisms of tolerance that may be operative in the species. Accumulation of osmoprotective chemicals might have led to succulence in

Table 2. Growth and salinity tolerance of *Casuarina equisetifolia* clones

Clone	IDW (g)	FDW-T (g)	FDW-C (g)	GR-T	GR-C	RGR	SURV (%)	TI
TNRM3	0.344	0.872	4.026	0.465	1.229	37.84	60	2270
CH3001	0.301	0.854	6.031	0.520	1.497	34.75	100	3475
CP5007	0.389	0.873	5.126	0.404	1.289	31.36	95	2979
CP3903	0.379	0.905	3.671	0.434	1.134	38.2	100	3827
CP4805	0.47	0.866	5.663	0.305	1.244	24.55	95	2332
TNRM8	0.663	0.865	4.400	0.133	0.946	14.07	90	1266
PY157	0.488	0.572	3.141	0.079	0.930	8.52	60	511
TNAM2	0.345	0.819	5.528	0.432	1.386	31.17	80	2493
TNAM1	0.472	0.772	2.507	0.246	0.834	29.51	60	1770
TNPP2	0.409	1.031	6.010	0.462	1.343	34.41	100	3442
TNPV2	0.435	0.771	5.752	0.285	1.290	22.12	85	1880
TNCN1	0.488	0.566	3.647	0.074	1.005	7.39	47.36	352
TNPK3	0.557	0.686	5.887	0.104	1.178	8.82	75	662
TNRM7	0.417	0.953	3.154	0.413	1.011	40.88	25	1022
TNPP4	0.319	0.647	3.463	0.352	1.191	29.61	100	2961
TNVM3	0.295	0.640	4.296	0.387	1.339	28.95	95	2750
TNRM4	0.385	0.801	4.161	0.365	1.189	30.74	95	2920
TNMT6	0.492	0.893	4.923	0.298	1.151	25.89	75	1942
TNCN2	0.333	0.679	3.514	0.355	1.177	30.17	45	1357
PYU2	0.22	0.608	2.997	0.508	1.305	38.93	60	2336

IDW: Initial dry weight; FDW-T: Final dry weight – treatment; FDC-T: Final dry weight – control; GR-T: Growth rate-treatment: GR-C: Growth rate-control; RGR: Relative growth rate; SURV: Survival; TI: Tolerance index

some clones, while in some clones the salt itself would have been accumulated in the older needles, which would be eliminated by dropping at some stage, thus excluding salt from the plant. Necrosis of tips and colouration were also found in different clones. Indications are that there may not be just one mechanism of tolerance, but many in isolation or in combination, varying among the sub-species and provenances of *C. equisetifolia*.

The clones identified as salinity tolerant can be used for planting in afforestation of salt-affected lands and to establish Special Purpose Clonal Seed Orchards. They can also be combined with productive clones, either by conventional breeding or non-conventional breeding procedures, employing biotechnological tools. Accumulation of proline does not appear to be the only mechanism operating in the species, and the mechanism of tolerance may vary among sub-species and provenances, which will require an in-depth study.

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Wood Property Variation in Selected Clones of *Casuarina equisetifolia* L. Grown in Karur District, Tamil Nadu for Pulp and Papermaking

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Abstract

Variation in wood physical (specific gravity), anatomical (fibre, vessel and ray morphology) and chemical (cellulose and lignin per cent) properties of 43 clones and 3 seedlots of *Casuarina equisetifolia* L. grown in Karur district, Tamil Nadu was studied to assess their suitability for pulp and paper making. Transverse discs representing each clone which were collected from the base of billets were converted to smaller specimens for undertaking studies on wood physical, chemical and anatomical properties. Estimation of specific gravity was undertaken using a precision balance and fibre morphology was studied using an image analysis system. Cellulose and lignin were estimated using standard procedures. Nested analysis of variance was carried out to find out inter and intra-clonal variation of clones. All the physical and anatomical properties except fibre lumen width, Runkel ratio, rigidity coefficient, flexibility coefficient and shape factor, showed significant difference between clones. Within clone variation was also significant for all the physical and anatomical parameters except specific gravity (oven dry). In order to assess the suitability of clones for pulp and paper making, specific gravity (oven dry), fibre length, Runkel ratio, shape factor, slenderness ratio, flexibility coefficient, rigidity coefficient, and cellulose and lignin content were considered. Among these, fibre length, slenderness ratio, flexibility coefficient, shape factor and cellulose and lignin content of clones were found to be within the acceptable range for pulp and paper making. For selecting the best clones suitable for pulp and paper making, clones were grouped into four clusters by carrying out hierarchical cluster analysis on the basis of all physical, anatomical, chemical and growth parameters. Cluster 4 (one clone) and cluster 2 (11 clones) were found to be suitable for pulp and paper making in comparison to other clusters.

1 Introduction

Casuarina equisetifolia L. is indigenous to the tropics and subtropics of Southeast Asia and Western Pacific regions, including northern Australia (Ogata et al., 2008). It is considered an important multi-purpose tree on account of its utility in nitrogen fixing, windbreaks, soil erosion control, suitability for fuel wood, poles, pulp and paper production. The suitability of wood for paper pulp makes it a promising raw material for the manufacture of paper for writing, printing and wrapping. It can also be used to prepare hard boards and chip boards. Of late, interest in this species has been on the rise amongst the farmers of several south Indian States including Tamil Nadu on account of its fast rate of growth and demand from the pulp and paper industries, fetching attractive prices on a fairly short rotation. It was in this back drop that the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore initiated tree improvement programmes in *C. equisetifolia*. In order to widen the genetic base of clones used in farm forestry, IFGTB conducted clonal testing with a large collection of clones.

In order to better understand the suitability of Casuarina clones as raw material for pulp and paper production, scientific knowledge on physical, anatomical and chemical properties of the wood is necessary. By understanding wood properties that determine wood quality, better selection criteria could be developed for specific end uses in the tree improvement programmes. In this regard, the present study aimed to find out wood property variation of selected clones of *C. equisetifolia* for getting the most superior clones in terms of wood properties, both for breeding and for processing for specific end uses.

2 Materials and Methods

2.1 Materials

The experimental material consisted of wood samples of 43 clones and three seedling origin materials collected from a clonal trial located in Mayiladumparai, near Kulithalai, Tamil Nadu (latitude 10° 55' N, longitude 78° 25' E; inland, red soil), The spacing adopted in the trial was 3m × 1.5m with 3 ramets per clone and 6 replications. The trees were three years old at the time of felling.

Table 1. Minimum, maximum and average values of specific gravity at three conditions of all materials

Condition	Minimum	Maximum	Mean
Fresh weight	1.047 ±0.01 (clone 1)	1.296 ±0.06 (clone 11)	1.161±0.03
Air dry	0.635 ±0.01 (clone 85)	0.869 ± 0.01 (clone 56)	0.794±0.01
Oven dry	0.594 ±0.02 (clone 85)	0.830 ±0.01 (clone 69)	0.741±0.01

2.2 Methodology

Billets of length 1 m were cut from the basal position of one tree (ramet) each selected randomly from the clones. Transverse discs of 6 cm thickness were collected from the base, middle, and top positions of each billet. For undertaking studies on wood physical and anatomical properties, these discs were cut into two transverse halves. One half was used for estimation of wood specific gravity and the other half for studying anatomical properties. For specific gravity measurements, five wood blocks of dimensions 2×2×2 cm were taken from one half of the transverse discs. Specific gravity measurement was estimated on fresh, air dry and oven dry weight basis using a specific gravity module attached to a precision electronic balance (Schimadzu AUY 220). For fibre morphological studies wood shavings were taken from the other half of the transverse disc and then maceration was carried out. Maceration of the wood samples was done using Jeffrey's method (Sass, 1971). For maceration, Jeffrey's solution was used and it was prepared by mixing equal volumes of 10 per cent potassium dichromate and 10 per cent nitric acid. Microscopic examination and quantification of macerated fibre were undertaken using an Image Analyser (Labomed-Digi 2). For vessel and ray morphology study, sections were taken from specimens of size less than 1 cm^3 using microtomy. Sections were then stained with safranin for 5 minutes. Excess stain was removed by washing sections successively in 70, 90 and 95% ethanol solution. Thin sections were further dehydrated using acetone and then kept in xylene for two hours for getting rigidity. Sections taken out from xylene were permanently mounted on microscopic slides with coverslips using DPX. Cellulose content of the wood was estimated following Sadasivam and Manickam (1992). Estimation of insoluble lignin was undertaken using Micro-Klason technique (Whiting et al., 1981). Nested ANOVA was performed to analyse the variation between clones. Pearson's bivariate correlation coefficient was calculated to examine the inter-relationship between and among anatomical and physical properties.

3 Results and Discussion

3.1 Specific gravity

Specific gravity (air dry, fresh weight and oven dry basis) differed significantly among clones at 1% significance level. The overall average specific gravity (air dry), specific gravity (fresh weight) and specific gravity (oven dry) of clones were 0.794, 1.161 and 0.741 respectively. Average whole tree specific gravity (air dry) ranges from 0.635 (clone 85) to 0.869 (clone 56), specific gravity (fresh weight) from 1.047 (clone 1) to 1.296 (clone 11) and specific gravity (oven dry) from 0.594 (for clone 85) to 0.830 (clone 69) (Table 1). Similar to the present study, researchers have studied specific gravity variation of clones of various species such as *Populus* spp., *Eucalyptus*

Table 2. Minimum, maximum and average values of fibre dimensions and fibre derived ratios

Character	Minimum	Maximum	Mean
Fibre length (μm)	1225.60 ±37.16 (clone 45)	1726.49 ±52.88 (clone 4)	1452.41 ±12.64
Fibre width (μm)	20.69 ±0.50 (seedling origin 90)	29.61 ±1.53 (clone 61)	25.19 ±0.15
Fibre lumen width (μm)	6.57 ±0.43 (clone 69)	12.53 ±1.35 (clone 45)	9.63 ±0.14
Fibre wall thickness (μm)	5.99 ±0.59 (clone 29)	9.34 ±0.45 (clone 56)	7.63 ±0.60
Runkel ratio	1.16 ±0.09 (clone 74)	2.83 ±0.24 (clone 69)	1.90 ±0.04
Slenderness ratio	97.72 ±6.78 (clone 45)	162.70 ±9.76 (clone 4)	118.31 ±0.96
Rigidity coefficient	52.81 ±1.94 (clone 74)	72.35 ±1.30 (clone 69)	62.01 ±0.47
Flexibility coefficient	54.16 ±2.63 (clone 69)	95.42 ±4.04 (clone 74)	75.49 ±0.87
Shape factor	0.64 ±0.03 (clone 74)	0.86 ±0.01 (clone 69)	0.74 ±0.01

Table 3. Minimum, maximum and average values of ray and vessel parameters

Character	Minimum	Maximum	Mean
Ray height (µm)	365.25 ±23.00 (clone 68)	591.50 ±39.75 (clone 1)	463.79 ±6.12
Ray width (µm)	25.50 ±1.09 (clone 33)	44.50 ± 2.0 (clone 48)	31.69 ±0.42
Ray frequency (no/mm^2)	10 ±0.51 (clone 44)	23 ± 1.29 (clone 86)	14 ±0.20
Vessel length (µm)	352.65 ±21.72 (clone 62)	546.59 ±37.22 (clone 45)	440.36 ±29.77
Vessel diameter (µm)	114.25 ±4.91 (clone 3)	176.00 ±4.55 (clone 85)	151.72 ±1.63
Vessel area (µm^2)	12037 ±846 (clone 3)	36470 ±3225 (clone 62)	26779 ±542
Vessel frequency (no/mm^2)	5 ±0.20 ;5 ±0.37 (clone 55 &61)	13 ±1.45 (clone 11)	7 ±0.14

spp., *Dalbergia* spp. in order to assess wood quality (Kauba et al., 1998; Rao et al., 2002). Grzeskowiak et al. (2000) reported significant differences in basic density in two *E. grandis* x *E. camaldulensis* hybrid clones. Pande and Singh (2009) found significant inter-clonal variation along radial directions of the clonal ramets of *Eucalyptus tereticornis*. Sreevani and Rao (2013) reported that basic density differed significantly between the clones of *E. tereticornis* developed by ITC Bhadrachalam.

While explaining the variations in growth, anatomical, physical and chemical properties of *Eucalyptus* spp. Kulkarni (2002) concluded that when different clones of the same species were planted in a new site, some of the clones performed well in terms of superior growth, anatomical, physical and chemical properties while some did not perform well in these respects. This was because of the resemblance of site conditions of these clones with their site of their origin. The findings of Kulkarni (2002) in *Eucalyptus* spp. is in line with the present study, i.e. significant differences in average specific gravity of different clones in the same site might be due to the resemblance of site conditions of some of these clones with their site of their origin.

Specific gravity is a complex feature which is influenced by cell wall thickness, the proportion of the different kind of tissues, and the percentages of lignin, cellulose and extractives (Valente et al., 1992). A study in *Eucalyptus* spp. found that density differences are largely driven by changes in cell wall thickness and vessel size (Downes et al., 1997). The present study found significant variation in vessel size, vessel and ray frequency, cellulose and lignin content, cell wall thickness. These variations contribute to between tree variation of specific gravity of clones.

3.2 Fibre characteristics

Fibre parameters like fibre length, fibre width and fibre cell wall thickness significantly differed between clones at 1% level whereas differences in fibre lumen diameter were non-significant. Average whole clone fibre length for different clones ranged from 1225.6 µm (clone 45) to 1726.5 µm (clone 4); fibre diameter from 20.69 µm (clone 90) to 29.61µm (clone 61); fibre lumen width from 6.54µm (clone 69) to 12.53 µm (clone 45); fibre cell wall thickness from 5.73 µm (clone 4) to 9.34 µm (clone 56) (Table 2). Previously Varghese and Sivaramakrishna (1996) reported that the mean fibre diameters and fibre wall thickness were 4.0-10.8 µm and 2.1-3.3 µm respectively in 10-year-old *C. equisetifolia* trees grown in India. On the other hand, El-Osta et al. (1981) reported greater mean fibre diameter (13.8 µm) and fibre wall thickness (5 µm) in *C. equisetifolia* in Egypt. According to Mahmood (1993), the average fibre length of *C. equisetifolia* grown in Pakistan was 978.28±48.2 µm, fibre diameter 14.81±4.9 µm and wall thickness 6.95±2.8 µm. The average fibre length of *C. equisetifolia* grown in India was 1.08 mm and mean average diameter as 11.0 µm (Guha and Madan, 1963). A comparison of the values obtained in the present investigation with the works referred above showed that the mean values of fibre parameters of any clone were higher than what had been reported for trees by others.

The clone effect on the examined fibre parameters was highly significant except for fibre lumen width. The clone effect was reflected in differences in means among clones for all fibre morphological parameters. For example, clone 4 showed the highest fibre length, whereas clone 61 showed the highest fibre diameter. Fibre lumen width and fibre length was the lowest for clone 45. Similar to the present study, Chauhan et al. (2001) reported that inter clonal differences were significant in *Populus deltoides* for fibre dimensions. Sreevani and Rao (2013) found that fibre length, fibre lumen diameter and fibre wall thickness significantly differed among clones of *E. tereticornis* whereas fibre diameter was non-significant.

Table 4. Correlation between wood properties

	SG. AD	SG. FW	SG. OD	FL	FW	FLW	FCWT	RR	SR	RCO	FCO	SF
SG.AD	1											
SG.FW	.125**	1										
SG.OD	.485**	.129**	1									
FL	.114**	.018	.052	1								
FW	-.031	.022	.036	.062	1							
FLW	-.003	-.002	.014	.086*	.481**	1						
FCWT	-.025	.035	.031	.036	.503**	-.157**	1					
RR	.042	.021	.073	-.006	-.232**	-.609**	.584**	1				
SR	.103**	-.002	.008	.599**	-.718**	-.302**	-.380**	.162**	1			
RCO	-.023	.021	.007	-.020	-.194**	-.538**	.738**	.869**	.118**	1		
FCO	.007	-.014	-.004	.078*	.234**	.955**	-.378**	-.690**	-.124**	-.614**	1	
SF	.010	.030	.016	-.053	-.269**	-.871**	.505**	.829**	.162**	.785**	-.932**	1

SG.AD - Specific gravity (air dried); SG.FW - Specific gravity (fresh weight); SG.OD - Specific gravity (oven dried); FL - Fibre length; FW - Fibre width; FLW - Fibre lumen; width FCWT - Fibre wall thickness; RR - Runkel ratio; SR - Slenderness ratio; RCO - Rigidity coefficient; FCO - Flexibility coefficient; SF - Shape factor

Among various ratios and factors studied i.e. Runkel ratio, slenderness ratio, rigidity coefficient, flexibility coefficient and shape factor, only slenderness ratio showed significant inter clonal variation. Mean Runkel ratio ranged from 1.159 (clone 74) to 2.830 (clone 69); slenderness ratio from 97.716 (clone 45) to 161.72 (clone 4); rigidity coefficient from 51.309 (clone 4) to 72.532 (clone 69); flexibility coefficient from 54.161 (clone 69) to 95.421 (clone 74); and shape factor from 0.638 (clone 74) to 0.860 (clone 69) (Table 2). The variations in fibre morphological parameters resulted in subsequent variation in fibre derived ratios and factors of clones.

3.3 Vessel and ray characteristics

All the vessel and ray parameters showed significant inter-clonal variation. Among the 46 accessions, average ray height ranged from 363.25 µm (clone 68) to 591.5 µm (clone 1); ray width ranged from 25.5 μm^2 (clone 33) to 44.5 µm (clone 48); ray frequency from 10 (clone 44) to 23 (clone 86) (Table 3); vessel length from 352.65 µm (clone 62) to 546.59 µm (clone 45); vessel area from 12037 μm^2 (clone 3) to 36470 μm^2 (clone 62); vessel diameter from 114.25 µm (clone 3) to 176µm (clone 85); and vessel frequency from 5 (clone 55 and clone 61) to 13 (clone 11) (Table 3). As mentioned earlier, similarity of site to their origin of site and genetic factors are the driving factors for the variations in vessel morphology among clones. Significant variations in vessel frequency, vessel element diameter and vessel length was reported by Shashikala and Rao (2005) among the clones of *E. tereticornis*. Similarly, Ramirez et al. (2009) reported wide range of variation in 7-year-old *E. globulus* clones for vessel frequency, vessel area and vessel coverage among the clones. Huda et al. (2012) reported significant difference in ray proportion among poplar clones. Lack of literature related to inter clonal variation in ray morphology limited the discussion of the same.

3.4 Chemical properties: Cellulose and lignin content

Student t-test was done for analysing the variation in cellulose and lignin content of the clones (only the top 15 clones were taken for lignin analysis). Results showed significant differences among clones in cellulose and lignin contents. Cellulose per cent ranged from 41.6% for clone 56 to 58.8% for clone 1. Average cellulose content of all the clones pooled together was 46.4%. Lignin per cent among clones ranged from 21.65% for clone 56 to 29.35% for clone 51. Average lignin content of the top 15 clones pooled together was 26.3 %. A study by Parthiban et al. (2011) on pulpwood characterization of 25 Casuarina hybrid (*C. junghuhniana* x *C. equisetifolia*) clones showed that the lignin content ranged from 24.2 to 27.8 per cent among clones.

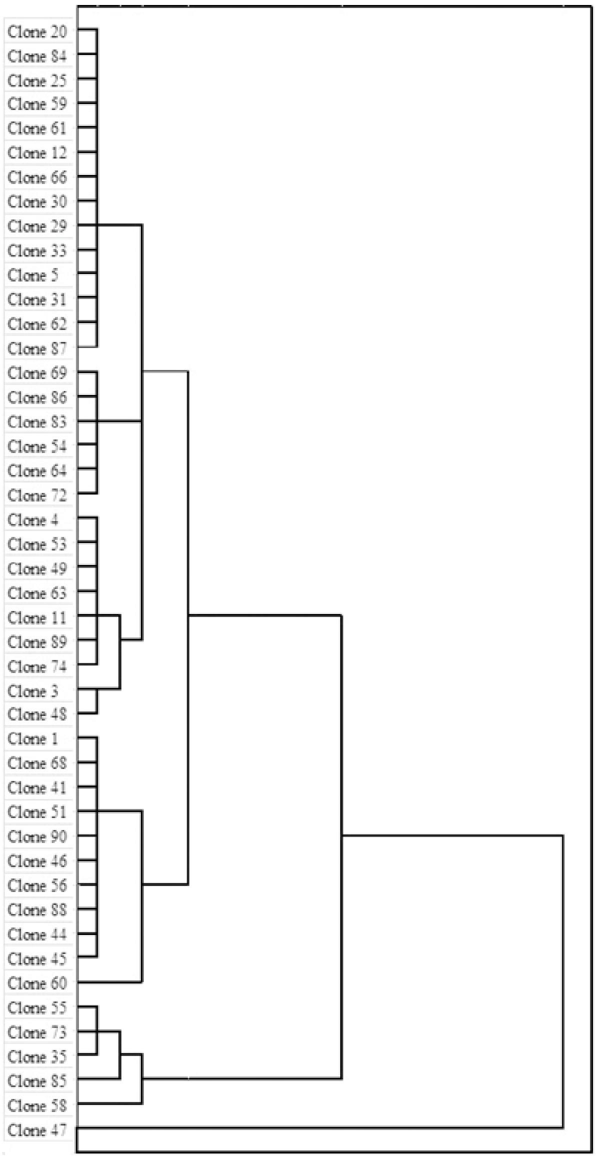
3.5 Inter relationship between wood properties

Table 4 gives correlation coefficient between different wood properties of 46 clones. Oven dry specific gravity and air dry specific gravity were positively related. Scientifically, these two density parameters differ only because of the change in moisture content. Fibre length was positively related to fibre cell wall thickness and was negatively related to fresh weight specific gravity and fibre lumen width. Any increase in fibre length contributes to proportionate increase in fibre cell wall thickness, which in turn reduces the fibre lumen width. Increase in lumen width allows wood to hold more water which results in increase in fresh weight of the wood. Fibre cell wall thickness was positively related to air dry specific gravity

Table 5. Origin of clones and seedling materials of *Casuarina equisetifolia*

Clone No.	Clone Code	Origin
1	TNRM 5	Rameswaram, TN
3	TNRM 8	Rameswaram, TN
4	TNPP 1	Pudupattinam, TN
5	TNPP 2	Pudupattinam, TN
11	TNVM 2	Vedaranyam, TN
12	TNVM 3	Vedaranyam, TN
20	TNIPT 16	International provenance trial, Neyveli
25	TN 111	Tamil Nadu Forest Department
29	APKKD 6	Andhra Pradesh
30	APVSP 14	Andhra Pradesh
31	JKCE 8	Odisha
33	TNCS1	Cudallore, TN
35	TNPV 4	Pudukottai, TN
41	APSKLM 25	Andhra Pradesh
44	CE 2003/4	International provenance trial, Puducherry
45	CE 2002/1	International provenance trial, Puducherry
46	CE 2002/2	International provenance trial, Puducherry
47	CE 2003/5	International provenance trial, Puducherry
48	CE 329	Provenance-progeny trial, Karunya
49	CE 2003/3	International provenance trial, Puducherry
50	CE 398	Provenance-progeny trial, Karunya
53	CE 332	Provenance-progeny trial, Karunya
54	CE 268	Provenance-progeny trial, Karunya
55	CE 80	Provenance-progeny trial, Karunya
56	CE 243	Provenance-progeny trial, Karunya
58	CE 327	Provenance-progeny trial, Karunya
59	CE 219	Provenance-progeny trial, Karunya
60	CE 224	Provenance-progeny trial, Karunya
61	CE 9	Provenance-progeny trial, Karunya
62	CE 128	Provenance-progeny trial, Karunya
63	CE 83	Provenance-progeny trial, Karunya
64	CE 303	Provenance-progeny trial, Karunya
66	CE 100	Provenance-progeny trial, Karunya
68	CE 281	Provenance-progeny trial, Karunya
69	CE 276	Provenance-progeny trial, Karunya
72	CH 2803	Chengalpattu, TN
73	CH 0905	Chengalpattu, TN
74	CH 2602	Chengalpattu, TN
83	TCR 060101	Tiruchendur, TN
84	TCR 120102	Tiruchendur, TN
85	TCR 120203	Tiruchendur, TN
86	JKCE 6	Odisha
87	CH 3001	Chengalpattu, TN
88	Seedling Origin	IFGTB CSO
89	Seedling Origin	IFGTB CSO
90	Seedling Origin	IFGTB CSO

Fig. 1. Dendrogram using average linkage (between groups)showing different clusters of clones



and negatively correlated to fibre lumen width. Increase in fibre cell wall thickness increases total biomass of wood that causes increase in the air dry specific gravity of wood. Usually, fibre with high lumen width will have low cell wall thickness. Fibre lumen width and fibre diameter were positively related to each other. Similar pattern of correlation between these variables was observed in a study by Taylor (1973) on *Eucalyptus grandis*. He observed a strong correlation between wall thickness and basic density (r=0.77). Fibre length was positively correlated with fibre diameter and wall thickness. Sreevani and Rao (2013) studied variation in basic density, fibre and vessel morphology of *E. tereticornis* clones and found positive correlation between lumen diameter (r= 0.615) and wall thickness and between fibre diameter (r=0.627) and wall thickness. The various correlations, as found in the present study, were suggestive of complex inter-relationship existing between wood properties in these newly introduced clonal materials.

3.6 Screening of different plus trees on the basis of wood properties and growth

In this study, hierarchical cluster analysis of *C. equisetifolia* clones, considering growth, anatomical, physical and chemical parameters was carried out. The 46 accessions could be grouped in to four clusters (Fig. 1). Cluster 1 was found highly divergent from the other two clusters. Cluster 4 having only one clone (clone 47), showed higher values for majority of growth, anatomical, physical and chemical properties. In the case of pulp and paper making properties (ratios and factors) also, cluster 4 showed acceptable ranges of values compared to others. While considering growth, physical, anatomical and chemical properties, Cluster 4 was followed by cluster 2, cluster 1 and cluster 3 in terms of its suitability for pulp and paper making. Cluster 4 included only clone 47 while cluster 2 included clone 1, clone 41, clone 44, clone 45, clone 46, clone 51, clone 56, clone 60, clone 68, seedling origin 88 and seedling origin 90. Out of these 12 clones, ten clones viz. clone 1, clone 41, clone 44, clone 45, clone 46, clone 47, clone 51, clone 56, clone 60 and clone 68 were listed as superior clones by IFGTB based on the growth performance.

4 Conclusion

All the wood properties showed significant difference between clones except fibre lumen diameter, Runkel ratio, rigidity coefficient, flexibility coefficient and shape factor. Cellulose and lignin content was significant among clones. Air dry specific gravity was positively influenced by fibre length. All the fibre parameters were correlated to each other and strongly influenced all the ratios and factors derived from it. Among four clusters classified based on hierarchical cluster analysis, cluster 4 (one clone) and cluster 2 (11 clones) ranked first and second in terms of superior physical, chemical and anatomical characters. Out of these twelve clones, ten clones exhibited superior growth characteristics as well.

Acknowledgement

Clones used in this study were selected by A. Nicodemus from provenance and progeny trials and R.S.C. Jayaraj, Ashok Kumar and A. Balasubramanian, from local plantations. The authors are thankful to them for making the clones available for the study.

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Variation in Early Growth Among Seed Sources and Clone of Casuarina Tested in North Coastal Andhra Pradesh, India

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Key words: clone, growth, pulpwood, seed orchard unimproved seedlot

Abstract

The BILT Tree Tech Limited (BTTL) is involved in developing farm forestry plantations of pulpwood species including Casuarina. A field test was conducted to demonstrate the superiority of genetically improved seeds of Casuarina from seed orchards in Andhra Pradesh State, India. Two seedlots from seed orchards (one each of *Casuarina equisetifolia* and *C. junghuhniana*), a locally obtained unimproved seedlot and a widely planted commercial clone (*C. junghuhniana x C. equisetifolia* hybrid) were included in the trial. Assessment of one year's growth showed significant differences between the four accessions with *C. junghuhniana* seedlot recording the best growth followed by seed orchard seedlot of *C. equisetifolia*. Deploying seed orchard progeny in the farm forestry plantations of BTTL is expected to increase farm income to farmers and secure pulpwood raw material availability to the Company.

1 Introduction

Casuarina wood is an important pulpwood raw material for papermaking to Ballarpur Industries Limited (BILT) in central India. BILT Tree Tech Limited (BTTL) is the farm forestry arm of BILT, involved in developing plantations of pulpwood species to produce raw material on a sustainable basis since its inception in 1989. BTTL's farm forestry programme is currently operating in several districts in the States of Andhra Pradesh, Chattisgarh, Madhya Pradesh, Maharashtra, Odisha, Telengana and Uttar Pradesh. The Company has planted more than 30,000 hectares of plantations of Acacia, Casuarina, Eucalyptus and Leucaena benefiting more than 38,000 farmers. BTTL produces site-specific high quality planting stock in its state of art nursery facilities and supplies to farmers and follows up with technical assistance in planting and managing of plantations. It also facilitates financial assistance for small and marginal farmers from bank and/or government agencies. BTTL assures market linkage for the wood produced in these plantations with BILT. BILT purchases the pulpwood at a pre-decided support price or the market price whichever is higher at the time of harvest. Hence farmers find BTTL as a dependable organization for their farm forestry needs which resulted in expansion of the Company's operating area.

BTTL has started expanding its Casuarina plantation programme in the State of Andhra Pradesh recently and created assets like seed orchards with clones procured from the Institute of Forest Genetics and Tree Breeding (IFGTB). In order to demonstrate the superiority of seed orchard-produced seeds over the currently used seeds, a gain test plot was established using two seed orchard seedots obtained from IFGTB along with a local unimproved seedlot and the commercially planted clone. The early results obtained from this test is presented in this paper which will be useful to motivate the farmers to use genetically improved planting material.

2 Materials and Methods

Seedlings raised from seed orchard-derived seeds of *C. equisetifolia* and *C. junghuhniana* were obtained from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, India. Seedlings raised from the unimproved locally collected seeds of *C. equisetifolia* and the widely planted hybrid clone (*C. junghuhniana x C. equisetifolia*) were included as benchmark accessions. These four entries were planted in a randomized block design with four replications and 49 trees per accession in each replication at a spacing of 1 x 1 m. The trial was planted in September 2012 at Achutapuram village, Vishakha patanam District, Andhra Pradesh State. All trees were measured for height and diameter growth at the age of one year. The data was subjected to ANOVA and DMRT.

3 Results and Discussion

All four accessions recorded a high survival of 95% and above at one year's age but their growth differed significantly. The best growth was observed for seed orchard progeny of *C. junghuhniana* recording a height of 4.9 m and 4.3 cm diameter followed by *C. equisetifolia* seed orchard progeny with 4.7 m height and 3.69 m diameter (Table 1). Some of the best trees of *C. junghuhniana* recorded 7 m height and 7 cm diameter growth. The commercial clone ranked third and the unimproved seedlot showed the poorest growth of all entries. The height growth of *C. junghuhniana* seedlot was 17 and 48% better than the clone and unimproved seedlot respectively. Similarly the diameter was 32% better than the clone and

Table 1. Survival and growth of Casuarina seed sources and clone at the age of one year in Andhra Pradesh, India.

Seed source / clone	Survival (%)	Height (m)			DBH (cm)		
		Min.	Max.	Mean	Min.	Max.	Mean
C. junghuhniana Seed orchard	97	2.5	7.0	4.9_a	1.3	7.0	4.33_a
C. equisetifolia Seed orchard	98	1.5	6.1	4.7_b	1.6	5.7	3.69_b
Casuarina clone	95	2.2	6.6	4.2_c	1.3	4.5	3.25_c
C. equisetifolia Unimproved seedlot	98	1.1	4.3	3.3_d	1.0	3.8	2.26_d

Mean values subscripted with different letters are significantly different (P<0.01) by Duncan Multiple Range Test.

90% better than the unimproved seedlings. The seed orchard progeny of *C. equisetifolia* also recorded 12 to 63% better height and diameter growth than the clone and local seedling accessions. Growth was highly variable among the trees of unimproved seed source.

Although the results presented here are based on one-year growth, it is still valuable because in India Casuarina is grown in very short rotations of 2 to 4 years. The faster growth of seed orchard-derived seeds of both *C. junghuhniana* and *C. equisetifolia* indicate potential of genetically improved seeds for increasing pulp wood production in Casuarina plantations. *C. junghuhniana* has been reported to be faster growing than *C. equisetifolia* in inland areas of south India (Nicodemus et al., 2005; 2015) which is in agreement with the results obtained in the present study. Since the see orchards were developed with highly adaptable and fast growing provenances and subjected to systematic thinning of inferior trees, their progeny has performed significantly better than the unimproved local seed source as reported previously (Varghese et al., 2011). Use of seed orchard seed in BTTL's farm forestry programme is likely to increase the farm income from plantations to the farmers and secure raw material supply to the Company.

4 Conclusion

Seedlings from seed orchards of *Casuarina equisetifolia* and *C. junghuhniana* are significantly faster growing than commercial clone and local seedlot and have the potential to increase pulp wood production from plantations and farm income to tree farmers.

Acknowledgement

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Mini-cutting Technique for Large-scale Clonal Propagation of *Casuarina junghuhniana*

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Key words: clone, fertigation, hardening, mini-hedges, rooting, sand bed

Abstract

The extent of clonal plantations of Casuarina is fast increasing in south India benefiting both farmers and paper industries with increased wood production. A large number of clonal plants are required every year to establish new plantations. An innovative technique of producing shoot cuttings from mini-hedges planted in raised sand beds inside polyfilm chambers has increased cutting production per unit space and time and also enhanced rooting response compared to conventional field-grown hedges. Providing nutrients through inline drip laterals ensures absorption by the plants resulting in fast growth and increased shoot production. The high level of juvenility in the cuttings ensured rooting response of around 90% without the treatment of root-inducing hormone. Rooting occurred on all sides of the stem and showed tap-root like growth providing anchor to the clonal plants after field planting. The new technique is cost-effective and improved the efficiency of clonal propagation and made high quality planting material affordable to smallholding farmers.

1 Introduction

Although *Casuarina equisetifolia* has been cultivated in India for more than a century, its value increased substantially after the paper industry started using it as a pulpwood raw material. In recent years, *C. junghuhniana* has gained popularity among farmers in south India owing to its many desirable characteristics in terms of growth rate, cultivation practices and marketability. Widely grown in short rotations of 3 to 4 years, it has recorded faster growth than *C. equisetifolia* both in coastal and inland areas (Varghese et al., 2011; Nicodemus et al., 2015). It is also tolerant to drought, not affected by the blister bark disease and possessing coppicing ability (Nicodemus et al., 2003). Since Casuarina wood is an important pulpwood raw material for the Tamil Nadu Newsprint and Papers Limited (TNPL), the Company is actively engaged in expanding its cultivation area through farm forestry programmes and to increase the productivity through various tree improvement activities.

TNPL produces more than two million seedlings and about a million clonal plants of Casuarina for supplying to farmers every year. While genetically improved sources like seed orchard derived seeds are used for raising the seedlings, high-yielding clones are deployed for the clonal forestry programme. The propagation techniques have also been constantly improved to enhance the quality of the plants and to reduce the production cost so that they become affordable to smallholding farmers. One such innovation in clonal propagation is use of cuttings derived from mini-hedges grown indoor under regulated climatic conditions and nutrient supply. This paper describes the techniques involved in rooting of cuttings derived from mini-hedge and compares their advantages with the conventional method involving field-grown hedges.

2 Clones for Mass Propagation

The mini-cutting technique was standardized with two of the most extensively grown clones of Casuarina: Clone CJ 1 is a male hybrid between *C. junghuhniana* x *C. equisetifolia* introduced from Thailand by the Tamil Nadu Forest Department (Thirawat, 1953); clone CJ 9 is a selection from the Noelmina River provenance of *C. junghuhniana* by the Institute of Forest Genetics and Tree Breeding, Coimbatore. These two clones constitute more than 90% of clonal plantations of Casuarina in India.

3 Field-grown Hedges

Under the conventional cutting production method, ortets are planted on the ground at a spacing of 1 m and grown under intensive cultivation to achieve fast growth. Six-month old trees are cut at 15 cm from the base to induce coppice shoot production. One month old coppice shoots are used for clonal propagation. The coppice growth is maintained as hedges for a period of two years after which plants are uprooted and replanted with new ortets.

4 Indoor Mini-hedges

In order to save space and exert control over nutrient supply, stock plants are grown in troughs (3 x 1 x 0.3 m) made of fibre reinforced plastic (FRP). These troughs are filled with gravel for 10 cm height and overlaid with river sand for 20 cm. These troughs are provided with drainage facility to avoid excess water stagnation. The troughs are kept in poly house covered on the top with ultra violet resistant low density polyethylene (UVLDPE) and insect proof nylon mesh on all the four sides. A facility for drip irrigation is provided with four laterals running throughout the length of the trough. The misting facility installed in the poly house about 2 m height over the troughs provides regular misting over the stock plants.

Table1. Fertigation requirement for Casuarina mini-hedges raised in sand bed

Nutrient	Quantity (mg plant^{-1})	Frequency
Di Ammonium Phosphate (DAP)	30	Daily
Urea	50	Daily
Potash	50	Daily
Potassium nitrate	20	Weekly
Potassium sulphate	20	Weekly
Complex (19:19:19)	20	Weekly
Mono ammonium phosphate	20	Weekly
Calcium ammonium nitrate	20	Weekly

Five months old ramets produced from the selected clones are planted in the troughs to serve as stock plants for continuous and large-scale cuttings production at a spacing of 10 cm. Each trough can hold 300 plants at this spacing. Water is provided twice a day through the inline drip laterals and schedule of macro and micronutrients provided to the plants is given in Table 1. Plants were pruned from the base to induce development of new apical shoots. Eight to 10 cm long shoots were collected 10 to 15 days after pruning and used for rooting. The cuttings were treated in Carbendazim (2 g L^{-1}) and planted in 90 cc root trainers containing decomposed coir pith. They were kept in mist chambers maintained with a temperature of 32 to 35 °C and a relative humidity of 85 to 90%. Water spray was given for 45 seconds at 30 minutes' interval. Plants were shifted to hardening chambers providing 50% shade after 35th day and provided with water spray for 15 seconds every two hours for the next 15 days.

After hardening, the plants were shifted to open sunlight and maintained for the next 30 days before supply for field planting. Sprinkle watering was provided for 15 minutes twice a day and each plant was given 0.5 g of NPK 19-19-19 fertilizer. Insect and pathogen attack was prevented through application of Carbendazim or Dithane M45 or Triazophos or Metasystox at 2 g L^{-1}.

5 Clonal and Seasonal Variation in Rooting

The duration required for rooting was found to be the same for both the clones. But clone CJ 9 showed higher rooting response (around 90%) compared to CJ 1 (around 85%). Number of roots and length of root and shoot were also higher for CJ 9 than CJ 1. Rooting response was lower during January to April compared to other months of the year. Rooting of mini-hedge cuttings were higher (80 to 94%) than that of field-grown hedges (45 to 56%) for the clone CJ 1 (Fig. 1). A comparison between the salient aspects of the two system of cutting production is given in Table 2.

The increased rooting response may be attributed to high level of juvenility in the indoor-grown mini-hedges (Titon et al., 2006). Mini-hedge cuttings do not require treatment with root-inducing hormone as reported for Eucalyptus species (Assis, 1992). Cuttings from mini-hedges produced adventitious roots from all around the stem which developed tap-root like growth providing good anchor to the clonal plants for establishment in the field.

6 Conclusion

Shoot cuttings of Casuarina produced from indoor-grown mini-hedges showed high level of rooting response without the treatment of root-inducing hormone. Mini-hedges required less space and produced larger number of cuttings

Fig. 1. Seasonal variation in rooting of shoot cuttings of Casuarina from field and indoor-grown hedges

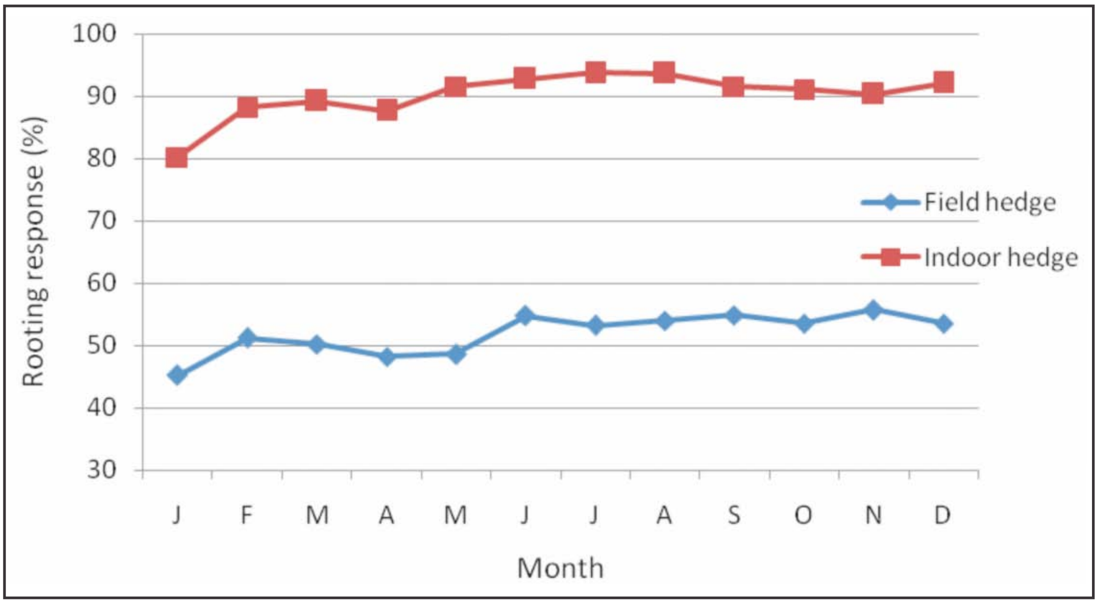

Table 2. Salient features of field-grown and indoor mini-hedges of Casuarina

Description	Field hedge	Mini hedge
Spacing between plants	1 m	10 cm
First harvest after planting	after 6 months	after 3 months
harvesting interval	20 days	10 days
Maintenance cost	High	Low
Shoots production m^{-2} $year^{-1}$	1183	6460
Collection of cuttings	Difficult	Easy
Rooting hormone	Required	Not required
Rooting initiation period	20 days	13 days
Root system	Forming lateral roots in one side of stem which leads to lodging during heavy wind	Forming lateral roots in all sides of the stem like tap root and it helps to withstand during heavy wind
Rooting response	45-56%	80-94%
Nutrient uptake of plants	Poor and influenced by the soil and other environmental factors	Required nutrients are directly provided to the plants

in a shorter period compared to the conventional field-grown hedges. This new technique is cost-effective and substantially increases the quality of clonal planting material making them affordable to smallholding farmers.

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Clonal Variation in Growth, Stem Form and Wood Density among Intra- and Inter-specific Hybrids of *Casuarina equisetifolia* and *C. junghuhniana* in South India

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Abstract

Twenty six intra-and inter-specific hybrid clones of *Casuarina equisetifolia* and *C. junghuhniana* were tested in coastal and inland locations of Tamil Nadu, India for growth, stem form, bark thickness and wood density along with 12 benchmark accessions. Significant differences were found among the clones for all the traits assessed at the age of three years and hybrid clones recorded substantially higher values for these characters than the benchmark accessions in both the locations. No adverse correlation was found between growth and other traits assessed and moderate positive correlation was observed between volume and wood density. Growth and bark thickness were higher in the coastal site compared to the inland site and a reverse trend was observed for wood density. Ranking of clones for growth and wood density differed in the two test locations indicating high levels of genotype-environment interaction for these characters. In general, clones with *C. equisetifolia* as the maternal parent were the fast growing in coastal site and those with *C. junghuhniana* as mother performed better in the inland location. The new clones have the potential to substantially improve wood production in commercial plantations apart from widening the genetic diversity of germplasm pool currently available for Casuarina in India.

1 Introduction

Systematic and long-term breeding programmes for *Casuarina equisetifolia* and *C. junghuhniana* have been implemented in India since 1995. Genetically improved seeds and field-tested clones derived from a broad-based international germplasm of these programmes have been deployed in commercial plantations which substantially increased wood production (Nicodemus et al., 2011a). Large-scale cultivation mostly by farmers and a steady market as pulpwood for paper industries which face acute shortage of wood raw material necessitate that the productivity of Casuarina plantations is further increased through tree improvement. In addition, new varieties with specific combinations of traits for managing insect and disease attack and reduced rainfall greatly influenced by climate change also need to be developed. Extensive field testing of a large number of provenance and landrace accessions of *C. equisetifolia* has shown that no accession possessed all the desirable characters in terms of adaptability, growth and stem form (Pinyopusarerk et al., 2004; Nicodemus et al., 2011a). Earlier studies have also showed that there is no adverse correlation between growth, stem form and wood traits (Pinyopusarerk et al. 1996; Nicodemus et al., 2015) which provides scope to simultaneously select provenances and/or individuals suitable for different end uses and also combine them through hybrid breeding.

All species of the genus *Casuarina* possess the same number of chromosomes (2n=18) and polyploidy is not known to occur (Barlow, 1983). A few of them have been reported to be naturally hybridizing both in natural environment (Wilson and Johnson, 1989) and when brought together under plantations (Badran et al., 1976; Wang et al., 1984). Flowering at a relatively young age (two years in some land races), dioecious sexual system, unisexual flowers which do not need emasculation and vegetative propagation of reproductively mature shoots of selected parents make control pollination of Casuarina easier than many other tropical trees. As a result active hybridization programmes involving different species of *Casuarina* are being implemented in China and India, the largest Casuarina-growing countries (Nicodemus et al., 2011b; Zhang et al., 2014). Since clonal forestry of Casuarina is well-developed in both these countries, the focus is to select the best hybrid clones through multilocation field testing and release them for large scale commercial planting. The present study reports variation in growth, stem form and wood density among intra- and inter-specific clones of *C. equisetifolia* and *C. junghuhniana* tested in coastal and inland sites in Tamil Nadu State of South India.

2 Materials and Methods

2.1 Study material

Twenty six individuals were selected from two hybrid progeny trials in coastal and inland locations of Kerala and Tamil Nadu States. These progeny trials included 35 hybrid families of different combinations involving *C. equisetifolia* and *C. junghuhniana* as parents. More details of these trials

Table 1. Pedigree of hybrid clones and benchmark accessions of Casuarina tested in South India

Clone Number	Clone ID	Parent clones Female	Male
1	CHK-1	CJ12	CE5
2	CHK-2	CJ12	CE5
3	CHK-3	CJ10	Open
4	CHK-5	CE7	Open
5	CHK-6	CE7	Open
6	CHK-7	CJ12	CE5
7	CHK-8	CE11	CJ14
8	CHK-9	CJ12	CE5
9	CHK-10	CJ12	CE5
10	CHK-11	CJ12	CE5
11	CHK-12	CJ12	CE35
12	CHK-13	CJ12	CE5
13	CHK-14	CJ12	CE5
14	CHK-15	CJ12	CE5
15	CHV-2	CJ12	CE35
16	CHV-4	CE7	Open
17	CHV-5	CE26	CJ35
18	CHV-7	CJ12	CJ2
19	CHV-8	CE33	CJ14
20	CHV-11	CE7	Open
21	CHV-12	CE26	CJ35
22	CHV-14	CE26	CJ35
23	CHV-15	CE26	CJ35
24	CHV-16	CE26	CJ35
25	CHV-18	CE26	CJ35
26	CHV-19	CE33	CJ14
27	CHNH	Natural hybrid clone	
28	CJ-8	CJ clone	
29	MTP-1	CE clone	
30	MTP-3	CE clone	
31	CE-102	CE clone	
32	CE-219	CE clone	
33	CE2003/3	CE clone	
34	CE-1	CE clone	
35	CE-2	CE clone	
36	CE-3	CE clone	
37	CE-220	CE clone	
38	--	CJ SSSO Seedling	

CE – *C. equisetifolia*; CJ – *C. junghuhniana*; Open-open pollination

and the results obtained are discussed elsewhere (Nicodemus et al., 2011b). The 26 individuals shortlisted for clonal testing constitute the best 1% of trees selected through an index of height, diameter at breast height (DBH) and stem straightness at the age of 2 years. They were felled at 15 cm from the ground level to produce coppice shoots. One-month old coppice shoots were rooted to produce the required number of ramets for clonal testing. Twelve accessions of seedlots and clones currently under cultivation in South India were included as benchmark to evaluate the hybrid clones. Details of all accessions tested are provided in Table 1.

2.2 Trial design and establishment

Two trials were established in contrasting locations. The coastal trial was located in Chellanchery, Cuddalore District, Tamil Nadu (Lat.: 11^{o} 45'; Long.: 79^{o} 40'; Alt.: 09 m; rainfall: 900 mm; sandy clay loam soil) with 33 accessions and the inland trial in Coimbatore (Lat.: 11o 02'; Long.: 76o53'; Alt.: 411 m; rainfall: 600 mm; red soil) with 35 accessions planted in a randomized complete block design during the year 2010. Each trial had five replications each having 3 trees per accession planted at a spacing of 2 x 1.5 m. Both the trials were planted during monsoon rains adopting standard land preparation and planting methods (Nicodemus, 2009). The coastal trial was provided with irrigation once a week whereas the inland trial received watering once a month.

2.3 Assessment and data analysis

All trees were assessed for growth (height and diameter at breast height - DBH) and stem straightness annually up to the third year. Wood samples collected from trees felled at the age of 3 years were used to determine wood density through water displacement method. Thickness of the bark was measured at a height of 1.3 m from ground level using a bark gauge. Data were subjected to analysis of variance using Genstat, 15^{th} Edition and by following the methods described in Williams and Matheson (1994).

3 Results

Survival at the age of three years was more than 90% for all accessions in both the locations and is not discussed further. Highly significant (P=0.000 to 0.038) differences in growth, stem straightness, bark thickness and wood density were found among the hybrid and other clonal accessions in both the test locations.

3.1 Clonal variation

In general the hybrid accessions showed substantially higher growth than the benchmark accessions in both the locations (Table 2). Many hybrid clones both in the inland and coastal sites showed better growth than the best benchmark accession. Clone number 9, a progeny of the cross CJ 12 x CE5 recorded the best growth in inland site with 14.2 m height and 7.43 cm DBH and a full score of 4 for stem straightness at the age of three years. At the coastal site clone number 22 (CE 26 X CJ 35) showed the site's best growth of 12.3 m height and 9.45 cm DBH with straight

Table 2. Growth and stem straightness of Casuarina hybrid clones and benchmark accessions tested in South India at 3 years age

Clone Number	Coimbatore (Inland) Height (m)	DBH (cm)	Volume index	Stem St'ness	Chellancheri (Costal) Height (m)	DBH (cm)	Volume index	Stem St'ness
1	11.3	6.68	506	4	10.7	6.84	500	4
2	12.5	6.05	457	4	12.3	8.26	898	4
3	11.8	5.09	307	4	12.1	8.92	955	2
4	10.7	5.62	337	4	11.7	8.01	748	3.3
5	9.7	4.56	201	4	11.3	7.85	699	3.7
6	12.3	5.83	420	4	10.3	6.58	447	4
7	8.8	4.67	192	3				
8	11.3	5.83	386	4	10.5	7.32	563	4
9	14.2	7.43	781	4				
10	12.1	5.73	394	3.3	10.1	6.31	399	4
11	9.5	5.52	289	4	11.2	6.86	528	4
12	11.7	4.77	266	4	12.1	8.81	938	4
13	12.1	5.62	379	3.7	12.8	8.71	972	3.7
14	12.3	6.68	551	4	12.2	8.65	911	4
15	9.1	5.21	243	3.3	11.3	7.75	681	4
16	9.7	5.52	294	4	12.7	8.71	960	4
17	10.7	5.73	350	3.7	11.8	6.35	476	4
18	9.7	5.31	272	3	11.6	7.71	721	4
19	9.7	5.31	272	4	11.5	7.82	759	4
20	10.1	5.83	340	4	10.8	7.22	561	4
21	10.7	5.41	312	4				
22	8.7	4.67	189	3.7	12.3	9.45	1101	4
23	11.1	5.31	310	3.7	11.1	7.81	675	3.7
24	10.3	4.77	236	4	8.1	5.15	212	4
25	10.5	5.52	320	4	11.5	7.27	608	4
26	11.3	5.83	386	4	10.2	6.95	555	3.9
Mean	10.8	5.55	344	3.81	11.3	7.55	669	3.8
27	7.80	4.03	178.7	4.0	10.82	7.47	673	3.9
28					9.91	6.37	445	3.9
29	8.93	4.93	244.7	3.7	8.95	5.66	360	3.8
30	6.33	3.40	86.0	3.7	9.61	6.49	455	3.8
31	8.27	4.77	286.6	4.0				
32	10.21	4.78	261.5	3.4	10.40	7.40	666	4.0
33	9.67	5.02	255.0	3.6	10.00	7.42	664	3.6
34					9.88	6.86	543	3.1
35	8.29	4.29	165.9	4.0				
36	9.70	5.10	299.1	4.0	9.30	6.32	421	4.0
37					10.38	7.11	569	3.6
38	9.90	4.78	259.4	4.0	10.36	7.45	728	3.8
Mean	8.79	4.57	226.33	3.8	9.96	6.86	552	3.8
Overall mean	10.24	5.27	310.1	3.8	10.77	7.27	655	3.8
LSD (clone)	2.24	1.37	231.2	0.45	3.9	2.2	499.6	0.7
P	0.00	0.00	0.00	0.00	0.038	0.001	0.014	0.00

Table 3. Bark thickness and wood density of Casuarina hybrid clones and benchmark accessions tested in South India at 3 years age.

Clone Number	Coimbatore (Inland) Bark t'kness (mm)	Coimbatore (Inland) Wood density (g cm^{-3})	Chellancheri (Coastal) Bark t'kness (mm)	Chellancheri (Coastal) Wood density (g cm^{-3})
1	2	0.727	2.3	0.619
2	2	0.745		
3	2.7	0.749	4	0.646
4	1.8	0.737	2.7	0.633
5	1.3	0.721	3.7	0.636
6	2	0.676	4	0.61
7	2	0.636		
8	1.7	0.678	3	0.596
9	2.3	0.729	3.7	0.569
10	2.3	0.719	4	0.645
11	1.7	0.717	4.3	0.639
12	1.3	0.713	2.7	0.635
13	2	0.744	3.3	0.602
14	3	0.714	3.3	0.638
15	2.7	0.663	3.7	0.645
16	2.7	0.639	3	0.641
17	2.3	0.691	3.3	0.663
18	2	0.657		
19	2.3	0.674		
20	2.8	0.691	4	0.628
21	2.3	0.765		
22	2	0.646	2.7	0.671
23	2.3	0.637	3	0.624
24	2	0.664	2.3	0.659
25	2	0.704	3.3	0.637
26	1.7	0.68	3.0	0.60
Mean	2.1	0.697	3.32	0.632
27			4	0.646
28			3	0.668
29	1	0.674	3	0.641
30			2.3	0.654
32	2	0.723	3	0.628
38			4.3	0.686
Mean	1.7	0.698	3.27	0.651
Overall mean	2.1	0.697	3.3	0.637
LSD (clone)	0.8	0.11	1.0	0.07
P	0.000	0.000	0.001	0.026

stems.

Bark thickness and wood density varied significantly (P=0.000 to 0.023) among the accessions in both the test locations. Hybrid clones showed slightly thicker bark (inland: 2.1 mm; costal: 3.32 mm) compared to the benchmark entries (inland: 1.5 mm; coastal: 3.3 mm) in both the trials (Table 3). The mean wood density of hybrid and benchmark accessions was similar in the inland site but

Table 4. Pearson correlation values between different traits of Casuarina hybrid clones tested in inland (above diagonal) and coastal (below diagonal) and between coastal and inland locations (along the diagonal, underlined) at three years age

Trait	HT	DBH	VOL	SST	BT	WD
HT	0.268	0.745	0.845	0.134	0.412	0.278
DBH	0.867	0.152	0.946	0.147	0.469	0.155
VOL	0.900	0.966	0.059	0.170	0.450	0.204
SST	-0.132	-0.302	-0.234	-0.102	-0.108	0.247
BT	0.153	0.061	0.086	-0.155	0.126	0.050
WD	0.082	0.131	0.095	-0.037	0.065	-0.008

higher for the latter in the coastal site. But only two of the five benchmark entries were studied for wood density due to small size of the trees of other entries (Table 2). Clones that showed the fastest growth also possessed wood density similar to or higher than the site mean. Fifty per cent of the clones in the inland site showed a wood density of above 0.7 g cm^{-3} and six clones in the coastal site showed wood density higher than 0.64 g cm^{-3}. There was no adverse correlation between the growth and other characters studied except stem straightness (Table 4). In particular, growth and wood density showed positive and moderate correlation.

3.2 Across-site variation

Between the two test locations, growth and bark thickness was substantially higher in the coastal site as revealed by the mean values for different characters studied (Table 2). Contrastingly wood density was more in the inland site (0.697 g cm^{-3}) compared to that of the coastal site (0.637 g cm^{-3}; Table 3). Ranking of clones was found varying across the locations for growth, bark thickness and wood density. Some of the best clones in one location recorded below average growth in the other location. This interaction between clones and site is further supported by low levels of correlation for different traits across the two locations (Table 4).

4 Discussion

The fast growth and desirable levels of stem form and wood density shown by the hybrid clones indicate their potential to substantially increase wood production and their suitability for end products like paper and poles. Earlier studies with the interspecific hybrid families showed them possessing desirable combination of parental characters like early adventitious rooting and resistance to blister bark disease apart from fast growth (Kannan et al., 2012; Nicodemus et al., 2013). These characters of the hybrids help in selecting hybrid clones with a desirable combination of characters suitable for a given end use and planting environment resulting in higher plantation productivity than the parent species. A simple and cost-effective clonal propagation technology available for Casuarina facilitates mass multiplication of the best clones and large-scale deployment in commercial plantations.

The differences in the performance of various hybrid clones in the coastal and inland locations indicate presence of high levels of G x E interactions. Hybrids having *C. equisetifolia* as the maternal parent performed better in coastal site and those with *C. junghuhniana* has the female parent were better adapted to the inland site as reported for the hybrid progeny tests (Nicodemus et al., 2011b). Adequate multilocation testing is a prerequisite for the commercial exploitation of hybrid vigour observed for different characters of economic importance. Particularly the coast-to-inland trend reported for growth and wood characters of Casuarina has to be considered for choosing clones for planting in different sites (Varghese and Sivaramakrishna, 1996).

In India, clonal forestry of Casuarina is in its early stages and involves a limited number of clones. A major risk involved in expanding the clonal plantations is the treat of blister bark disease caused by *Trichosporium vesiculosum*. Since there is no effective control measure reported for this disease, it can cause severe damage to the clonal plantations with low levels of genetic diversity. The availability of interspecific hybrid clones involving the disease resistance species, *C. junghuhniana* help in widening the diversity of clones available for clonal plantations with reduced risk of disease incidence. The principal end uses of Casuarina wood in India are pulpwood raw material for papermaking and poles for rural construction and scaffolding. While the stem straightness of the new selections has been found at desirable levels for its suitability as poles, their wood properties particularly the pulping characters have to be studied further to identify the most suitable accessions for pulpwood plantations.

5 Conclusion

Interspecific hybrid clones of *C. equisetifolia* and *C. junghuhniana* showed faster growth and desirable levels of stem straightness, bark thickness and wood density compared to various benchmark accessions currently used for planting in Tamil Nadu State of south India. Their deployment in clonal plantations will benefit the tree farmers through substantial increase in wood production and farm income. More field testing to improve the efficiency of clone-site matching and study of pulp wood properties for end use matching is essential for maximum exploitation of the potential of the new hybrid clones.

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G x E Interaction in Clones of *Casuarina equisetifolia*

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Key words: genotype, growth, multilocation testing, regression, stability

Abstract

Eighty-seven clones of *Casuarina equisetifolia* were subjected to stability analysis to understand the GxE interaction. Multilocational field tests were conducted in three locations in Tamil Nadu for this purpose. Stability analysis was performed using the data on tree height, diameter at breast height (DBH) and volume index obtained at 2.5 years of age. A high yielding genotype with unit regression coefficient (bi=1) and the deviation from regression not significantly different from zero (s^{-2}di=0) was considered as the stable one. Clones which possessed high mean (general mean + two SE) only were considered for classification and characterization for adaptability. When the data for tree height, DBH and volume index were subjected to stability analysis, the variance due to clone x growth period interaction was significant for all these characters. Therefore, further analyses were carried out for these characters and stability parameters worked out. Clones CH 3001 and CE 2003/5 were included in group I (bi around unity, s^{-2}di around 0) when stability parameters for tree height was considered and hence are the most stable clones with respect to tree height. Clones CE 224 and TNVM 3 though recorded high mean values, were found unpredictable over growth periods due to the significant deviation from regression. Clones CE 268, CE 224 and CE 2003/5 exhibited stability for DBH. Five clones namely, CE 220, CH 3001, CE 243, CE 224 and CE 2003/5 proved to be stable across the three locations with respect to volume index. Clones CE 243, CE 9 and TNPP 1 were found suitable for planting in sites with stress or favourable conditions. They were placed in group II (bi significantly deviating from unity, s^{-2}di around 0) when analyzed for DBH or volume index.

1 Introduction

A special concern in tree improvement and genetic testing relates to genotype x environment interaction which means that the relative performance of clones, families, provenances or species differs when they are grown in different environments. Because of the possible presence of G x E interaction, it is always advisable that genetic tests be established in multiple environments (Zobel and Talbert, 1984). In tree improvement, superior performing genotypes in a range of environments are highly desired (Hanson, 1970). Though stability analyses have widely been conducted in agricultural crops, only limited studies have been reported in tree species. The importance of selecting stable genotypes by testing the available selections at different growth phases, fertility regimes or locations have been highlighted by several researchers (Ataga, 1993; Ades and Garnier-Gere, 1996; Song and Zhang, 1997; Larochelle et al., 1998; Sonesson and Eriksson, 2000; Warrier and singh, 2011) as G x E interaction can reduce genetic gains or make a tree breeding programme more complex and expensive.

The Institute of Forest Genetics and Tree Breeding, Coimbatore has identified and assembled a large number of clonal accessions of *Casuarina equisetifolia* over the past two decades and is a focal point for Casuarina research in India. A clone bank consisting of 275 accessions of *C. equisetifolia* selected from various parts of the country is in place at present (Latitude 11° 01' N, Longitude 77° 02' E). Eighty-seven clones selected from this clone bank were subjected to stability analysis.

2 Materials and Methods

Multilocational field tests were conducted with 87 select clones and three seed raised progeny in three locations in Tamil Nadu namely, Mayiladumparai, Karur district (Latitude: 10° 52.080' N and Longitude: 78° 27.376' E, inland red soil), Moorthipalayam, Karur district (Latitude: 11° 01.933' N and Longitude: 78° 01.121' E, Sodic soil) and Sirugramam, Cuddalore district (Latitude: 11° 47.390' N and Longitude: 79° 27.339' E, inland casuarina growing region).

Stability analysis was performed using the data on tree height, diameter at breast height (DBH) and volume index obtained at 2.5 years of age. The analysis was carried out following the method suggested by Eberhart and Russel (1966). Considering Y_{ij} as the mean observation of i^{th} clone in j^{th} environment, the stability model described by them is as follows

$Y_{ij} = m + B_i I_j + \delta_{ij}$ (i = 1,2……t and j = 1,2, …..s)

(t = clones; s = growth periods)

where,

Y_{ij} = Mean of i^{th} clone in j^{th} growth period

m = Mean of all the varieties

b_i = Regression coefficient of the i^{th} clone on the environmental index which measures the response of the clone to varying growth periods

I_j = The environmental index which is defined as the deviation of the mean of all the clones in j^{th} growth period from the overall mean

$$= \frac{\sum_i Y_{ij}}{t} - \frac{\sum_i \sum_j Y_{ij}}{ts} \quad \text{with } \sum_j I_j = 0$$

and δ_{ij} = Deviation from regression of the i^{th} clone at j^{th} growth period

Two parameters of stability were calculated namely, (a) regression coefficient which is the regression of the performance of each clone under different growth periods on the growth period means and (b) mean square deviations from linear regression.

The regression coefficient is estimated as

$$bi = \sum_j Y_{ij} I_j / \sum_j I_j^2$$

where,

$\sum_j Y_{ij} I_j$ is the sum of products and

$\sum_j I_j^2$ is the sum of squares

Mean square deviations (Sd^{-2}) from linear regression is estimated as

$$\frac{\sum_j \delta^2_{ij}}{(s-2)} - \frac{S^2_e}{r}$$

where,

$$\sum \delta^2_{ij} = \left[\sum_j Y^2_{ij} - \frac{Y^2_i}{t} \right] - \frac{(\sum_j Y_{ij} I_j)^2}{\sum_j I^2_j}$$

and S^2_e = Estimate of pooled error

3 Results and Discussion

3.1 Pooled ANOVA

The results of the pooled ANOVA for the three characters studied are presented in Table 1. The sources of variation namely, clone, environment, clone x environment, environment + (clone x environment) and environment (linear) were significant at 5 per cent level for tree height, DBH and volume index.

3.2 Mean performance and stability parameters

The mean performance and stability parameters were worked out (Data not shown).

3.2.1 Tree height

The means for tree height ranged from 5.6 m to 9 m. Clone TNCN 1 recorded the minimum value whereas clone TNVM 3 showed the maximum value. The grand mean over the three locations was 7.2 m and 40 clones registered higher values than the grand mean.

The regression coefficients (bi) varied between 0.07 and 1.62. All the 'bi' values were found to be around unity.

The deviation from regression ($s^{-2}di$) values ranged from -0.293 to 3.119. The $s^{-2}di$ values for 11 clones were not significantly different from zero.

3.2.2 Diameter at breast height

The grand mean for DBH was 4.3 cm over the three locations and the means ranged from 3.1 cm (TNCN 1) to 5.8 cm (CE 2003/5). Forty-three clones recorded higher values than grand mean.

The 'bi' values ranged from 0.232 (CE 9) to 1.566 (CE 2003/4). Except five clones namely, TNPP 1, CE 243, CE 9, CH 2602 and TCR 120203 all others registered unit regression coefficient.

Clone CH 2803 recorded the minimum value (-0.213) for deviation from regression ($s^{-2}di$) whereas, the maximum value (1.299) was registered by clone CE 327. Seventy-seven clones recorded $s^{-2}di$ values around zero.

3.2.3 Volume index

The means of volume index varied between 6836.35 and 32527.75 cm^3 with a grand mean of 17082.68 cm^3.Clone TNIPT 5 recorded the minimum value whereas, clone CE 2003/5registered the maximum mean value for volume index. Forty-one clones registered higher values than the grand mean.

The values for regression coefficient (bi) varied from 0.27 (CE 88) to 1.76 (CE 243) and all the clones except three

Group	Mean	Regression Coefficient 'bi'	Deviation from Regression '$s^{-2}di$'
I	High	Around unity	Around zero
II	High	Significantly deviating from unity	Around zero
III	High	Significantly deviating from unity	Significantly deviating from zero
IV	High	Around unity	Significantly deviating from zero

Table 1. Pooled analysis of variance for phenotypic stability

Source	DF	Tree Height	DBH	Volume Index
Clone	89	1.247*	0.955*	87009762.418*
Environment	2	180.069*	149.077*	10918451120.622*
Clone x Environment	178	0.417*	0.272*	29226506.891*
Environment + (Clone x Environment)	180	2.413*	1.925*	150217892.3370*
Environment (Linear)	1	360.138*	298.159*	21836867040.000*
Clone x Environment (Linear)	89	0.339	0.230	33158667.865
Pooled Deviation	90	0.490	0.309	25013581.113
Pooled Error	810	0.293	0.213	2044867.000

* Significant at 5 per cent level

recorded values around unity. They were TNPP 1, TN 111 and TCR 060101.

The s^{-2}di values for 84 clones were found around zero. The minimum and maximum values were registered by clones CP 0401 and CE 327 respectively.

In the present study, stability parameters were estimated using the model proposed by Eberhart and Russel (1966). According to them, a high yielding genotype with unit regression coefficient (bi=1) and the deviation from regression not significantly different from zero (s^{-2}di=0) is considered as the stable one. Clones which possessed high mean (general mean + two SE) only were considered for classification and characterization for adaptability (Cavalcanti and Gurgel, 1973). The high-yielding clones were further classified into four groups following the methodology suggested by Mehra and Ramanujam (1979).

Clones in group I will be highly stable over the growth phases. An above or below average response could be expected from clones falling in group II and they will be suited for stress or favourable growth phases. Groups III and IV may be ignored as the behaviour of the clones falling in these groups will be unpredictable. When the data for tree height, DBH and volume index recorded from three locations at 2.5 years of age were subjected to stability analysis, the variance due to clone x growth period interaction was found significant for all these characters. Therefore, further analyses were carried out for these characters and stability parameters worked out.

Clones CH 3001 and CE 2003/5 were included in group I when stability parameters for tree height was considered and hence are the most stable clones with respect to tree height (Table 2). Two clones (CE 224 and TNVM 3) though recorded high mean values, were found unpredictable over growth periods due to the significant deviation from regression. Clones CE 268, CE 224 and CE 2003/5 exhibited stability for DBH. Five clones namely, CE 220, CH 3001, CE 243, CE 224 and CE 2003/5 proved to be stable across the three locations with respect to volume index. Clones CE 243, CE 9 and TNPP 1 were found suitable for planting in sites with stress or favourable conditions. They were placed in group II when analyzed for DBH or volume index. All the three seed raised progenies exhibited their stability for DBH and volume index and were placed in Group I.

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Table 2. Grouping of clones based on stability parameters

Characters	Group I	Group II	Group III	Group IV
Tree Height	CH 3001 CE 2003/5	Nil	Nil TNVM 3	CE 224
DBH	CE 268 CE 224 CE 2003/5	CE 243 CE 9 TNPP 1	Nil Nil	 Nil
Volume Index	CE 220 CH 3001 CE 243 CE 224 CE 2003/5	TNPP 1		

All the three seed raised progenies exhibited their stability for DBH and volume index and were placed in Group I.

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Developing Interspecific Hybrids Involving Three Species of *Casuarina*

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Key words: control pollination, compatibility, growth, provenance, tree form, variation

Abstract

Control pollination experiments were conducted involving three species of *Casuarina* viz., *C. equisetifolia, C. junghuhniana* and *C. cristata* to determine the crossability between them and to produce desirable cross combinations. Parent accessions for the crossing programme were selected from the second generation progeny trials for *C. equisetifolia* and *C. junghuhniana* and in a species-provenance trial for *C. cristata* based on growth and stem straightness. The initial fruit set for different cross combinations ranged from 14.4 to 76.6% but reduced to 2 to 57% when the fruits matured five months after pollination. Interspecific crosses involving *C. cristata* resulted in fruitset only when the species was used as the male parent due to differences in the size of style and pollen tube across species. Viable seed set ranging from 75 to 94% and seed germination from 3.6 to 91% were obtained for different crosses indicating compatibility among the three species and the possibility to produce site and end use specific hybrids.

1 Introduction

Casuarina is one of the four genera of the family Casuarinaceae which includes all the widely cultivated members of the family in the tropics and subtropics. In India, *Casuarina equisetifolia* was introduced during the late 19th century and grown extensively for fuel wood, poles, charcoal and pulp wood. Casuarina trees are also commonly planted in windbreaks, agroforestry systems and soil rehabilitation programmes. Apart from *C. equisetifolia*, the other significant introductions into India include a male hybrid clone of *C. junghuhniana* x *C. equisetifolia* from Thailand during 1951 (Thirawat, 1953; Pinyopusarerk and House, 1993) and *C. junghuhniana* provenances from Timor and Wetar regions of Indonesia (Nicodemus et al., 2005). These taxa are currently under cultivation in Andhra Pradesh and Tamil Nadu States. Reports on introduction of other species of *Casuarina* into India are not many and only limited information is available on their performance. *C. cunninghamiana* was introduced in the western Karnataka and Maharashtra States but was not found to be superior to local *C. equisetifolia* (Amanulla et al., 2001). All species of *Casuarina* possess the same chromosome number (2n=18) and reported to be hybridizing both in natural and introduced environments (Badran et al., 1976; Wang et al., 1984; Wilson and Johnson, 1989). Interspecific hybrids of *C. equisetifolia* and *C. junghuhniana* have shown faster growth and better stem straightness and disease resistance than the parent species in South India (Nicodemus et al., 2011). Since India has diverse environments in terms of rainfall, soil type and agricultural systems and plantations are raised for a range of products and services, hybrids combining desirable traits across provenances and species will have the potential to increase productivity and value of plantations. The present study was undertaken with the objective of conducting control pollination experiments involving three species of *Casuairna*, viz. *C. equisetifolia, C. cristata* and *C. junghuhniana* and to determine the most compatible combination(s) of species.

2 Materials and Methods

2.1 Tree selection and pollen collection

The parent trees of *C. equisetifolia* and *C. junghuhniana* were selected from 4-year old second generation progeny trials located at Karur and Salem in Tamil Nadu State, India respectively. In the case of *C. cunninghamiana* and *C. cristata*, parent accessions were selected in a species-provenance trial located at Karunya, Tamil Nadu. Details of selected species and clones are given in Table 1 and the salient morphological and desirable end-use characters are listed in Table 2. The control pollination experiments were carried out in two locations: a species-provenance trial established in 2002 at Karunya, Coimbatore, India (Lat.: 11° 00' N; Long.: 76° 58' E; Alt.: 300 masl) and a clone bank planted during 2000 in Forest Campus, Coimbatore (Lat.: 11° 01' N; Long.: 96° 93' E; Alt.: 410 masl). Male inflorescences were harvested from the selected trees at least one hour before anthesis (around 06.00 hours) in clean Petri dishes and brought to the lab. The dishes were incubated at 35 °C for one hour and the pollen that was shed in the dishes were harvested and passed through 25µm sieve to remove anther lobes and other debris and collected in Eppendorf tubes and stored at 4 °C.

2.2 Controlled pollination

Secondary branches with female inflorescences were covered with pollination bags three days before anthesis when flower buds start appearing pinkish in colour. Needles in the branch were trimmed to facilitate pollen application and to avoid rubbing of needles to the flowers. A

Table 1. Details of parent trees of *Casuarina* species selected for involving in control pollination experiments

Clone ID	Location	Sex	Height (m)	DBH (cm)	Stem St'ness
C. equisetifolia					
CE18	Second generation progeny trial, Karur, Tamil Nadu	Female	13	9.5	4
CE40		Male	12.5	7.9	4
CE166		Female	12	9.8	4
CE164		Female	13	9.3	4
CE183		Male	12	6.5	4
	Mean of selected trees		12.5	8.6	4
	Overall mean		9.8	6.3	3.5
C. junghuhniana					
CJ23	Second generation progeny trial, Salem, Tamil Nadu	Female	12.5	6.5	3
CJ38		Male	13.5	9.5	3
CJ55		Female	11	9.0	4
CJ85		Female	12.5	7.9	4
CJ89		Male	12.5	10.2	4
	Mean of selected trees		12.4	8.6	3.6
	Overall mean		9.3	5.9	3.1
C. cunninghamiana (Rollingstone provenance; CSIRO Number 13519)					
CU16	Species-provenance trial, Karunya Tamil Nadu	Female	20	16.4	4
Trial mean			15.2	11.00	3.03
C. cristata (Dalby Moonie provenance; CSIRO Number 17757)					
CR18/1	Species-provenance trial, Karunya Tamil Nadu	Male	12	14.8	4
CR18/2		Female	14	15.0	4
	Mean of selected trees		13	14.9	4
	Trial Mean		15.2	11.00	3.03

coiled wire was tied around the branch as scaffolding to prevent of the bag touching the flowers and to facilitate aeration inside the bag. The pollination bag was placed around the wire-scaffolding and tagged with details of the cross. Each branch had 10 to 15 inflorescences and pollen was applied to the fully expanded stigma using a fine hairbrush between 7.00 and 8.00 hours. The bags were retained for the next 2 weeks or until the stigma dried up. One secondary branch in each female parent was tagged and observed for fruit set under open-pollinated conditions. Details of parental combinations used in control pollination experiments are given in Table 3. Control pollination could not be conducted in *C. cunninghamiana* due to lack of flowering during the period of experiment. While no emergence of female inflorescences were observed, male inflorescences developed up to a certain stage but did not matured into functional flowers. Data was subjected to analysis of variance (ANOVA) by using the software GenStat (15^{th} Edition) and following the methods of Williams and Matheson (1994).

3 Results

3.1 Controlled pollination success

The extent of fruit set showed highly significant ($P<0.0001$) variation among different cross combinations ranging from 14.4 to 76.6% at 45 days after pollination and 2 to 57% five months after pollination (Table 3). Among the 17 cross combinations performed involving different species, three crosses involving *C. cristata* resulted in no fruit set. There was a reduction in the overall initial fruit set (31.3%) as they matured five months after pollination (19.7%). The

Table 2. Morphological and desirable end use characters of *Casuarina* species

Species	Morphology	Desirable end use character
C. equisetifolia	Leaf teeth in whorls of 7- 8; bark smooth, brownish in colour	Good stem form, pulping traits
C. junghuhniana	Leaf teeth in whorls of 9 – 12; bark fissured, light grey in colour	Fast growth, drought and disease resistance, coppicing ability
C. cunninghamiana	Leaf teeth in whorls of 8-10; bark dark grey in colour	Good clear bole, narrow crown, disease resistance
C. cristata	Leaf teeth in whorls of 9-16; bark dark brown to blackish in colour	High wood density and fiber dimensions and disease resistance; frost tolerance

highest final fruit set of 56.9% was obtained with the cross CJ23xCJ89. In general, *C. equisetifolia* as a female parent recorded the high percentage of fruit set ranging from 12.2 to 38.3% five months after pollination, followed by *C. junghuhniana* (2 to 25.3%) and *C. cristata* (2.8%). Fruit set was higher in open pollinated inflorescences than controlled pollination in *C. junghuhniana* (46.4%) and *C. cristata* (12.56%), but *C. equisetifolia* recorded lower fruit set (20.80%) under open-pollination than most of the controlled crosses after five months.

Among the interspecific crosses, CE166 crossed with *C. cristata* showed the highest value of fruit set (38.3%) followed by CE166 x CJ 38 (35.9%), CE166 x CJ89 (34.2%), CE18 x CJ38 (23.1%) and CE18 x CJ89 (21.1%). Another accession of *C. equisetifolia* (CE164) crossed with *C. cristata* (CR18) recorded the lowest percentage of fruit set (12.2%). *C. junghuhniana* as a female parent recorded average fruit set for interspecific combinations of *C. junghuhniana* x *C. cristata* (15.9%) and *C. junghuhniana* x *C. equisetifolia* (11.2%). Inter specific cross combination of *C. cristata* (female) with *C. equisetifolia* and *C. junghuhniana* (male) did not result in fruit set.

3.2 Hybrid seed set and germination

The analysis of variance showed highly significant ($P<0.0001$) variation in seed set and germination potential of different crosses (Table 4). Seed set was generally high (>75%) for all crosses. Among the seven inter specific cross combinations, *C. equisetifolia* as maternal parent crossed with *C. junghuhniana* as male parent showed the highest seed set (93.8%) and *C. cristata* as a male parent had the lowest seed set (75.1%) and also had the highest rate of aborted seeds (Table 4). However the interspecific cross CJ85 x CR18 recorded high seed set (84.2%). Aborted seeds either failed to germinate or died within 3 days of germination.

Seeds of different crosses showed high level of variation in germination ranging from 3.6 to 88.7% (mean = 45.2%; Table 4). Among the various combinations, *C. junghuhniana* (CJ85) crossed with *C. cristata* (CR18) had the highest germination (88.7%). Even though the crosses between *C. equisetifolia* and *C. cristata* had the high seed set, the germination was low (22.8 and 23.5%). Germination was also poor (3.6%) for the cross between *C. equisetifolia* (166) and *C. junghuhniana* (38) among the different cross combinations (Table 4).

4 Discussion

The extent of fruit set, seed production and germination obtained with different interspecific cross combinations confirms the possibility of developing hybrids with desirable traits by involving *C. equisetifolia, C. junghuhniana* and *C. cristata*. Each of these species have unique adaptability and end use characters and hybridization through control pollination is the best way to make different combinations of these characters to suit the planting environment and end use requirements. Since vegetative propagation of Casuarina is simple and cost-effective, mass multiplication of selected hybrid individuals and their deployment in commercial plantations can easily be accomplished. Further availability of genetic resources across species is also an insurance to face challenges in the future triggered by events like climate change and outbreaks of pest attack. The difference in fruit set involving different species and clones may be influenced by the efficiency of the hand pollination techniques adopted and the choice of genotypes involved as female and male parents. In the previous studies involving *C. equisetifolia* and *C. junghuhniana*, fruit set obtained through control pollination was quite lower than the open pollination (Nicodemus et al., 2011). It was observed that the pollination success was affected by hand pollination techniques, damage caused to the flowers while applying pollen on the stigma, and high temperature and relative humidity inside the pollination bags. In the present study, instead of paper bags cloth bags were used to facilitate aeration inside the bags and placing of a wire coil inside the pollination bag prevented physical damage to the inflorescences. Zhang et al. (2014) reported a novel approach of using pollination chambers to increase fruit set in control pollination programmes. They adopted a factorial mating design inside closed chambers which functioned like pollination bags and increased fruit set and seed germination from 7 to 89.9% and 8.15 to 51.8% respectively compared to conventional controlled pollination method involving bags.

Selection of suitable species for deploying as maternal and paternal parents is essential to overcome the reproductive

Table 3. Fruit set percentage in different cross combinations of *Casuarina* species

Female	Male	Fruit set (%)	
		45 days	5 months
CE166	CJ38	63.40	35.96
CE166	CJ89	39.53	34.26
CE166	CR18/1	54.40	38.33
CE18	CJ38	29.93	23.16
CE18	CJ89	76.66	21.20
CE164	CR18/1	34.40	12.20
CE166	Open pollination	22.13	20.80
CJ85	CE183	14.43	11.23
CJ85	CJ38	23.63	11.06
CJ85	CR18/1	23.06	15.96
CJ55	CJ38	28.03	25.36
CJ55	CJ89	34.70	17.36
CJ55	Open pollination	52.03	46.40
CJ23	CR18/1	0.00	0.00
CJ23	CJ89	33.20	2.00
CJ23	CJ89	59.86	56.96
CR18/1	CJ38	0.00	0.00
CR18/2	CJ89	0.00	0.00
CR18/1	CR18/2	23.20	2.80
CR18	Open pollination	14.43	12.56
	Mean	31.36	19.77
	LSD	4.967	2.208
	P	<0.0001	<0.0001

barriers that may prohibit creating desirable cross combinations. Crosses involving *C. cristata* as the male parent for crossing with *C. equisetifolia* and *C. junghuhniana* resulted in high percentage of fruit set and seed germination. But when used as female parent with other species as paternal parents, no fruit set was obtained. The structural differences in the female flowers among different species could be a reason for failure in fertilization and fruit set. The size of the *C. cristata* flower was the largest among the four species studied. The structural barrier is reported to be unilateral and is due to the pollen tubes of small-flowered species being unable to grow in the full length of the style of large-flowered species (Potts, 1988; Gore et al., 1990). Such structural barriers prevented fertilization in crosses involving *C. equisetifolia* with large-sized flower as the female parent and *C. junghuhniana* with small-sized flower as male parent but in the reciprocal crosses resulted in fruit and seed set. A similar pattern of crossability barriers was reported in *Eucalyptus* species. Gore et al. (1990) found that *E. globulus*

Table 4. Seed set and seed germination of interspecific crosses of *Casuarina* species

Cross combination		Seed set (%)		
Female	Male	Normal	Aborted	Germination (%)
CE166	CJ38	93.81	3.32	23.08
CE166	CJ89	88.58	23.106	19.16
CE166	CR18	75.086	25.266	23.5
CE18	CJ38	91.56	8.15	3.67
CE18	CJ89	90.11	8.98	10.69
CE164	CR18	76.71	22.22	22.87
CJ85	CJ183	88.58	11.12	91.32
CJ85	CJ38	84.68	14.06	81.19
CJ85	CR18	84.24	14.14	88.73
CJ55	CJ38	84.54	14.25	88.03
Mean		84.48	14.76	45.22
LSD		1.668	0.955	1.222
F	<0.0001	<0.0001	<0.0001	

markedly differed in flower size and the F_1 hybrid can only be produced using the smaller-flowered *E. nitens* as the maternal parent since the style of *E. globulus* being too long for the short pollen tube of *E. nitens* to reach the ovary. In the present study, production of hybrid progeny involving *C. cristata* can only be achieved by using it as the male parent whereas *C. equisetifolia* and *C. junghuhniana* can be used as both female and male parents.

The fruit maturation percentage and seed germination was found low in both control and open pollination five months after of pollination. This reduction shows that different species of *Casuarina* might have post-fertilization barriers occurring during the fruit maturity period as reported for different *Eucalyptus* species (Griffin et al., 1987; Ellis and Sedgley, 1992).

5 Conclusion

Hybridization among different species of *Casuarina, C. equisetifolia, C. junghuhniana* and *C. cristata* is feasible thorough control pollination. Species that have large-sized flowers like *C. cristata* better function as male parents than as maternal parents. Studies on induction of flowering are needed in shy-flowering species like *C. cunninghamiana* to facilitate control pollination in such species. The cross compatibility among different species of *Casuarina* provides scope to produce site and end-use specific hybrids to meet the commercial and livelihood needs.

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Early Growth Evaluation of New Introductions of *Casuarina equisetifolia, C. junghuhniana* and their Hybrids in Odisha State, India

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Key words: seed orchard seed, shelterbelt, productivity, field testing

Abstract

A new set of genetically improved planting material of *Casuarina equisetifolia, C. junghuhniana* and their hybrids were tested in two locations of Odisha State, India for identifying the fast-growing accessions for large-scale planting. A seedlot from the seedling seed orchard located in Karunya, Tamil Nadu State recorded 28% better height growth than the local seedlot. Most of the clones of *C. junghuhniana* and the interspecific hybrids had trees that were 7 to 45% taller than the local seedlot of *C. equisetifolia*. These preliminary results indicate the potential of new planting material for increasing productivity of Casuarina plantations in Odisha State. Systematic multilocation testing of more number of clonal and seedlot accessions drawn from the ongoing breeding programmes is essential to realize their full potential.

1 Introduction

Casuarina equisetifolia is the most preferred tree for establishing shelterbelts along the 480 km long coastline in Odisha State (formerly known as Orissa) which is located on the eastern coast of the Indian peninsula. It was introduced in the State during 1916 mainly to control the movement of sand dunes and to provide a source of fuel wood for the coastal population. Since then the tree has been extensively planted by both government agencies and farmers for it multiple end uses. Odisha coast is also prone to frequent cyclones and Casuarina shelterbelts play an important role in protecting human habitations and agricultural fields. In the recent years paper industries have been promoting Casuarina as a pulpwood species and encourage farmers to grow it as a short rotation crop. Despite the extent of cultivation and the socio-economic and environmental benefits of Casuarina, tree improvement efforts to improve productivity are limited. An international provenance trial was established in Balukhand, Puri District during 1995 and the overall results showed that natural provenances from the South East Asia with better growth than local and other seed sources tested (Pinyopusarerk et al., 2004). Breeding programmes undertaken in the neighbouring States of Andhra Pradesh and Tamil Nadu with similar set of seed sources significantly improved growth and form characteristics. In order to benefit from the output from such programmes, a field testing experiment was taken up involving seed orchard seeds and clones of *C. equisetifolia* and *C. junghuhniana* and their hybrids in two locations in Odisha State.

Table 1. Height growth of clones and seed orchard seedlot of *Casuarina equisetifolia* tested in Odisha State, India at three years age

Accession Number	Source	Height (m)
CE - 1	Clonal Test, Tamil Nadu State	4.57
CE - 2	Clonal Test, Tamil Nadu State	4.72
CE - 3	Clonal Test, Tamil Nadu State	4.16
CE - 4	Clonal Test, Tamil Nadu State	3.89
SSO Seedlot	SSO, Karunya, Tamil Nadu State	5.89
Local Seedlot	Odisha State	4.60
Mean		4.64
LSD		0.831
P		0.004

2 Materials and Methods

2.1 Testing clones and seed orchard seeds of *C. equisetifolia*

Four clones (CE 1 to CE 4) and a seedlot from seed orchard (Karunya, Coimbatore, Tamil Nadu) of *C. equisetifolia* were obtained from the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore for establishing the trial. The local seed source of *C. equisetifolia* was included as a control entry. The trial was established at Chandipur, Balasore District during 2012. Sixteen trees of each accession were planted in four replications at a spacing of 2.5 x 2.5 m using a randomized block design. Height measurement was recorded at the age of three years.

2.2 Testing clones of *C. junghuhniana* and interspecific hybrids

A clonal test was established in Arabandh Reserve Forest, Nilgiri Wildlife Range, Balasore Division with 19 clones and one local seed source of *C. equisetifolia*. They included 10 clones of *C. junghuhniana* and nine clones of interspecific

Table 2. Height growth of clones of *Casuarina junghuhniana* and interspecific hybrids tested in Odisha State, India at two years age

Hybrid Clone	Pedigree		Height (m)
	Male	Female	
CJ 1			3.29
CJ 5			2.61
CJ 10			3.02
CJ 15			3.30
CJ 16			4.14
CJ 18	*C. junghuhniana*		4.11
CJ 19			3.36
CJ20			3.19
CJ23			4.29
CJ 30			3.17
CHV 2	CJ12	CE35	3.94
CHV 4	CE7	Open	3.74
CHV 8	CE33	CJ14	3.93
CHV 19	CE33	CJ14	4.00
CHK 6	CE7	Open	3.79
CHK 7	CJ12	CE5	2.68
CHK 8	CE11	CJ14	3.20
CHK 9	CJ12	CE5	3.32
CHK 11	CJ12	CE5	3.29
Local seedlot	*C. equisetifolia*		2.95
Mean			3.34
LSD			0.721
P			<0.001

hybrids involving *C. equisetifolia* and *C. junghuhniana* selected from coastal (4 clones) and inland (5 clones) locations. The trial was planted in a randomized block design with four replications, each having five plants of each accession planted at a spacing of 2.5 x 2.5 m. Height growth was measured at the age of two years. Data from both the trials were subjected to ANOVA to test the significance of growth differences between the accessions.

3 Results and Discussion

Significant differences were observed between the height growth of different accessions of *C. equisetifolia* ($P=0.004$) *C. junghuhniana* and their hybrids ($P<0.001$) tested in the two locations. At Chandipur, the *C. equisetifolia* seedlot from the Seedling Seed Orchard (SSO), Karunya recorded the best height growth of 5.89 m compared to 4.6 m of the local seedlot (Table 1). Although clone CE 2 showed better growth than the local seedlot it was not significant. The 28% gain in height growth obtained from the SSO seedlot over the local unimproved seedlot shows the potential of genetically improved seed to increase productivity of Casuarina plantations in Odisha. This result is consistent with reports from other parts of India where seed orchard seed is tested with local unimproved seed sources (Nicodemus et al., 2011). Since Casuarina trees attain reproductive maturity at ages from 3 to 5 years, establishing seed orchards with seedling and clonal accessions from ongoing breeding programmes implemented by IFGTB and other agencies will increase wood production in the short term.

Except two clones, all clones of *C. junghuhniana* and hybrids recorded better height growth than the local seedlot of *C. equisetifolia* at the age of two years (Table 2). The range of height growth among the 10 clones *C. junghuhniana* was large from 2.6 to 4.3 m against 3 m of the local seedlot. The best growth was recorded by clone CJ23 which was 45% taller than the local source followed by clones CJ 16 (40%) and CJ 18 (39%). The four hybrid clones from the coastal location showed consistently better performance than the local seedlot with height growth ranging from 3.7 to 4 m and those from the inland location recorded 2.7 to 3.8 m. The average gain in height with the hybrid clones from costal location was 32% over the local seedlot and 36% for the best clone (CHV 19). Despite selected from the inland location, clone CHK 6 recorded 28% better growth than the local seedlot. The better gains in growth of clones compared to the seed orchard seed indicates that productivity of Casuarina plantations in Odisha can substantially be increased through clonal plantations. The growth difference among the clones shows that adequate testing is essential to match the clones with a given planting site. Since the test location is close to the coast, clonal accessions selected from a similar location performed well. Multilocation testing of more number of clones is needed to select the best suited clones for different planting environments in Odisha.

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Isolation, Characterization and Expression Profiling of Glucanase from *Casuarina equisetifolia*

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Key words: abiotic stress; antifungal; PR-2; recombinant protein

Abstract

β-1,3-glucanases (EC 3.2.1.39) hydrolyze β-1,3-glucosidic linkages are found in diverse forms of life including bacteria, fungi, algae and higher plants. Plant β-1,3-glucanases, included under pathogenesis-related (PR) proteins, PR-2 are potential antifungal enzymes. In the present study the gene encoding β-1,3-glucanase was isolated from the needles of *Casuarina equisetifolia* and was designated as *CeGlu1*. The full length coding domain sequence of the gene was 948 bp and encoded for a putative protein with 315 residues and harbored the conserved domain of glycosyl hydrolase. The theoretical pI and molecular weight of the translated product were 8.9 and 34.33 kDa respectively. The expression of *CeGlu1* under biotic (fungal elicitor) and abiotic (heat, salt, wound and drought) stress conditions was analyzed using qRT-PCR. The gene was significantly up-regulated by fungal elicitor, wound and heat stresses while down-regulation of the gene was observed during high salt and drought conditions. Further, *CeGlu1* was cloned into pET-28a vector and expressed in *Escherichia coli*. Antifungal property of the recombinant protein was investigated against *Fusarium verticillioides*. This is the first report on isolation of glucanase gene from *C. equisetifolia*, and the detailed functional analyses of *CeGlu1* will help in understanding its specific role in defence against pathogens in Casuarina.

1 Introduction

Defense related genes are activated in plants in response to pathogen invasion which leads to synthesis of pathogenesis related (PR) proteins. These PR proteins exemplify induced resistance in plants. There are 17 families of PR proteins described till date and plant β-1,3-glucanases are included in the PR-2 family (van Loon and van Strien, 1999; Ferreira et al., 2007). β-1,3-glucanases are broadly categorized into five classes depending on their amino acid sequences, among which three classes of glucanase are pathogen inducible (Jin et al., 1999; Selitrenikoff, 2001). PR-2 proteins have been documented widely from the plant kingdom and in forest tree species, they are reported in poplar, *Jatropha curcas*, *Picea glauca*, *Fagus sylvatica* and *Castanea sativa* (Veluthakkal and Dasgupta, 2010). In plants, apart from their largely recognized role in defense against fungal pathogens, β-1,3-glucanases are also involved in diverse physiological and developmental processes. As a defense protein, β-1,3-glucanase acts against fungal pathogens by hydrolyzing (1,3) β-glucans, which are one of the major structural component of fungal cell wall. The effect is more towards the hyphal apex where the glucans are widely exposed (Selitrenikoff, 2001). It is also envisaged that glucanases have an indirect effect on plant defense through the formation of polysaccharide elicitors that regulate expression of other PR and defense – related genes (Klarzynski et al., 2000). β-1,3-glucanases have been functionally characterized in both plant and bacterial system. The expression of plant glucanase in bacterial system has been documented in sugarcane (Su et al., 2013), rice (Akiyama et al., 2004) and barley (Malehorn et al., 1993).

In the present study, we have isolated the full length β-1,3-glucanase (*CeGlu1*) from needle tissues of fungal elicitor treated *Casuarina equisetifolia*. The heterologous expression of the gene and its expression pattern during biotic and abiotic stress conditions were also elucidated.

2 Materials and Methods

2.1 Plant material and elicitor treatment

Cuttings of *Casuarina equisetifolia* subsp. *equisetifolia* (CSIRO seedlot number 19129 from Lakei Sibur Bako, Malaysia) were collected from the *Casuarina* species trial maintained by the Institute of Forest Genetics and Tree Breeding at Panampally Research Station, Kerala, India. The cuttings were rooted and maintained in the vegetative propagation complex for bioassay studies. The bioassay was conducted as described by Mohan and Manokaran (2001).

2.2 Isolation and sequence analysis of full length β- 1, 3-glucanase gene

Total RNA was isolated from forty eight hours fungal elicitor treated needles using in- house protocol (patent pending) and cDNA was synthesized. Degenerate primers were designed from glucanase sequences of *Fagus sylvatica* (Acc. No. DQ124218) followed by 3' and 5' RACE (Rapid amplification of cDNA ends). All the products were cloned, sequenced and assembled to generate contigs using the sequence assembly program CAP3 (Huang and Madan, 1999).

2.2.1 *In silico* characterization of *CeGlu1*

The sequences were edited and their similarity to existing sequences in database was analyzed by BLASTn and BLASTx (www.ncbi.nlm.nih.gov/blast/Blast.cgi) against the GenBank non-redundant database for nucleotide and translated sequences. The coding domain sequence (CDS) of the gene was analyzed using ORF Finder program (http://www.ncbi.nlm.nih.gov/projects/gorf/orfig.cgi), while the theoretical pI and molecular weight was calculated using the online tool available at http://web.expasy.org/cgi-bin/compute_pi/pi_tool. Protein domains were identified employing Conserved Domain Database (www.ncbi.nlm.nih.gov/cdd) and using InterPro (www.ebi.ac.uk/Tools/pfa/iprscan). Signal peptide prediction was done with SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP).

2.2.2 Phylogenetic analysis

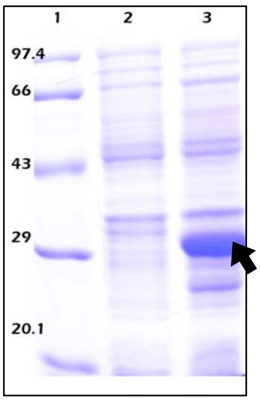
Phylogenetic analysis of the translated product was conducted using the software MEGA 4.1 (Beta 3) version (Kumar et al., 2008). Peptide sequences used for phylogenetic analysis were downloaded from GenBank and aligned using ClustalW and phylogenetic analyses was performed using Neighbour-joining method implemented in MEGA 4.1 software using complete deletion of gaps and missing data and p-distance model. Phylogenetic tree architecture confidence was evaluated by 1000 bootstrap replications.

2.3 Expression profiling of glucanase in biotic and abiotic stresses

2.3.1 Real-time quantitative RT- PCR analysis of gene transcripts during pathogen elicitation

Total RNA was isolated from needles harvested from 24 hr and 48 hr fungal elicitor treated cuttings while needles harvested from water treated cuttings served as control. The quality of RNA was checked on a 1% agarose gel and concentration was determined spectrophotometrically using Picodrop spectrophotometer (Picodrop Limited, Saffron Walden, UK). mRNA was purified from the RNA samples and first strand cDNA was synthesized. The cDNA concentrations were quantified and 200 ng of cDNA was used for the analysis. Primers for real-time PCR were designed using PRIMER3 program (Rozen and Skaletsky, 2000) and ubiquitin gene was used as internal control. qRT-PCR was carried out in Applied Biosystems ABI 7700 sequence detection system using the SYBR green chemistry. Quantification of the target gene expression was done employing the comparative CT method (Livak and Schmittgen, 2001). For each sample, average CT values from triplicate reactions of the target gene were normalized to CT values obtained for ubiquitin.

Fig. 1. SDS-PAGE showing induction of recombinant *CeGlu1*



1. Protein ladder 2. Total cell protein from *Escherichia coli* transformed with pET 3. Total cell protein from *E.coli* transformed with pET-CeGlu1.

2.3.2 Real-time quantitative RT- PCR analysis of gene transcripts during abiotic stresses

C. equisetifolia needles were subjected to salt, osmotic, heat and wound stresses. For establishing the stress conditions needles were kept in Petri plates containing NaCl solution (salt stress, 1500 mM), PEG solution (osmotic stress, 40%) and in polypropylene tubes containing distilled water preheated to 50 °C (heat stress). Wound stress was induced by making fine incisions on the needles prior to keeping in Petri plates containing distilled water. Except those under heat stress, needles were incubated at room temperature. Total RNA from each sample was isolated after 24 hr of incubation and cDNA was synthesized and qRT-PCR was performed as described in section 2.3.1.

2.4 Heterologous expression of *CeGlu1* in *E. coli*

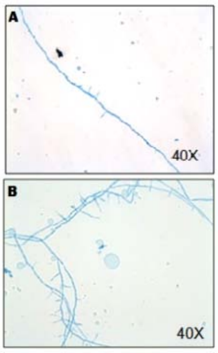
pET 28 a(+) plasmid was used for expression of *CeGlu1* in *Escherichia coli*. Glucanase gene devoid of the signal peptide was amplified with primers having sites for *Nde*I and *Not*I. The amplified fragment was digested with *Nde*I and *Not*I and further ligated into *Nde*I- *Not*I-digested pET plasmid. Gene expression was induced in *Escherichia coli* BL21(DE3) cells. Overnight grown transformed bacterial culture was diluted to a factor of 1:100 and incubated at 37ºC in presence of appropriate antibiotics. At an OD_{600}= 0.6, expression of *CeGlu1* was induced by adding 1 mM IPTG and the bacterial suspension was further incubated at 37°C for 4 h. The cells were harvested and stored at -20 °C until purification.

Purification of the protein was carried out by Ni-NTA affinity chromatography (Ni-NTA Spin Kit, Qiagen, GmbH, Hilden, Germany). The frozen pellet was lysed and purified under denaturing conditions (7-8 M urea). The eluted product was dialyzed to facilitate refolding. All the protein samples were analyzed by 12% SDS-PAGE under reducing conditions (Laemmli, 1970) and post electrophoresis gels were stained with Coomassie brilliant blue.

2.5 *In vitro* antifungal assay

To characterize the recombinant protein for antifungal properties, hyphal mass of *Fusarium verticillioides*

Fig. 2. Antifungal activity of the recombinant *CeGlu1*



A. control B. glucanase treated *Fusarium verticillioides* hyphae.

suspended in ½ MS medium was mixed with 50 µl of the dialyzed protein and incubated for 24 hr. Hyphal suspension mixed with dialysis buffer served as the control. After incubation, hyphae from each sample were stained with lactophenol cotton blue and observations were made under light microscope.

2.6 Spot assay

To study the response of *E. coli* cells transformed with pET28a-*CeGlu1* or vector alone (pET28a) to PEG, NaCl and heat/cold stress, spot assay was performed as described by Guo et al. (2012). After inducing with 1 mM IPTG at OD_{600}= 0.6, the cells were further grown for 12 h at 37 °C. The cultures were then diluted to OD_{600}= 0.6 and further to 10^{-3} and 10^{-4} cells. From each dilution 10 µl was spotted on the LB plates containing NaCl (250 mM, 500 mM and 750 mM) and PEG (15%, 30% and 45%). LB plates were infiltrated with PEG according to Van der Weele et al. (2000). Observations were made after 16 hr of incubation at 37 °C. For heat/cold stress studies LB plates spotted with each dilution were incubated at 22 °C, 28 °C and 45 °C. All plates were supplemented with 30 mg L^{-1} kanamycin.

3 Results and Discussion

3.1 Isolation and sequence analysis of *CeGlu1*

The cDNA pool amplified using glucanase-specific primers designed from *Fagus sylvatica* (Acc. No. DQ124218) yielded a product at 750 bp and the sequence showed similarity with plant glucanases. Subsequently, 3' RACE and 5'RACE were performed and contigs were generated. A 1 333 bp full length glucanase gene was obtained which comprised of a 948 bp CDS, 56 bp 5' UTR and 329 bp 3'UTR. The CDS was designated as *CeGlu1* and was submitted to GenBank with Accession no. JQ316122.1. The sequence showed similarity to glucanases from *Fagus sylvatica* and *Prunus persica* (*Gns1*). The CDS coded for a putative protein of 315 aa with predicted pI and molecular weight of 8.92 and 34.33 kDa respectively. The sequence harbored a conserved domain of glycosyl hydrolases family 17. Sequence similarity and theoretical pI of the deduced amino acid sequence suggested that the isolated glucanase is a basic isoform and belong to the class I glucanases. Gene encoding basic β-1,3-glucanases with similar domains have been isolated and characterized in another tree species like *Prunus persica* (Thimmapuram et al., 2001), while two acidic glucanase with glycosyl hydrolases family 17 domain were isolated from sugarcane infected with *Sporisorium scitamineum* (Su et al., 2013). Signal peptide prediction tool revealed a signal peptide cleavage site between 32nd and 33rd residues in the sequence, indicating that the gene product was a secreted protein. Phylogenetic analysis with MEGA 4.1 revealed that *CeGlu1* formed a separate cluster and grouped with *Arabidopsis* with low confidence, which indicate the divergence of the *C. equisetifolia* sequence from the other known sequences in the database.

3.2 Real-time quantitative RT- PCR analysis

Quantification of the target gene expression was done with comparative CT method (Livak and Schmittgen, 2001). The Ct value was normalized using ubiquitin as the reference gene and control sample as the reference sample to find the relative expression of the gene transcript. During pathogen elicitation, the expression of glucanase transcripts showed 17.8 fold increase in 24 hrs compared with control sample which further elevated to 58.6 folds in 48 hrs of elicitation. This expression profile of *CeGlu1* during elicitation is characteristic of PR proteins with distinct and progressive up-regulation suggestive of systemic defense reaction (Dasgupta et al., 2013).

The expression of glucanase transcripts were also studied during various abiotic stresses like heat, salt, wound and drought. It was observed that under heat and wound stress, the expression of the glucanase gene transcript was up-regulated to two-fold and 18-fold compared to control respectively (Dasgupta et al., 2013). However, in both salt and drought stress, there was four-fold decrease in the transcript level of *CeGlu1* in comparison to the untreated sample. The up-regulation of the transcript during mechanical wounding is in agreement with studies in *Ziziphus jujuba* (Tian et al., 2007) and *Castanea sativa* (Schafleitner and Wilhelm, 2001).

3.3 Heterologous expression of *CeGlu1* and protein purification

CeGlu1 was cloned into pET vector devoid of the signal peptide for optimal expression in a heterologous system (Hwang and Hwang, 2011). High level of expression was observed when *CeGlu1* was induced with 1mM IPTG (Fig. 1). On analysis of the expressed protein, it was found that a considerable amount of the protein was present as inclusion bodies in the insoluble fraction. Ni-NTA affinity chromatography was employed in protein purification. Since the protein was in the insoluble fraction, the cells were lysed under denaturing condition. SDS-PAGE analysis of the eluted protein showed over-expression of the recombinant protein at 31kDa. Recombinant glucanases reported from barley (*ABG2*) and rice (*OsGLN2*) were expressed as active enzymes in *E. coli* (Malehorn et al., 1993; Akiyama et al., 2004), while a β-1,3-glucanase (*TaGluD*) isolated from wheat was over-expressed as inclusion bodies in the bacterial system (Liu et al., 2009).

3.4 Antifungal activity of *CeGlu1* and spot assay

Antifungal activity of the purified glucanase against *Fusarium verticillioides* was investigated by microtiter plate-based assay. Although there was no apparent lysis of the hyphal tips, alterations in the hyphal morphology was observed in treated samples in comparison to the control including extensive branching (Fig. 2). Abnormal branching of hyphae on treatment with antifungal proteins is also reported in other studies involving various phytopathogenic fungi (Bormann et al., 1999; Nielsen, 2002; Ghosh, 2009; Naing et al., 2014). The absence of typical cell lysis in treated hyphae may be attributed to effectiveness of the enzyme in synergistic combination with chitinases or other classes of glucanases than when applied individually (Ferreira et al., 2007).

Spot assays revealed that there was an increase in cell growth of cells harboring *pET-CeGlu1* compared to the control at 500 mM NaCl. There was no cell growth in both the samples at 750 mM NaCl or any concentrations tested for PEG till 16 hrs of incubation. Similar studies for the glucanase gene from sugarcane (*ScGluA1*) showed that the gene was functional in both NaCl and PEG stresses, while another glucanase gene (*ScGluD1*) gave contrasting results, exhibiting varying tolerance imparted by glucanases to different abiotic stresses (Su et al., 2013). In heat/cold stress treatment there was no marked difference between growth of control and gene expressed cells. Both samples recorded similar growth at 28 °C, while no cell growth was observed either at 22 °C or at 45 °C.

4 Conclusion

A gene encoding for a basic β-1,3-glucanase was isolated from the needles of *C. equisetifolia*. Gene transcription was considerably induced during fungal cell wall elicitation and wounding. The recombinant protein expressed in *E. coli* affected the hyphal morphology of *Fusarium verticillioides*. This is the first report on isolation and molecular characterization of a β-1,3-glucanase gene from *C. equisetifolia*. The ectopic expression of *CeGlu1* in synergism with other antifungal genes could be a viable option in generating crops with tolerance to broad spectrum pathogens.

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Contribution of Transgenic Plants to Our Knowledge of Actinorhizal Tree, *Casuarina glauca*

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Abstract

Casuarinaceae members are fast-growing multipurpose trees which do not require chemical fertilizers due to their symbiotic association with the nitrogen-fixing actinomycete *Frankia* and with mycorrhizal fungi that contribute to improved phosphorous and water acquisition by the root system. Casuarinaceae can grow in difficult sites, colonize eroded lands and improve their fertility, allowing the subsequent growth of more demanding plant species. A genetic transformation procedure based on *Agrobacterium tumefaciens* was developed for actinorhizal tree species *Casuarina glauca*. Transgenic trees were obtained within six to nine months following genetic transformation of epicotyl fragments with the strain of *A. tumefaciens* C58C1. Transgenic Casuarinas are important tools for the basic molecular knowledge of the symbiotic process established between *Frankia* and *Casuarina*. Transformed plants are used for dissecting the roles of candidate genes in plant root infection and nodule development, and for comparing regulatory mechanisms of legume and actinorhizal symbiotic genes. Besides, in some countries such as India and China, Casuarina plantations are faced with a number of problems including abiotic stresses such as saline soils and biotic stresses linked to diseases and pests. Developing gene transfer techniques in Casuarinaceae trees is therefore a major issue to contribute to the genetic improvement of these valuable tropical tree species.

1 Introduction

Casuarina glauca is an actinorhizal tree whose natural habitat is centered to Australia, consisting of a narrow belt extending from Benga in New South Wales to Rock-Hampton in Queensland (National Research Council, 1984). It has been successfully introduced into India, China, Israel, Cyprus, Kenya, Malawi, South Africa, Egypt and Florida. This tree can survive on difficult sites where other species of *Casuarina* fail because of salinity or high soil moisture. It can reach 20 m height after 12 to 14 years, even on saline water tables.

In the field, *C. glauca* bears nitrogen-fixing root nodules induced by the soil actinomycete *Frankia* and exhibits a nitrogen-fixing potential of about 300 kg N ha^{-1}, a value comparable to those found in legumes. Casuarina roots also form associations with endo- and ecto-mycorrhizal fungi, which facilitate the uptake of minerals such as phosphorus and increase water absorption due to the extension of the rooted soil volume (Duponnois et al., 2003). As a consequence, these actinorhizal trees are capable of growing in poor and disturbed soils and are important elements in plant communities worldwide. They are used in forestry for timber and pulpwood production; they are also planted to stabilize desert and coastal dunes, protect field crops and restore degraded soil sites (Diouf et al., 2008).

In the recent years, there has been a renewed interest in developing approaches to transfer nitrogen fixation ability in cereals such as maize, wheat and rice (Charpentier and Oldroyd, 2010). Biological nitrogen fixation by *Frankia* offers a natural means of providing fixed nitrogen to actinorhizal plants, thus preventing the ecological problems resulting from the overuse of chemical fertilizers. Because nitrogen is a critical element for plant growth and development, the understanding of the molecular basis of actinorhizal root nodule symbiosis is a major issue to extend nitrogen-fixation to non-nodulating crops such as cereals (Santi et al., 2013).

In 2002, the first transgenic plants of *C. glauca* were reported following the use of a disarmed strain of *Agrobacterium tumefaciens* (Smouni et al., 2002). This result has contributed to major breakthrough in the knowledge of root endosymbioses. In this paper, we summarize the major steps involved in a successful transformation of *C. glauca* by *A. tumefaciens*, together with the necessary tests to prove that foreign genes have been transferred into the plant. The potential of transgenic plants for the functional characterization of key symbiotic genes and for the genetic improvement of Casuarina trees are discussed.

2 Factors Involved in a Successful Genetic Transformation by *Agrobacterium tumefaciens*

Agrobacterium tumefaciens is a gram negative soil bacterium that causes the plant crown gall disease when inoculated into dicotyledonous plants. More than thirty years ago, it was discovered that this bacterium has the ability to transfer a well-defined DNA segment called the T-DNA, from its tumor-inducing (Ti) plasmid DNA to the genome of wounded plant cells. Disarmed *A. tumefaciens* strains in which the native T-DNA has been replaced with valuable genes are the most efficient vectors used to-day for the production of transgenic plant species (Tzfira and Citovsky, 2006). Many protocols adapted for the genetic transformation of various plant species have been developed over the past decades; however, there are still a limited number of forest trees that can be genetically transformed (Castellanos-Hernandez et al., 2011).

The development of a reliable transformation protocol depends on the establishment of an efficient regeneration procedure, a high transformation rate of the regenerable cells, and an effective selection of the transformed cells. In preliminary experiments, explants of *C. glauca* including cotyledons, hypocotyls, epicotyl fragments and mature zygotic embryos were grown in the presence of different concentrations of kanamycin ranging from 25 mg L^{-1} to 200 mg L^{-1} (Le et al., 1996). The concentration of 50 mg L^{-1} was found appropriate to select transformed cells of *C. glauca.* Concentrations higher than 100 mg L^{-1} caused immediate browning of the explants and negatively affected the differentiation of transgenic buds and shoots. To inhibit *Agrobacterium* growth after the cocultivation stage, cefotaxime at a concentration of 250 mg L^{-1} was chosen. Although this concentration was compatible with the regeneration of transgenic plants from calli growing on nutrient medium with hormones, we found that removing this antibiotic six months after cocultivation with the agrobacteria improved bud differentiation and shoot elongation.

The transformation procedure of *C. glauca* developed in 2002 was mainly based on the protocol used to obtain transgenic plants of *Allocasuarina verticillata* (Franche et al., 1997). Experiments were conducted with epicotyl fragments excised from 45-day-old plants and genetically transformed with the *A. tumefaciens* strain C58C1 (pGV2260; pBIN19GUSint) (Vancanneyt et al., 1990). After three days of cocultivation at 25 °C on a nutrient medium containing 0.5 µM naphtaleneacetic acid (NAA) and 2.5 µM 6-benzylaminopurine (BA), the plant material was further grown on the same nutrient medium with the addition of kanamycin at 50 mg.L^{-1} and cefotaxime at 250 mg L^{-1}. To prevent untransformed zones in the initial callus, selection pressure with kanamycin was maintained several months until rooting, whereas cefotaxime was removed after 5 to 6 months. One to five shoots were obtained within six to nine months on 20% of the putatively transformed calli. About 70% of these shoots were rooted after a three-day treatment with 10 µM indole-3-butyric acid (Smouni et al., 2002). Nodulation ability of the transgenic *C. glauca* plants was found similar to that of the non-transformed plants.

3 Molecular Characterization of Transgenic Plants

To establish that plants growing on kanamycin were genetically transformed, some molecular tests were necessary. PCR experiments using primers to the *GUS* and *nptII* genes revealed that 89% of the calli growing on kanamycin contained both the ß-glucuronidase gene and the selection marker. In 8% of the remaining calli, PCR amplifications were positive only for the *GUS* gene, thus indicating that the *nptII* gene located near the left border of the T-DNA had been deleted during the transfer from the bacteria to the host plant. Amplification was neither obtained with the *GUS* nor the *nptII* primers in 3% of the calli; it can be concluded that these plant cells have escaped antibiotic selection. To demonstrate that the sequences amplified with the *GUS* and *nptII* primers were not due to a contamination linked to residual agrobacteria in the transformed calli, additional experiments were set up using the *virD1* primers. Since the virulence (*vir*) region is not transferred to the plant cells, the absence of *vir* amplification contributes to establish that the antibiotic resistant calli do not contain significant amount of residual agrobacteria.

Southern blot analyses were performed to provide additional evidence for gene transfer into the plant cells, and to determine the T-DNA copy number that integrated into the host genome (Franche et al., 1997). The pattern of hybridization with a *GUS* probe, to the plant DNA digested with a restriction enzyme that recognizes a unique cleavage site in the T-DNA, was consistent with the integration of only one copy in 80% of the transformed lines. Two to three copies were seen in the remaining lines. No hybridization was found in the non-transformed control *C. glauca* calli.

4 Transgenic RNAi Plants for the Functional Characterization of Symbiotic Genes

RNA interference (RNAi) refers to several different types of gene silencing mediated by small, dsRNA molecules. In model plant species such as Arabidopsis and rice, RNAi has been routinely used to characterize gene function and to engineer novel phenotypes (Eamens et al., 2008).

In order to determine if RNAi could down-regulate plant genes from the tropical Casuarinaceae trees, gene constructs containing hairpin RNA (hpRNA) directed towards the *GUS* reporter gene were first introduced by *A. rhizogenes* in the root system of stably transformed *35S-GUS A. verticillata* plants (Gherbi et al., 2008a). Two different hpRNA constructs were tested; the first one, pHKN30, contained a sequence of 582 nt at the 3' end of the *GUS* coding region, and the second one, pHKN31, included a 325 nt sequence at the middle of the reporter gene. Quantitative analysis of GUS activity in the cotransformed root system from the composite plants established that, in 100% (pHKN30) and 70% (pHKN31) of the *GUS-hpRNAi* roots, there was a 90% reduction in reporter gene activity (Gherbi et al., 2008b). Histochemical analysis revealed that approximately 70% of the *35S-GUS-hpRNAi* roots exhibited no blue staining. Further qPCR analysis confirmed that the level of *GUS* transcripts was down regulated from 46 to 94% in the RNAi roots.

The RNAi strategy was then used to down-regulate two symbiotic genes from *C. glauca, CgSymRK* and *CgCCaMK* (Gherbi et al., 2008b; Svistoonoff et al., 2013). *CgSymRK is* orthologous to the receptor-like kinase gene *SymRK* required for nodulation in legumes, and *CgCCaMK* encodes a calcium and calmodulin dependent kinase related to legume symbiotic CCaMKs. Quantitative RT-PCR analyses revealed a reduction in endogenous symbiotic genes expression ranging from 40 to 94% in the *RNAi-CgSymRK* and *RNAi-CgCCaMK C. glauca* plants. Down regulation of these genes strongly affected the infection and nodulation process by *Frankia*, thus pointing the universal role of *SymRK* and *CCaMK* in actinorhizal and rhizobial endosymbioses. These data underline the value of hpRNA constructs for the analysis of gene function in actinorhizal trees.

5 Potential of Transgenic Plants for the Genetic Improvement of Casuarina Trees

Conventional breeding of forest trees is often hindered by long generation times and self-incompatibility mechanisms. Genetic engineering can provide means to rapidly incorporate foreign genes or to manipulate endogenous genes to improve elite tree genotypes chosen from breeding programs (Dubouzet et al., 2013).

The gene transfer procedure based on *A. tumefaciens* paves the way for genetic engineering of *C. glauca* (Zhong et al., 2013). Strategies can be developed to engineer this nitrogen-fixing tree to resist major pathogens, such as *Rhizoctonia solani*, and insect pests, such as *Limantria xylina*. Transgenic trees that are more tolerant to adverse edaphic conditions, such as salt, drought and cold, would also be very valuable in tropical regions. Other aspects may include modification of lignin content and/or composition to obtain trees that are more suitable for industrial uses (Baucher et al., 2003; Studer et al., 2011). Paper production has more than tripled in the last 35 years and the paper industry suffers from the high cost of removing lignin from cellulose, which also has a negative environmental impact. Another goal linked to lignin modification could be to prevent Casuarina wood from splitting when it dries. This is currently a major drawback for the use of Casuarina wood for the manufacture of furniture.

To our knowledge, no transgenic Casuarina trees have yet been planted in the field. Additional information on the stability of transgene expression in field-grown *C. glauca* exposed to changing environments is thus needed to determine the real potential of genetic engineering for the introduction of valuable new traits in this tropical tree family. Furthermore, to prevent an uncontrolled escape into the environment, efforts should be made to obtain sterile transgenic Casuarinaceae trees that do not form fertile pollen or seeds (Ahuja, 2009).

6 Conclusion

Although research carried out in the area of genetic engineering of forest trees is not comparable to the numerous data obtained in annual crops, they are being increasingly used for research and many developmental processes are being studied at the molecular level. *C. glauca* is one of the forest tree species that could benefit from the successful genetic transformation by *A. tumefaciens*. Transgenic *C. glauca* plants have already proven to be valuable tools to improve the knowledge on the actinorhizal infection and nodulation process by *Frankia*. In the future, genetic transformation of *Casuarina* species will probably help to accelerate the process of genetic improvement through the introduction of valuable traits.

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Signalling and Communication in the Symbiosis of *Casuarina-Frankia*

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Abstract

Two nitrogen-fixing root nodule symbioses between soil bacteria and plants have been described: one between rhizobium and legumes and the other between *Frankia* and actinorhizal plants. Legume and actinorhizal nodules differ in their ontogeny and structure. However, phylogenetic studies based on *rbcL* gene sequence analysis have shown that all plants able to enter a root nodule symbiosis belong to the same clade, suggesting thus that they share a predisposition for symbiosis. The molecular bases of this predisposition are so far unknown. Although several genetic components of the host-symbiont interaction have been identified in legumes, the genetic basis of actinorhizal symbiosis remains largely unknown. In actinorhizal root nodule symbiosis, major developments in *Frankia* and plant signalling have been (i) the determination of the genome sequences and the absence of *nod* genes in three *Frankia* strains, (ii) the presence of a symbiosis receptor kinase (*SymRK*) in *Casuarina glauca* and *Datisca glomerata* that determines the development of actinorhizal nodules as well as endomycorhizae and the presence in *C. glauca* and *Alnus glutinosa* of homologs of the whole common symbiotic signaling cascade (iii) the recent observation that *Frankia* induces Ca spiking in root hair cells of actinorhizal plants. All these studies have led to the emergence of a vision of a diversity of bacterial symbiotic signalling determinants and the presence of an evolutionarily partly conserved pathway on the plant side. The evolutionary pattern emerging from these studies reinforces the hypothesis of a common genetic ancestor of the Fabid (Eurosid I) nodulating clade with a genetic predisposition for nodulation.

1 Introduction

Nitrogen and phosphorus availability are frequently major limiting factors in plant growth and development. Indeed, most crop plants including cereals are unable to exploit atmospheric nitrogen for their nutrition. The high cost of synthetic nitrogen fertilizers, together with the negative environmental impact of fertilizer use, has recently led to a renewed interest in the environment-friendly use of biological nitrogen fixation. A relatively small group of plants are able to form efficient symbiotic associations with soil bacteria capable of converting atmospheric nitrogen into ammonium. In all cases, these so-called endosymbioses involve the formation of specialized root organs known as nodules containing intracellular N-fixing bacteria. Two kinds of associations lead to the formation of Root Nodule Symbioses (RNS): 1) those involving Gram-negative proteobacteria called rhizobia, able to interact with most leguminous plants and 2) those involving the Gram-positive filamentous actinobacteria *Frankia*, able to interact with ~260 species collectively called actinorhizal plants (Vessey et al., 2005).

Actinorhizal species are of particular interest ecologically, since they generally possess high rates of N-fixation and are able to grow in poor quality soils. Certain plants can even resist environmental stresses including high salinity, extreme pH and heavy metal contamination. *Casuarina* spp. have potential as commercial wood products including lumber. Casuarina trees are well suited for fuelwood production in tropical and subtropical countries because of their high growth rate resulting from their capacity to adapt to poor soils and to establish symbioses with the actinomycete *Frankia* and with mycorrhizae (Diagne et al., 2013). A growth rate of 3 to 5 m height per year has been reported in the plantations in India. Although Casuarina wood has high density, the green wood moisture content is relatively low, making the energy value considerably higher than that of other tree species. Casuarina produces high-quality fuelwood that burns readily even when green, has low ash content, and makes an excellent charcoal. In India Casuarina is widely used in farm forestry for poles, firewood charcoal and pulpwood. Because of its multipurpose nature, it has been widely and rapidly accepted by the farmers in South India (Uma et al., 2011).

Symbiotic interactions between *Frankia* and Casuarina are not well understood at a molecular level. Molecular data shows that all plants able to form nitrogen fixing nodules belong to the same clade (Fabids). Root nodule symbioses (RNS) probably evolved independently at least 6 times in different plant lineages of Fabids about 60 Ma ago (Doyle, 2011). Thus, it has been hypothesized that a common ancestor of these plants developing root nodule symbiosis (RNS) could have acquired a genetic predisposition for nodulation (Soltis et al*.,* 1995)*.* Whilst the nature of this predisposition is still a mystery, comparative studies between legumes, *Parasponia* and actinorhizal plants are beginning to provide important information about the common molecular and cellular mechanisms used by all nodulating plants. RNS share many features with arbuscular mycorrhiza (AM), an ancient symbiotic association between plants and Glomeromycota which appeared ~400 Ma ago. AM are found in ~80% of plant species and play a crucial role in improving nutrient uptake and in particular phosphate, as well as providing protection against pathogenic fungi. In legumes, many genes required for rhizobial nodulation are also required for AM suggesting that rhizobial nodulation recycled part of the ancestral program used by most plants to interact with AM fungi. Most genes required for rhizobial RNS are also present in actinorhizal species (Hocher et al., 2011) and at least one of them (*SymRK*) plays a crucial role in actinorhizal nodulation, thus demonstrating that all three endosymbioses share common genetic elements (Gherbi et al., 2008; Markmann and Parniske, 2009).

In the present paper we summarize recent knowledge about the molecular dialogue between *Frankia* and Casuarina roots leading to the development of nitrogen fixing nodules.

2 Casuarina Flavonoids: Signaling Role in the Symbiotic Interaction

In legume, flavonoids function as inducers of rhizobial *nod* gene expression (*nod* genes are associated with Nod signal biosynthesis in rhizobia) and as chemo-attractants to concentrate the compatible rhizobium at the root surface. In addition, major role thought to be played by flavonoids once the bacteria enter the plant is the modulation of auxin transport during the initiation of nodule primordia (Hassan and Mathesius, 2012).

In Casuarina, RNA interference was used to silence chalcone synthase, which is involved in the first committed step of the flavonoid biosynthetic pathway. Transformed flavonoid-deficient hairy roots were generated and used to study flavonoid accumulation and further nodulation. Knockdown of chalcone synthase expression reduced the level of specific flavonoids and resulted in severely impaired nodulation. Nodule formation was rescued by supplementing the plants with naringenin, which is an upstream intermediate in flavonoid biosynthesis. These results provide, for the first time, direct evidence of an important role for flavonoids during the early stages of actinorhizal nodulation. These data point to a role for flavonoids in the early stages of the infection process of Casuarina roots with *Frankia* (Abdel-Lateif et al., 2012; 2013). Additional studies are still needed to establish what are the flavonoid changes in the plant that enable efficient *Frankia* nodulation.

3 *Frankia* Symbiotic Signaling Molecules are Still Unknown

The genetics of *Frankia* is in its infancy. These bacteria are slow growing, and genetic tools are only beginning to be developed. At present, there is no known system for gene transfer for *Frankia*. No phages, R-plasmids, transposable elements, or conjugative plasmids have been identified, and standardized mutagenesis protocols have not been firmly established. To gain insight on the actinorhizal symbiosis, three *Frankia* isolates were initially sequenced. Several *nodC* and *nodB* distant homologs were seen, these were spread all around the genomes. Furthermore, they did not form a SYM-island and finally no *nodA* gene could be detected (Normand et al., 2007). The group of Philippe Normand (CNRS/ Lyon I University, France) is in the process of identifying the *Frankia alni* (ACN14 strain) Root Hair Deforming Factor (RHD) through a bio-assay guided strategy that involves HPLC fractionation, size fractionation, enzymatic digestion, temperature treatment, solvent fractionation, acid hydrolysis followed by mass spectrometry and nuclear magnetic resonance. In previous work, a partial characterization had been done and showed that factor had some similarities with the Rhizobium Nod factor (thermostability, size) but also major differences (polarity, resistance to most chitinases). This compound is present at very low concentration.

In conclusion, little is known about *Frankia* signaling molecules, but it is clearly very different from that known in Rhizobia.

4 Several Casuarina Genes are Homologs of the Legumes Symbiotic Signaling Cascade

Genetic screens in the model legumes *Medicago truncatula* and *Lotus japonicus* have provided new insights in the molecular components of the symbiotic signaling pathway. Using map-based approaches, several of the corresponding genes were identified (Oldroyd and Downie, 2008). The earliest genes isolated are the receptor-like kinase with LysM domain NFR1/NFR5, LYK3/NFP that are hypothesized to bind Rhizobia Nod Factors (NFs). Other signaling genes that act downstream NFs perception have been identified. Interestingly, these genes are also required for AM formation suggesting the existence of common symbiotic genes that are shared among plant endosymbioses. Several regulatory elements involved in transcriptional changes inducing early nodulation program have also been identified (Oldroyd, 2013).

Although several genetic components of the host-symbiont interaction have been identified in legumes, the genetic basis of actinorhizal symbiosis is unknown. Gherbi et al. (2008) demonstrated that the receptor like-kinase gene

SymRK, which is required for nodulation in legumes, is also necessary for actinorhizae formation in *C. glauca*. This indicates that both types of nodulation symbiosis share genetic components. We have also shown that SymRK is involved in AM formation in *C. glauca* and can restore both nodulation and AM symbioses in a *L. japonicus symrk* mutant. In addition, transcriptomic analysis showed that most of the genes of the common "SYM" pathway described for Arbuscular Mycorhizal (AM) and legumes-rhizobium symbioses were identified in *C. glauca* and *Alnus glutinosa*. This pathway contains a receptor-like kinase, nuclear pore proteins and potassium channels required for the induction of calcium oscillations. A putative calcium and calmodulin-dependent protein kinase (CCaMK) is also present and might thus recognize calcium "actinorhizal signatures". Interestingly, this analysis also revealed the presence of genes linked to a "NOD"-specific pathway (not shared with AM symbiosis) used by legumes in their symbiosis with rhizobium (Hocher et al., 2011).

Taken together these data reinforce the hypothesis of a common genetic ancestor of the Fabid (Eurosid I) nodulating clade with a genetic predisposition for nodulation (Hocher et al., 2011).

5 Conclusion

Ongoing research by the actinorhizal community has led to major advances in our knowledge of symbiotic genes. The major challenge in the coming years will be the isolation of the receptor for the signal molecules emitted by *Frankia* in response to contact with the root system of the host, and the biochemical characterization of these molecules. Expected data will highlight the diversity of molecules and molecular mechanisms underlying endosymbiotic root symbioses and will pave the way towards transferring nodulation to cereals.

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Agrobacterium-mediated Transformation of Chitinase Gene from Actinorhizal Tree, *Casuarina equisetifolia* in *Nicotiana tabacum*

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Abstract

Genetic transformation of plants offers the possibility of testing hypotheses on the function of individual genes as well as the exploitation of transgenes for targeted trait improvement. Cloning of a full-length class I chitinase (*CeChi1*) from *Casuarina equisetifolia* was reported earlier (Veluthakkal and Dasgupta, 2012). In the present study, tobacco was used as a model system to functionally evaluate the potential of *CeChi1*. The *pUH-CeChi1* construct was introduced into tobacco by *Agrobacterium* –mediated transformation and the putative transformants were confirmed for stable gene integration, transcript expression and recombinant protein production using PCR, qRT-PCR, antifungal assays and *in planta* analysis. The expression of chitinase gene in putative transgenic line (T2) was found to be 3.8-fold greater than that of the untransformed tobacco. The result of RT-qPCR also denoted the stable expression of chimeric *CeChi1* in transgenic tobacco plants without silencing phenomena and growth retardation. The *in vitro* antifungal bioassay using the total proteins from transformed plantlets caused lysis of hyphal tips of targeted pathogenic fungi *viz. Trichosporium vesiculosum*, *Fusarium oxysporum* and *Rhizoctonia solani*, characteristic of class I chitinase enzyme. *In planta* bioassay of transformed tobacco revealed reduced symptoms when compared to untransformed tobacco plants which showed marked diseased symptoms. The study revealed that the class I chitinase isolated from *C. equisetifolia* can act as a new gene resource in future transformation programs for conferring tolerance to disease caused by fungal pathogens.

1. Introduction

Chitin, one of the most abundant biopolymer in nature next to cellulose and lignin, is a major structural component of the cell wall of many pathogenic fungi. Plant chitinases are hydrolytic enzymes (EC 3.2.1.14) that catalyze the hydrolysis of chitin, a polymer of N-acetyl-D-glucosamine. They are believed to play an important role in plant defense against infection by pathogens. Chitinases have been isolated from many plant species, especially dicots like *Arabidopsis thaliana* (Samac et al., 1990), bean (Awade et al., 1989), winged bean (Esaka and Teramoto 1998), cucumber (Metraux et al., 1989), pea (Chang et al., 1995), and soybean (Yeboah et al., 1998). With its potential antifungal properties, it is possible to constitutively over-express a chitinase gene in transgenic plants and confer enhanced resistance to one or more fungal pathogens. Several studies on ectopic expression of chitinase in plant system are reported (Dana et al., 2006; Xiao et al., 2007). In the present study the class I chitinase *(CeChi1)* isolated from the needle tissues of *Casuarina equisetifolia* challenged with fungal elicitors (Veluthakkal and Dasgupta 2012) was ectopically expressed in tobacco, to functionally validate the antifungal property of the encoding protein.

2. Materials and Methods

2.1 Plant materials and culture condition

Seeds of *Nicotiana tabacum cv. Samsun* were surface sterilized, germinated and grown *in vitro*. The meristematic tissues of the emerging shoots were cut and maintained on MS medium containing 1.0 mg/l BAP by sub-culturing every two weeks prior to transformation.

2.2 Agrobacterium mediated leaf disc transformation

Agrobacterium tumefaciens strain LBA4404 bearing the binary vector *pUH-CeChi1* (Katiyar-Agarwal et al., 2002) was used for transformation. The T-DNA region of the binary vector contained a hygromycin phosphotransferase gene (HPT) under the control of the CaMV 35S promoter and the *CeChi1* gene under the control of the maize *Ubiquitin1* promoter. The modified transformation method described by Öktem *et al*. (1994) was used for transforming tobacco leaves using leaf disc transformation method. Leaf discs, which were not infected with *Agrobacteria* and cultured on selection media served as negative control. Leaf discs which were not co-cultivated and were placed on regeneration media were used as positive control.

2.3 Co-cultivation and regeneration of transformants

Tobacco leaf discs were incubated in the culture of *Agrobacterium* harboring pUH-*CeChi1* for 25-30 min with gentle shaking at regular intervals under sterile conditions. Subsequently, leaf discs were dried on sterile tissue paper and transferred back to pre-incubation medium and incubated in dark for 48-72 hrs, depending on the growth of bacteria on the periphery of leaf discs.

Leaf discs were removed from co-culture media and placed on MS selection media containing 300 mg L^{-1} cefotaxime and 25 mg L^{-1} hygromycin after washing with cefotaxime (250-300 mg L^{-1}) to remove excessive bacterial cells. Regeneration started after 15-20 days and leaf discs were transferred to fresh selection medium after every two weeks. When shoots reached 1-2 node stage, they were transferred to the rooting medium (MS media with 0.1 mg L^{-1} IBA).

2.4 Molecular analysis of putative transgenic plants

Genomic DNA from control and putative transgenic tobacco plants were isolated by Cetyl dimethyl Ethyl Ammonium Bromide (CTAB) method. Confirmation of gene transfer by PCR was carried out with specific primer pairs to amplify the *CeChi1* transgene (KpnF: 5'- GGAATT CCGGTACCAAAATGAGGTTTTGGATCTTTGC -3' and SacR: 5'- CCCGAGCTCCTACATGGTATCCACCAAGAGTCCATTG -3') and hygromycin (HygF: 5' – GCTTTCAGCTTCGATGTAGGAG – 3' and HygR: 5' – CACGCCATGTAGTGTATTGACC – 3') gene. The amplified PCR products were analyzed by electrophoresis on a 1% (w/v) agarose gel.

2.5 Transgene expression analysis by qRT-PCR

Two putative transformants (T1 and T2) were randomly selected from two independent events for further studies. Total RNA was extracted from tobacco leaves of untransformed control and the two putative transformants using the Plant RNA mini kit (Chromous Biotech, Bangalore, India) according to the manufacturer's instructions. Reverse transcription was performed with first strand cDNA synthesis kit (Fermentas, Hanover, MD, USA). Relative quantitative PCR reaction was performed using StepOne plus Sequence Detection System (Applied Biosystems, USA) and associated software using the SYBR green chemistry. Controls included non-RT controls and non-template controls (NTC; water template). Changes in gene expression of the transgene as relative fold difference between transgenic samples and control plantlets were calculated using the comparative Ct ($2^{-\Delta\Delta Ct}$) method (Livak and Schmittgen 2001; Schmittgen *et al.* 2000). β-actin from tobacco (TactinFP: 5'-CCTGAGGTCCTTTTCCAACCA-3'; TactinRP: 5'-GGATTCCGGCAGCTTCCATT-3') (Schmidt and Delaney 2010) was used as an endogenous control to normalize the data for differences in input RNA and efficiency of reverse transcription between the samples.

2.6 Extraction of total proteins and in vitro antifungal assay

Hundred mg of fresh leaves were harvested from untransformed and putative transformants (T1 and T2) and total protein was extracted in extraction buffer (100 mM Sodium acetate pH 5). The samples were centrifuged at 12,000 rpm for 10 min at 4°C and the supernatant was used for bioassay studies. The *in vitro* antifungal study was conducted in replicates by hyphal extension inhibition assay against the fungal isolates of *Fusarium oxysporum, Trichosporium vesiculosum* and *Rhizoctonia solani.*

2.7 *In planta* biosassay

Rhizoctonia solani was grown on PDA medium at 25 °C and after 1 week, the mycelial mat was harvested and spore suspension was prepared by flooding the petri dish with sterile water, agitating the surface gently with an inoculation loop, filtering and adjusting the resulting spore suspension to 2 x 10^6 spores ml/l. Tobacco plants (fifteen each of both untransformed and transformant T1) were sub-cultured onto fresh MS medium without antibiotics and allowed to root. Subsequently, 1 ml fungal suspension was injected into the medium with a sterile needle to allow fungal contact with the roots. Mock-inoculation was done by spraying sterile water onto the medium. The plants were then incubated at room temperature for 4 weeks and periodic (daily) observations were made for disease development.

3 Results and Discussion

Tobacco was used as a model system to functionally characterize *CeChi1* driven by the maize *Ubi* promoter. Tobacco transformation was conducted using the *Agrobacterium* mediated protocol due to its well established and highly efficient process for generating transgenics (Öktem *et al.* 1994).

3.1 Selection and regeneration of transformants

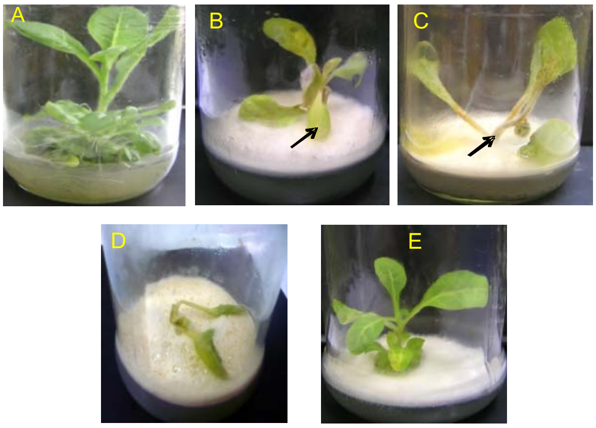
The transformed tissues grew normally in the presence of hygromycin, while non transformed cells died. Regeneration started as small green calli around the periphery of leaf discs after three weeks on selection media. The hygromycin resistant calli eventually showed signs of

Table 1. Transformation of tobacco with LBA4404 harboring pUH-CeChi1

No. of leaf disks infected	Disks for positive and negative control	No. of disks regenerated on selection media	Transformation efficiency (%)	Total plants produced
250	30 each	166	66.4	89

Transformation efficiency was calculated as the ratio of the number of tobacco disks with hygromycin resistant calli to the total number of leaf disks incubated with Agrobacterium.

Fig. 1. *In planta* evaluation of transgenic tobacco expressing CeChi1 against *Rhizoctonia solani*



A : Mock inoculated B : Untransformed control displaying symptoms of yellowing of leaf (After 4 weeks) (indicated by arrow); C : Untransformed control displaying symptoms of brownish water soaked lesions (After 4 weeks) (indicated by arrow); D : Untransformed control displaying symptoms of wilting; (After 4 weeks) E : Transgenic line T2 with reduced symptoms

organogenesis and produced small shoots from the calli after 2-3 weeks. Rooting started after 5-6 days of transferring the shoots to rooting media. A number of independent hygromycin resistant *CeChi1* transgenic tobacco lines were generated. All these hygromycin resistant tobacco plants showed normal phenotype. The transformation efficiency was recorded as 66.4 % (Table 1).

Gain-of-function can be achieved by increasing gene expression levels through the random activation of endogenous genes by transcriptional enhancers or following the expression of individual transgenes introduced by genetic transformation (Abdeeva et al. 2012). Several studies on using plant system to functionally characterize genes have been reported (Condori et al. 2009; Yasmin 2009; Vongpaseuth 2011; Yuan et al. 2012). In this paper, tobacco was used as a model system to functionally evaluate the antifungal potential of the CeChi1 from *C. equisetifolia.*

3.2 Molecular analysis of putative transgenic plants

Putative transgenics were confirmed first by selection on hygromycin media and subsequently by PCR and qRT-PCR. Transfer of the T-DNA into the putative transgenic was confirmed by PCR analysis. PCR analysis was conducted with ***CeChi1***, *hph* primers in combinations (KpnF and HygR; QChiFP and HygR). The ***CeChi1*** and *hph* specific primers amplified at 1.0 kb and 340 bp respectively in the putative transformant T2 confirming the transformation event. Amplification of the untransformed, T1 and T2 DNA using primer combinations (KpnF and HygR; QChiFP and HygR) yielded amplicons of size 2.0 and 2.5 kb in the putative transformant T2, whilst in T1 and untransformed tobacco DNA, no amplification was observed with any of these primer pairs. The results confirmed that the transformed tobacco T2 stably contained both, the *CeChi1* transgene and plant selectable *hygromycin* marker gene. The untransformed condition of T1 could be due to an escape during selection.

The cDNA synthesized from untransformed tobacco and putative transformant T2 were subjected to real time quantitative PCR analysis to detect the level of expression of the *CeChi1* transgene. qRT-PCR results indicated a 3.8 fold overexpression of *CeChi1* in the putative transgenic T2 in comparison to the untransformed control, confirming the expression of the transgene.

3.3 Extraction of total protein and *in vitro* antifungal activity of putative transgenics

Total protein was extracted from the transformed plant and an untransformed control. A fungal inhibition assay was conducted against three test pathogens including *Trichosporium vesiculosum, Fusarium oxysporum* and *Rhizoctonia solani*. A prominent inhibition zone was observed near the disc loaded with total proteins extracted from T2 when compared to the T1 and untransformed control. No inhibition was observed near the disc loaded with buffer-only.

3.4 Evaluation of *in planta* antifungal resistance

Putative transgenic line T2 and wild-type control plants were challenged with the fungal pathogen *R. solani* to assess level of disease tolerance of transgenic tobacco expressing *CeChi1*. The mock-inoculated plants showed no morphological or developmental abnormalities during the period of study (4 weeks) (Fig.1). In the control untransformed plants, symptoms were observed 4–5 days after infection, whereas no visible symptoms were found on the leaves of transgenic line T2, which remained green with no sign of disease development (Fig.1). The untransformed tobacco showed symptoms like water-soaked lesions starting from leaves and extending up the stem with lesion color changing to brownish black followed by severe wilting and yellowing of leaves characteristic of *R. solani* infection.

In the present study, *in vitro* and *in planta* bioassays were conducted to elucidate the role of *CeChi1* in transgenic plants. The hyphal extension of all three pathogens was inhibited by the total proteins derived from transformed plantlets confirming the stable expression and functionality of the transgene. Similar confirmation of transgene expression in tobacco was reported by Saiprasad et al. (2009) where in the efficacy of total protein derived from transgenic tobacco expressing *Tricoderma harzianum* endochitinase in inhibiting fungal growth was demonstrated. Guan et al. (2008) reported a similar inhibitory role in transgenic *Brassica* expressing chitinase against *Colletotrichum truncatum* and *Colletotrichum acutatum*. *In vitro* fungal bioassays using *Trichoderma viride* showed that extracts from transgenic potato lines co-expressing *Brassica juncea* chitinase (*BjCHI1*) and *Hevea* *brasiliensis* β-1,3-glucanase (*HbGLU*) inhibited fungal growth better than extracts from transgenic potato expressing either *BjCHI1* or *HbGLU*, suggesting a synergistic effect (Chye et al. 2005). The present study demonstrates that the *CeChi1* isolated from *C. equisetifolia* enhanced the tolerance of transgenic tobacco against *R. solani* and can be used as a gene source for future transformation studies to generate disease tolerant transgenic lines.

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Research on Cold Tolerance of Casuarinas in China

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Key words: physiological and biochemical response, gene expression, transcriptome

Abstract

Over 300,000 hectares of Casuarinas mainly *Casuarina cunninghamiana, C. equisetifolia* and *C. glauca* have been planted in five southern coastal provinces of China viz., Guangdong, Guangxi, Fujian, Hainan and Zhejiang. The climate in the casuarina planting area ranges from warm humid tropics in Hainan to cold subtropics in Zhejiang which experience subzero temperature in the winter. Cold tolerance of casuarinas has been studied by determining the physiological and biochemical response traits. Differences between species and between clones in cold tolerance have been observed. Plan is underway to investigate gene expression and molecular mechanism of cold tolerance using *C. cunninghamiana* as a pilot species.

1 Introduction

Casuarina species especially *C. equisetifolia*, *C. cunninghamiana* and *C. glauca*, form a common part of the landscape in the coastal area in southern China. They are environmentally and economically important. Casuarinas are planted as windbreaks to stabilize the moving sand, in agroforestry systems, and for rehabilitation programmes. Plantation-grown trees are harvested for a wide range of uses, such as fuelwood and charcoal and general construction, and are increasingly used as woodchips for paper pulp and veneer for plyboard. From the first introduction to Taiwan in 1897 (Yang et al., 1995), there are about 300,000 hectares of casuarinas at present in the southern provinces of Fujian, Guangdong, Guangxi, Hainan and Zhejiang (Zhong et al., 2011). The most widely planted species is *C. equisetifolia* which accounts for up to 90% of total casuarina area.

The climate of the Casuarina planting area in China ranges from warm and humid tropics in Hainan and part of Guangdong to subtropics in Zhejiang and northern part of Fujian. Early species and provenance trials showed that *C. cunninghamiana*, *C. equisetifolia* and *C. junghuhniana* are most suitable for the tropical region, while *C. glauca* and *C. cunninghamiana* from southern Australia are more adaptable in the subtropical region (Zhong et al., 2011). Cold tolerance is obviously a prerequisite for successful planting of Casuarinas in areas which experience extreme low or subzero temperatures in winter. In Taizhou, Zhejiang province (latitude 28° 29' N, longitude 121° 53' E), 18-month-old *C. equisetifolia* plants were found to survive absolute temperature of -6 °C (Zhang and Lu, 2002). However, Cao et al. (2011) exposed *C. equisetifolia* seedlings to low temperature stress and concluded that the critical low temperature for this species was -3 °C. Jiang (2011) found some *C. cunninghamiana* trees planted in the 1980s at Baohe Forest Farm in Hanzhong, Shanxi province (latitude 33° 20' N, longitude 106° 96' E, absolute minimum temperature -10°C) have survived the extreme cold temperature and grown to 30 cm in diameter. These observations indicate the ability of some Casuarina species to tolerate cold temperature stress to some extent. Understanding the physiological and biochemical response traits, and gene selection and expression under cold treatments can help cultivate Casuarinas successfully in cold subtropical region. This paper summarizes research conducted in China in this respect.

2 Physiological and Biochemical Responses Under Cold Stress

Physiological and biochemical responses of plants can reflect mechanism of cold regulation on plant tissues and cells level under cold stress (Hew et al., 1986). Electrical conductivity, percentage of electrolyte leakage, cell membrane permeability, malondialdehyde (MDA), superoxidase dismutase (SOD), peroxidase (POD), catalase (CAT) and chlorophyll fluorescence parameters are reliable indicators to determine the extent of cold tolerance.

Physiological and biochemical traits for establishing protocol of Casuarinas cold tolerance have been reported for some *Casuarina* species. Gao et al. (2011) used the percentage of electrolyte leakage to determine cold tolerance among *C. cunninghamiana*, *C. equisetifolia* and *C. glauca*, and found the temperature at corner point for *C. equisetifolia* to be -6.59 °C, more cold tolerant than the other two species, -5.74 – -6.45 °C (Table 1). Such results appear to contradict the field observations by Zhong et al. (2011) that *C. equisetifolia* would be least cold tolerant among the three species.

Wu et al. (2012) found that inoculation with mycorrhizal fungi significantly improved growth of 12-month old *C. equisetifolia* seedlings under temperature gradients from 25 °C to -8 °C. The inoculation appeared to have enhanced

Table 1. Curve equation of percentage of electrolyte leakage and temperature under low-temperature stress and the temperature at corner point of three *Casuarina* species

No.	Species	Seed source	Temperature at corner point (°C)
1	*C. glauca*	Royal National Park, NSW, Australia	-5.92
2	*C. equisetifolia*	Kilifi, Kenya	-6.59
3	*C. cunninghamiana*	Cowra, NSW, Australia	-6.27
4	*C. cunninghamiana*	Singleton, NSW, Australia	-5.74
5	*C. cunninghamiana*	Armidale, NSW, Australia	-6.45

the protection of enzyme activity, reduced cell membrane permeability and MDA content of the twigs leading to improvement in cold resistance (Table 2).

Based on the relative conductivity, soluble protein content, MDA content and SOD activity, He et al. (2011) observed variation in cold tolerance among three *C. glauca* clones which were subjected to low temperature gradient treatments from -2 to -11 °C. Two of the clones (C36 and C24) were propagated from parental ortets which survived field temperature of -6 °C at Hangzhou Bay, Zhejiang. The relative conductivity of clones was lower than the control, while the values of soluble protein content, MDA content and SOD activity were higher (Fig. 1). These results indicated that Clones 36 and 24 are more cold tolerant than the control.

Wu et al. (2013) also observed clonal variation in *C. equisetifolia* for cold tolerance under natural condition. The study examined the changes in chlorophyll fluorescence in the leaves (branchlets) of seven clones selected from a local plantation in Zhejiang. One clone exhibited the strongest cold tolerance to -4.5 °C by recording the highest level of actual photochemistry quantum yield and thus the greatest photosynthesis efficiency.

3 Proposed Research on Molecular Biotechnology

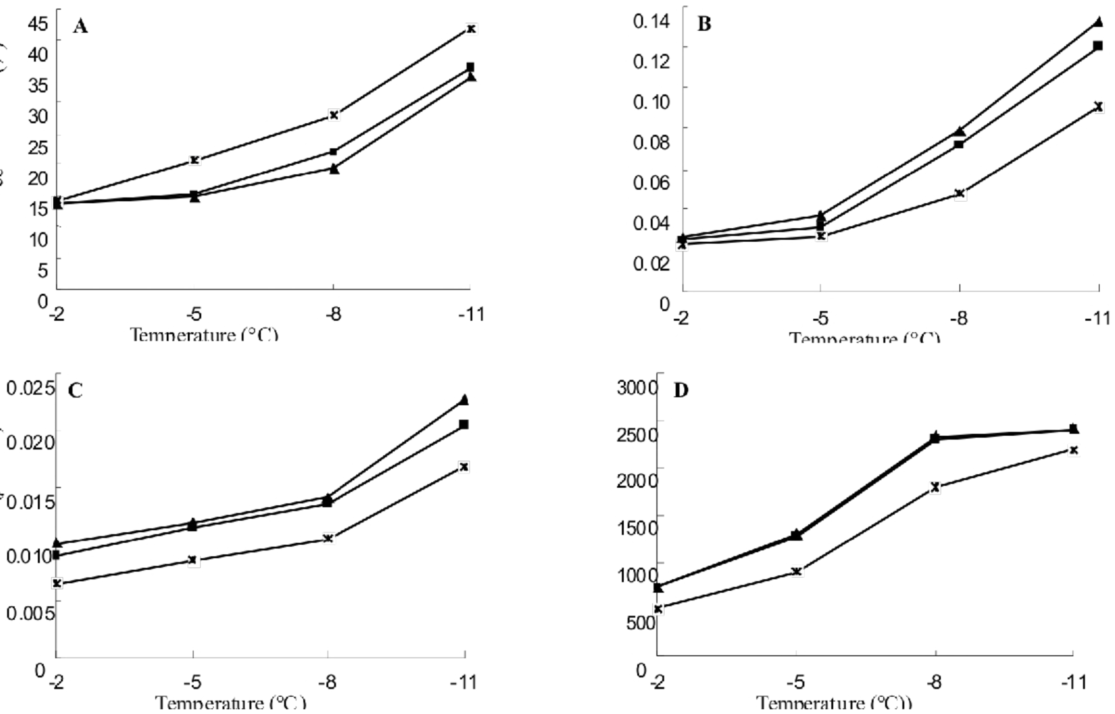
Molecular biotechnology could provide useful tools to ascertain signal transduction and gene expression regulation mechanism of the plant affected by low temperature stress (Thakur and Nayyar, 2013). To understand the gene expression and molecular mechanism of cold tolerance, we have clonally propagated cold tolerant *C. cunninghamiana* tree growing at Baohe Forest Farm in Hanzhong, Shanxi province, for a study on transcriptome which includes relative cold genes. Rooted cuttings are being raised in a nursery at the Research Institute of Tropical Forestry in Guangzhou. When the seedlings attain one year's age they will be subjected to cold stress (-5 to -10 °C) under simulated cooling condition in low temperature incubators set at different cooling regimes. The technology of RNA-Seq and DGE sequencing will be used for selection and annotation of different expressing genes, and analysis of GO function and pathway. Results of this research are expected to clarify the characteristics and mechanism of

Table 2. The effect of MDA of temperature stress on *Casuarina equisetifolia* inoculated with different mycorrhizal fungi (source: Wu et al., 2012)

Inoculation treatment	Temperature gradient (°C)				
	25	4	0	-4	-8
M_1	0.45Cc	0.49Cf	0.66Bbc	0.69Bc	0.84Ac
M_2	0.58Cb	0.60Cc	0.64BCc	0.67Bc	0.72Ac
M_3	0.52Cb	0.56BCcd	0.57ABde	0.58ABd	0.61Ad
M_4	0.37Cd	0.65Bb	0.67Bb	0.79Ab	0.83Ab
M_5	0.51Bb	0.53Bdef	0.55Bef	0.55Bd	0.74Ad
M_6	0.45Cc	0.50BCef	0.54Bf	0.56Bd	0.66Ad
M_7	0.51Cb	0.54Cde	0.59Cd	0.69Bc	0.78Ac
M_{CK}	0.63Da	0.72Ca	0.77Ca	0.88Ba	0.95Aa

NB. Values in each column with different lowercase letters indicate significant difference between inoculation treatments at P<0.05. Values in each row with different uppercase letters indicate significant difference between temperature gradients at P<0.05.

Fig. 1. Physiological responses of three *C. glauca* clones to cold temperature stress (A. relative conductivity, B. soluble protein content, C. MDA content, D. SOD activity). ▲ = Clone C36, ■ = C24, ✳ = control (source: He et al., 2011)



gene expression under different chilling methods, and the relationship of chilling injury molecular mechanisms associated with chilling stress types in *C. cunninghamiana*. The results will reflect the effects of different chilling phenomena under natural condition, including sudden cooling down, slow cooling down, and cooling followed by warming then cooling again. Overall, the technology of genetic transformation on Casuarinas is being developed rapidly (Laplaze et al., 2008; Jiang et al., 2011). Such technology will provide a new and rapid method for breeding cold tolerant Casuarinas by transferring foreign cold relative genes into casuarina plants.

4 Conclusion

Studies in China have demonstrated that *Casuarina* species such as *C. cunninghamiana*, *C. equisetifolia* and *C. glauca* can survive extreme low temperature of -5 to -10 °C. Differences between species and also between clones of the same species have been observed suggesting the need for more studies in order to understand genetic variation in the cold tolerance.

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Identification of Cinnamoyl CoA Reductase (CCR) Gene in *Casuarina equisetifolia*

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Key words: cinnamoyl CoA reductase, primer pairs, conserved domain

Abstract

Wood is primarily composed of lignin, cellulose, and non-cellulosic polysaccharides which associate to form a complex structure that is distinctive to woody perennials. Wood properties influence pulp and paper quality and are directly related to the cellulose, hemicellulose and lignin content. In the recent years, several research groups have worked on identification of lignin biosynthetic genes in tree species like *Populus*, *Eucalyptus*, *Betula* and *Acacia*. Cinnamoyl CoA Reductase (CCR) is the key gene involved in the first step of the phenylpropanoid pathway specifically dedicated to the monolignol biosynthetic branch. The present study aimed at isolating a fragment of CCR gene from *Casuarina equisetifolia*. Primer pairs targeting the conserved domain was designed from their orthologs from *Populus, Betula, Hevea* and *Leucaena* and amplified in the genomic DNA of *C. equisetifolia*. An amplicon of 850 bp was cloned and sequenced. Sequence analysis revealed significant similarity to CCR orthologs from other tree species. The sequence was deposited to GenBank with Accession No. JQ982980. This is the first report on isolation of CCR gene fragment from this species and the study will form the basis for initiating genomic research to understand wood formation in *C. equisetifolia.*

1 Introduction

Wood is the most abundant biological resource on earth and an important raw material for wood based industries (Kavi Kumar et al., 2013). Lignin is an aromatic heteropolymer and present in the secondary thickened plant cell walls with cellulose and hemicelluloses. It is the second most abundant biopolymer in the earth after cellulose. Lignin biosynthesis is very complex and involves several enzymes involved in phenyl propanoid pathway (Vanholme et al., 2010; Hua et al., 2012). In the past decade, cloning and characterization of genes involved in lignin biosynthesis and modification of lignin content and composition in plants through genetic transformation, have provided insight into the lignin biosynthesis pathway (Ghosh et al., 2014; Giordano et al., 2014). Analysis of lignin biosynthetic genes and the biochemical characterization of the enzymes will help to decode the ambiguous points in the lignin biosynthetic pathway. Cinnamoyl CoA reducase (CCR) is a key enzyme in lignin biosynthesis and its expression was associated with tissues undergoing lignification (Lacombe et al., 1997; Lacombe et al., 2000). In transgenic tobacco, a significant down regulation of CCR expression was shown to impact both lignin content and S/G ratio (Piquemal et al., 1998; Cook et al., 2012). These results suggest CCR as a relevant functional candidate gene to controlling variation of lignin quantity and quality.

Casuarina equisetifolia is a short rotation tree species with high growth rate and broad ecological adaptability. The wood is a suitable raw material for paper production (Gurumurthi et al., 2001). Besides paper industry, the wood is used for beams, boatbuilding, electric poles, fences, furniture, gates, house posts, mine props, oars, pavings, pilings, rafters, roofing shingles, tool handles, wagon wheels, yokes and extensively cultivated for fuel, erosion control, and also as a windbreak. Hence the present study aimed at isolating a fragment of the CCR gene from *C. equisetifolia*.

2 Materials and Methods

Plant materials used in the present study were collected from IFGTB Model Silviculture nursery, IFGTB campus. Young needles were collected and stored at -20°C until further use.

2.1 Genomic DNA isolation

Hundred and fifty microgram of young needles were used for isolation of genomic DNA using the DNAeasy mini kit (Quiagen). The extracted genomic DNA was quantified using Nanodrop and the quality of genomic DNA was checked through 0.8% agarose gel electrophoresis.

2.2 Designing of Primers and PCR program

Three sets of degenerated primers viz, Dgp-1 (F) athggigcngtitayatgga & Dgp-1 (R) ccaigcngcytgytcigc; Dgp-2 (F) gcigarcargcngcitgg & Dgp-2 (R) gtiggdatnggrtaytcigg; Dgp-3 (F) gaygaraaraayccimg & Dgp-3 (R) ckiggrttyttytcrtc were designed based on the consensus sequences of the CCR orthologs from *Betula luminifera* (FJ410450.1), *Hevea brasilensis* (HQ229954.1), *Populus tricocarpa* (AJ224986.1) and *Leucaena leucocephala* (EU195224.2). The primer pairs were amplified in genomic DNA using the program: initial denaturation at 94 °C for 3 min; denaturation at 94 °C for 30 sec; annealing of primers at 51 °C for 30 sec; primer extension at 72 °C for 1 min; and final extension at 72 °C for 7 min and the reaction was repeated for 34 cycles.

2.3 Amplicon cloning and sequencing

The amplicon size of 850 bp was confirmed following an electrophoresis in a 1.2% agarose gel and cloned into pTZ52R/T vector using InsTAclone kit (Fermentas). Positive colonies were selected by blue/ white screening and sequenced.

2.4 Data analyses

The DNA sequences were analysed using NCBI Blast (http:// blast. Ncbi.nlm.nih.gov/) and Biology work bench (Clustal W).

3 Results and Discussion

The degenerated primer pair combination viz, (Dgp-1 (F)& Dgp-2(R)), amplified at 850 bp (Fig-1) and the product was cloned and sequenced. The sequence was analyzed and it showed significant similarity to CCR orthologs from several tree species including *P. trichocarpa*, *P. tomentosa, P. tremuloides, P. nigra* (90% - 93%), *B. luminifera* (93%), *H. brasiliensis* (86%), *Pyrus pyrifolia* (90%), *Prunus persica* (83%), *Leucaena leucocephala* (78%), *Eucalyptus species* (76%) and *Corymbia citriodora* (76%). The Blast tree view widget (Fig-2) indicated the CCR genes from *B. luminifera* and *P. trichocarpa* were closely related to its ortholog from *Casuarina*. The sequence was submitted in NCBI and assigned the accession number JQ982980**.**

4 Conclusion

The present study highlights the amplification of a CCR gene fragment of 850 bp from *C. equisetifolia*. The sequence of the CCR gene fragment showed significant homology with its orthologs from other tree species suggesting that the gene is highly conserved across genera. This is the first report on isolation of CCR gene from the genus *Casuarina* and the study will form basis for future genomic studies in this species.

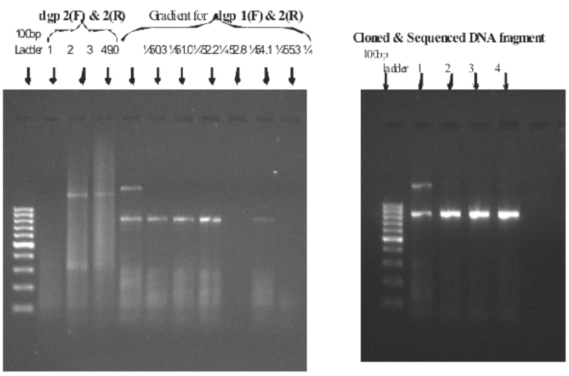

Fig-1. CCR gene specific profiles using degenerated primers in *Casuarina equisetifolia*

Acknowledgments

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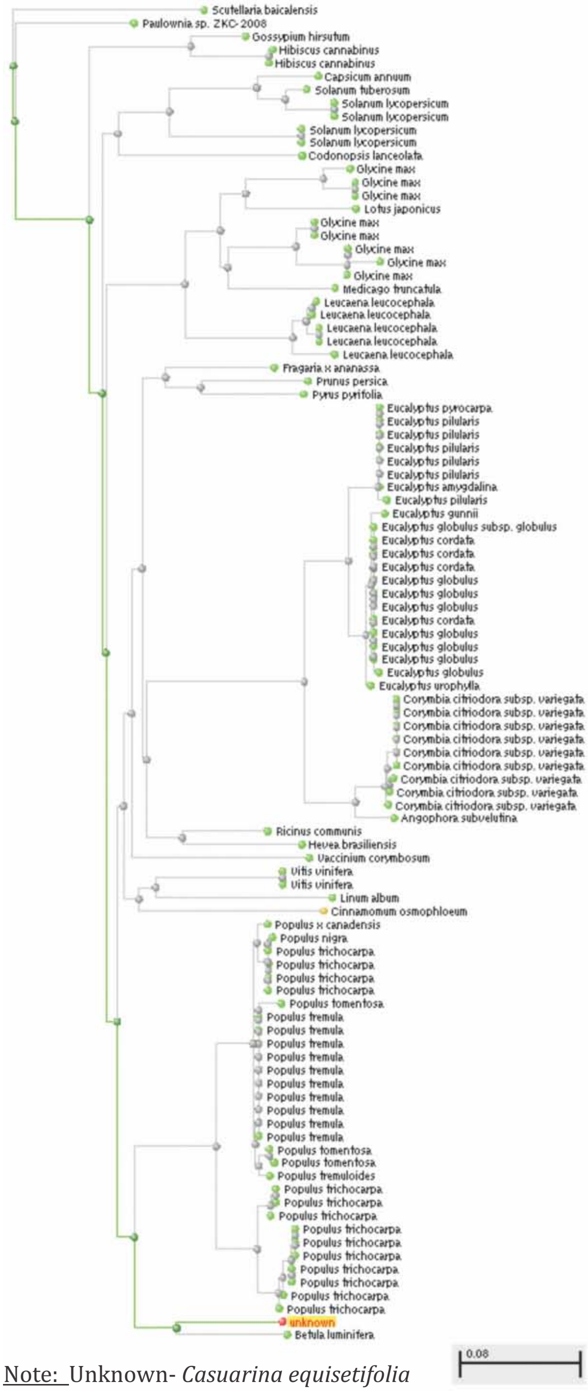
Fig-2. BLAST Tree view comparing CCR sequence from *C. equisetifolia* with their orthologs



Note: Unknown- *Casuarina equisetifolia*

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Selection and Evaluation of Casuarina Phenotypes for Windbreak Agroforestry System

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Key words: banana, branching traits, clones, wind damage, windbreak agroforestry system.

Abstract

Banana is the third important commercial crop in the western zone of Tamil Nadu State, India, covering an area of 30,000 ha and annual fruit production of 1.1 million metric tonnes. Banana growers are facing an annual loss of about USD 77,000 due to wind damages to the crop. In an attempt to address this recurrent problem, the Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, has taken up selection of phenotypes of Casuarina suitable for planting in windbreak agroforestry system. Twenty one phenotypes were selected by adopting 'point grading method' and assigning greater score for higher productivity and branching characters suitable for windbreak function. The selected phenotypes were propagated through rooting of shoot cuttings and a germplasm bank was established. On the basis of rooting ability and initial growth, ten clones were short listed and deployed in multilocation field testing. A widely cultivated male hybrid clone of *C. junghuhniana* x *C. equisetifolia* was used as check clone. Five clones of *C. junghuhniana viz.* CJ-6, CJ-8, CJ-9, CJ-17 and CJ-18 which registered superior values for growth and branching traits, were selected for planting in windbreak agroforestry systems.

1 Introduction

In the field of agriculture, drought, flood, extreme temperatures and strong winds are among the major natural causes for loss in crop and animal production. Probably since the beginning of agriculture, farmers have recognised the value of shelter provided by rows of trees or shrubs planted as windbreaks (Cleugh and Lahane, 2003). Windbreaks, planted as single or multiple rows of trees or shrubs in farm fields protect crops and livestock from strong wind, reduce wind erosion, improve irrigation efficiency, expand wildlife habitat, improve aesthetics, and provide marketable products (Angima, 2009). The effectiveness of a windbreak depends on the suitability of tree and shrub used and the planting density and spacing adopted (Wilkinson and Elevitch, 2000; Sackett et al., 2011). Planning of a windbreak is necessary to optimize its effectiveness and avoid future problems.

The nitrogen fixing ability and adaptability to grow in a wide range of soil and climatic conditions including moisture and nutrient limited sites makes Casuarina a preferred choice for commercial and environmental planting programs. The short rotation period of 3-4 years suits the average Indian farmer with a small landholding (Rawat et al., 2011). Its fast growth, straight stem, and light crown characteristics make it an ideal tree for agroforestry system (Viswanath et al., 2001; Saravanan et al., 2012). Badran and El-Lakany (1978) found *Casuarina* spp. suitable for growing as windbreaks and in salt and drought affected areas.

Banana is the third important commercial crop in the western zone of Tamil Nadu State, India. It is planted in about 30,000 ha and the annual fruit production is around1.1 million metric tonnes. The estimated loss due to wind damage for the banana growers is about USD 77,000 per year. The Institute of Forest Genetics and Tree Breeding (IFGTB) Coimbatore initiated selection of Casuarina phenotypes suitable for windbreaks. Certain phenotypes of *C. equisetifolia* and *C. junghuhniana* have shown unique branching characteristics which are ideal for the services sought from windbreaks (Nicodemus et al., 2001). Such phenotypes can be selected for developing windbreaks in banana-growing belt of the western zone of Tamil Nadu, which faces strong gusty winds during monsoon periods. The present study was carried out to select and evaluate the suitable phenotypes of casuarina for windbreak agroforestry system.

2 Materials and Methods

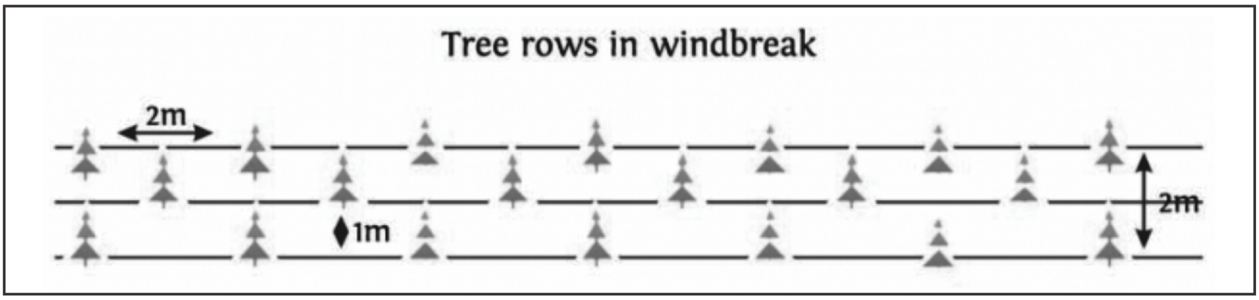
2.1 Selection of superior phenotypes

Twenty one phenotypes of Casuarina were selected from plantations in different locations of Tamil Nadu State viz., Mayiladuthurai, Karur, Tiruppur, Cuddalore, Erode, Neyveli, Tirupati and Coimbatore. 'Point grading method' adopted by Jayaraj et al. (1998) was used for selection of candidate phenotypes for windbreaks with modification by assigning greater score for more branchiness and other branch related traits. The candidate phenotypes were selected by comparing with the rest of the trees in each plantation in terms of height and diametrical growth as well as on the basis of branch characteristics.

2.2 Establishment of clonal bank

The branch cuttings collected from selected phenotypes were rooted and evaluated for rooting potential which showed large variation. After evaluating the rooting

Fig. 1. Planting configuration adopted in clonal trials in windbreak agroforestry system



potential, a clonal bank was established in the IFGTB campus. Early height growth rate was closely monitored on monthly basis for two years. Ten clones were selected based on the rooting ability and initial growth in clonal bank. These clones were further propagated to raise multilocation field trials for evaluating their superiority in terms of growth and branching traits under windbreak agroforestry systems.

2.3 Establishment of multilocation field trials

Three representative farm areas were identified where the damage to banana crop occurred earlier. The selected farms were located at Idikarai (latitude: 11° 07' N, longitude: 77° 58' E, altitude: 418 m, soil type: calcareous black soil), Kovilpalayam (latitude: 11° 10' N, longitude: 77° 07' E, altitude: 499 m, soil type: red gravely soil), and Narasimmanaicken Palayam (latitude: 11° 07' N, longitude: 76° 58' E, altitude: 325 m, soil type: deep black clayey soil).

2.3.1 Planting configuration

The shortlisted 10 windbreak clones were planted in three rows along the boundaries of a banana plantation as a windbreak agroforestry system. A widely planted hybrid clone of *C. junghuhniana* x *C. equisetifolia* and a seedling accession were used as benchmark. The spacing between the rows was 1 m and the spacing within rows was 2 m. Pattern adopted was zig-zag configuration (Quincunx planting method) as depicted in Fig. 1.

2.3.2 Data collection

Data has been collected on six traits *viz.* girth at breast height (cm), total height (m), number of branches up to 3 m height from the ground, branch length (cm), branch thickness in mm (mid-girth of branch) and branch angle in degrees (to the main stem) at the age of 1.5 years. Superiority of test clones over check clone was calculated for each of the six traits. Finally superiority percentage for the six traits was added by dividing cumulative superiority percentage by 100. A "Cumulative Superiority Index" has been developed for final ranking (Table 1).

Cumulative Superiority Index = cumulative superiority percentage for six traits / 100

Results and Discussion

Rooting ability of the initially selected 21 phenotypes varied from 40% to 94% with a mean of 83%. Previous

Table 1. Volumetric growth and branching traits of *Casuarina junghuhniana* clones under windbreak agroforestry system at 1.5 years age

Clone Number	Diameter (cm)	Total height (m)	Number of branches	Branch length (cm)	Branch angle (degrees)	Branch thickness (mm)
CJ-5	3.44 ±0.46	4.98 ± 0.72	41.8 ± 4.27	97.7 ± 14.46	74.5 ± 4.11	16 ± 2.96
CJ-6	3.23 ± 0.37	5.83 ± 0.49	46.3 ± 1.55	119.6 ± 12.17	77.4 ± 2.17	16 ± 0.58
CJ-7	3.08 ± 0.40	5.57 ± 0.59	41.3 ± 4.82	89.8 ± 13.65	72.1 ± 4.88	15 ± 0.88
CJ-8	3.60 ± 0.62	5.88 ± 0.76	43.5 ± 6.40	138.3 ± 19.24	66.3 ± 2.63	22 ± 1.33
CJ-9	3.35 ± 0.73	5.14 ± 0.75	39.8 ± 6.80	143.9 ± 29.86	68.1 ± 3.66	18 ± 2.40
CJ-17	3.81 ± 0.95	5.45 ± 0.68	40.8 ± 5.76	106.0 ± 17.48	82.0 ± 1.94	19 ± 2.19
CJ-18	3.72 ± 0.87	5.32 ± 0.50	43.3 ± 5.89	129.6 ± 15.45	68.1 ± 3.81	19 ± 2.31
CJ-20	2.97 ± 0.32	4.13 ± 0.52	38.3 ± 3.57	100.5 ± 18.82	66.7 ± 3.89	17 ± 1.53
CE-15	2.73 ± 0.72	3.82 ± 0.50	42.3 ± 1.18	110.0 ± 20.31	69.9 ± 2.70	18 ± 1.86
Seedlings	2.54 ± 0.35	4.23 ± 0.94	40.7 ± 3.06	91.4 ± 19.23	74.7 ± 4.58	18 ± 2.31
CJ-12 (Check clone)	2.31 ± 0.31	3.92 ± 0.43	40.5 ± 3.66	81.1 ± 12.55	62.5 ± 3.68	15 ± 2.00

Values presented are mean ± standard error

Table 2. Cumulative Superiority Index (CSI) of *Casuarina junghuhniana* clones for windbreak agroforestry system

Rank	Clone Number	Girth	Height	Number of branches	Branch length	Branch angle	Branch thickness	Cumulative Superiority Index (CSI)
1	CJ-8	56.01	49.99	7.4	70.5	6.1	44.4	2.34
2	CJ-18	61.19	35.63	6.8	59.7	9	26.7	1.99
3	CJ-17	65.30	38.91	0.6	30.7	31.2	23.3	1.90
4	CJ-9	45.41	31.22	-1.9	77.5	8.9	22.2	1.83
5	CJ-6	39.96	48.82	14.4	47.5	23.8	5.3	1.80
6	CJ-5	49.10	27.10	3.1	20.5	19.2	5.3	1.24
7	CJ-7	33.74	42.01	1.9	10.7	15.4	-3.3	1.00
8	Seedlings	10.04	8.02	0.4	12.7	19.5	20	0.71
9	CE-15	18.61	-2.59	4.5	35.7	11.8	17.3	0.85
10	CJ-20	-10.01	5.44	-5.6	24	6.7	12.9	0.33

Values are percentage superiority over value of benchmark clone CJ-12 mentioned in Table 1.

studies revealed that the vegetative propagation of *C. junghuhniana* for mass planting varied from 40% to 90%, depending on the time of year in India (Kondas, 1983). The overall mean height growth of 21 phenotypes was 1.90 m at 180 days after planting in clone bank, while the mean value for these ten selected clones recorded was 2.40 m. Mean height increased to 3.87 m at the end of second year for all the 21 phenotypes and at the same period, the selected 10 clones registered a mean height of 4.0 m.

Volumetric growth and measurements on branching traits are presented in Table 1. Large variation was observed in growth and branching traits among clones. The windbreak phenotypes registered girth values between 10.13 and 11.96 cm whereas the check clone had only 7.24 cm. Similarly, mean height ranged from 5.14 to 5.88 m for the selected clones compared to 3.92 m of the check clone. In India, *Casuarina* species and their hybrids grow up to 3 m per year (Zhong et al., 2011). Nicodemus (2009) reported a wide range of growth parameters in 4-year-old *C. equisetifolia* plantations, with height ranging from 12 m to 20 m and girth ranging from 25 cm to 50 cm. In Tamil Nadu, the reported overall mean annual increment of girth for *C. equisetifolia* was 5.4 to 11 cm per year and for height 3 to 6 m (Ravi et al., 2013). Growth rate observed in the present study is tending towards the maximum values reported for *C. equisetifolia* which can be attributed to the superiority of the clones as well as to the fact that trees were grown in irrigated condition along with banana crop. The "Cumulative Superiority Index" of different clones varied from 0.33 to 2.4 (Table-2). Five clones viz. CJ-6, CJ-8, CJ-9, CJ-17 and CJ-18 which registered the highest values for growth and branching traits are recommended for deployment in windbreak agroforestry systems as it is reported that the highly branched swinging twigs and the 10+ m height of Casuarina can effectively reduce the wind energy and velocity for a distance of 130 m (Sims, 1945).

Windbreak agroforestry offers a new scope for increasing industrial wood supply in Tamil Nadu which has around 4.89 million ha of cultivated area. If two-thirds of such area is brought under windbreak agroforestry system, around 3 million ha will get protection from wind damage. On an average 600 trees can be planted in a hectare as three rows of windbreak system all around the farm, thus approximately 1800 million trees can be planted. With an anticipated wood production of 8 tonnes ha^{-1}, 2.4 million tonnes of wood can be produced through windbreak agroforestry systems in a 3 years' harvest cycle. It is an important fact that windbreak agroforestry system will not replace the agriculture crops while increasing the tree cover and industrial wood supply in the State.

4 Conclusion

Clones of *Casuarina junghuhniana* showed large variation in growth and branching characters under windbreak agroforestry system offering scope to select for both productivity and protection from wind damage.

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Performance of *Casuarina equisetifolia L.* in Farm Forestry Plantation in Tamil Nadu, India

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Keywords: decomposing microorganisms, litter fall, litter decomposition, plantation forestry, nutrient cycling.

Abstract

Distribution, tree growth, biomass, litter fall, litter decomposition, nutrient return through litter, nutrient cycling and litter decomposing microorganisms were quantified in an age series of 1-, 2- and 3-year-old trees in high density plantation (ca.10,000 trees ha^{-1}) of *Casuarina equisetifolia* in farm forestry in coastal zone of Tamil Nadu, India. One-year old trees attained 2.6 cm DBH with a total height of 3.8 m and 0.0017 m^3 volume. Three-year-old trees had 6.5 cm DBH with a total height of 13.3 m, and volume of 0.0372 m^3. Litter fall ($t\ h^{-1}\ year^{-1}$) was recorded at the rate of 0.64 in one-year, 4.69 in two-year and 5.19 in three-year-old plantations. Nutrient turnover through litter was in the order of Ca> N> K> Mg>Na>P>Fe> Zn >Cu>Cr in all age series. The constant value of annual decomposition (k) was 1.83. Higher values for decomposition rate were recorded during rainy season. Seven fungal species were isolated and identified in litter accumulated in the three year old plantation.

1 Introduction

The substantial socio-economic importance of casuarinas (several members of the family Casuarinaceae) has ensured ongoing global interest in research and development of this group of nitrogen fixing trees. Increasing availability and profitability of off-farm employment as a result of economic and commercial development reduce the significance of agricultural production both in terms of sustenance and farm income. Casuarina is popular among farmers of Tamil Nadu State in India since it provides attractive returns (from tree crop and intercrop) within a short period by farmers. Income from Casuarina cultivation is on par with agricultural crops but it requires lower input cost. Analysis of secondary data collected by the Statistical Department, found substantial number (41%) of farmers had adopted casuarina in farm forestry system. Totally 62,373 hectares of Casuarina were cultivated in all districts during 2008-2009. Nearly 80% of farm forestry plantations are raised in, Cuddalore, Kancheepuram, Villupuram and Thiruvallur districts.

The present study was undertaken to understand the impact of high density plantation (ca. 10,000 trees ha^{-1}) of *Casuarina equisetifolia* in farm forestry in coastal zone of Tamil Nadu, India harvested in short rotations of two to four years. The distribution, tree growth, biomass, litter fall, litter decomposition, nutrient return through litter, nutrient cycling and litter decomposing microorganisms were quantified in an age series of 1-, 2- and 3-year-old trees.

2 Planting Material

In Tamil Nadu, Casuarina seedlings are raised commercially for income generation by farmers in Aandikuppam and Pillaiyarkkuppam in Villupuram districts. More than 80% farmers of Swamiyarpet village of Parangipettai region of Villupuram District commercially benefitted from supplying Casuarina seedlings to farmers of other areas throughout the year. In recent years the paper industry has been distributing quality planting material to farmers at low cost. Seeds from orchards developed by the Institute of Forest Genetics and Tree Breeding are now widely used for seedling production.

3 Tree Growth

Tree growth (height (m), diameter at breast height (cm) and volume (m^3)) of 1-, 2- and 3-year-old *Casuarina equisetifolia* plantation in farm forestry is presented in Table 1. The trees attained 2.6 cm diameter at the breast height (DBH) with a total height of 3.8 m and 0.0017 m^3 volume at 12 months after planting. In 24 months after planting it was 4.6 cm in DBH, 8.9 m of total height and 0.0118 m^3 of volume. When the trees attained harvestable age at 36 months, DBH was 6.6 cm with a total height of 13.4 m, and volume of 0.0372 m^3.

Increase in the growth of trees is dependent on the availability of light, moisture and nutrients. Therefore, regular irrigation helps making nutrients available in the rhizosphere and also beneficial microorganisms may be influenced by plant roots directly in tripartite association with *Frankia* and arbuscular mycorrizha (Reddell, 1990; Rajendran and Devaraj, 2004). Growth is also influenced by intensive silvicultural management practices such as superior genetic material, appropriate spacing, regular watering, weeding and manuring. However, the growth of trees was markedly higher while adopting systematic cultivation method using suitable biofertilizers recording

Table 1. Diameter at breast height (DBH, cm), height (m), and volume (m^3) of 1-,2-, and 3-year-old farm forestry plantation of *Casuarina equisetifolia*

Age (years)	DBH (cm)	Height (m)	Volume (m^3)
1	3.04 ±0.17	4.10 ±0.16	0.0026 ±0.0003
2	6.31 ±0.16	9.87 ±0.26	0.0247 ±0.002
3	7.40 ±0.16	13.88 ±0.26	0.0474 ±0.003

height ranging from 9.8 to 11.9 m and DBH from 5.3 to 7.4 cm at 2 years age (Rajendran and Devaraj, 2004).

4. Total Biomass

Biomass accumulation of tree components in 1, 2- and 3-year-old Casuarina plantations was recorded as 2.95, 11.11 and 18.92 kg per tree respectively under high density cultivation (ca. 10,000 trees per ha) (Table 2). In view of these results, it seems that fast-growing species like *C. equisetifolia* rapidly accumulate biomass and carbon during the early ages of its life span under well-managed farm forestry conditions. The above- and below-ground biomass allocation of 1-, 2- and 3-year-old plantations ranged from 86 to 91 % and 9 to14 % respectively. This shift in the accumulation of biomass and carbon may be related to intensive silviculture management such as superior genetic materials, appropriate distance, regular watering, weeding and manuring. Contribution of different tree components to the total biomass of a 3-year-old *C. equisetifolia* plantation in farm forestry is depicted in Fig. 1.

5 Litter Production

Seasonal variation of the litter fall on unit area basis is given in Table 3. The annual litter production was 0.64, 4.69 and 5.18 tonnes per hectare during first, second and third year respectively. Maximum litter fall was observed during

Fig. 1. Contribution of different tree components to the total biomass of a 3-year-old *Casuarina equisetifolia* plantation in farm forestry.

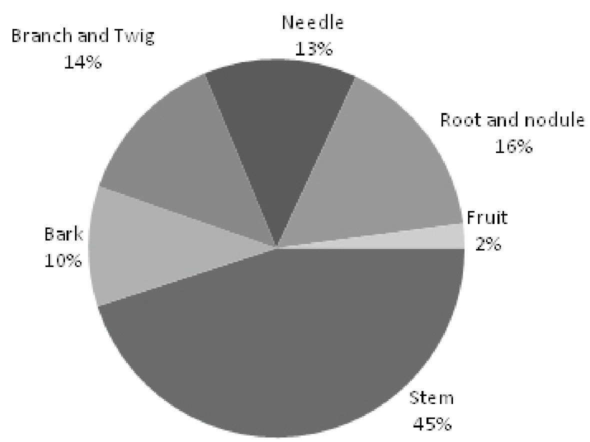

Table 2. Biomass (kg) of different components in 1-, 2 and 3-year-old *Casuarina equisetifolia* plantations in farm forestry

Age (year)	Stem	Bark Twig	Branch & Needle	Fruit	Root	Root	Nodule	AGB	BGB	Total Biomass
1	1.23±0.03	0.09±0.003	0.43±0.010	0.85±0.02	NA	0.36±0.02	0.002±0.000	2.59±0.06	0.36±0.10	2.96±0.07
2	4.68±0.20	1.12±0.120	1.49±0.210	2.57±0.16	NA	1.22±0.98	0.035±0.001	9.86±0.64	1.26±0.10	11.11±0.73
3	8.52±0.19	1.95±0.130	2.54±0.090	2.49±0.09	0.40±0.010	2.95±0.15	0.078±0.014	15.90±0.34	3.03±0.16	18.93±0.48

AGB-Above Ground Biomass; BGB- below Ground Biomass; ±Standard error. NA - Not assessed

Table 3. Monthly litter production (mean ± SE) in a 3 - year old *Casuarina equisetifolia* plantation in farm forestry

Month	Litter production (g m^{-2})		
	One year	Two years	Three years
January	_	35.45 ±0.924	41.49 ±1.390
February	_	43.66 ±1.106	42.26 ±1.344
March	_	37.09 ±0.268	43.23 ±0.879
April	_	53.48 ±0.397	51.29 ±1.169
May	_	57.28 ±0.499	60.44 ±0.465
June	_	60.25 ±0.748	64.49 ±2.055
July	13.99 ±0.372	40.36 ±0.701	46.45 ±1.604
August	13.79 ±0.662	22.24 ±0.315	36.28 ±0.234
September	11.88 ±0.335	28.96 ±0.399	28.20 ±0.676
October	10.13 ±0.537	23.49 ±0.322	35.77 ±0.677
November	08.01 ±0.456	32.23 ±0.375	30.55 ±0.422
December	06.41 ±0.481	35.29 ±1.159	38.40 ±0.425
Total	64.21	469.78	518.85

rainless summer months between April and June (40-45%). Biomass production is directly related to the availability of plant nutrients and growth rate of the species. The presence of a large number of trees in a unit area exerts pressure on the soil nutrients. However, some amount of nutrient is returned to the soil through litter fall. In the present study, litter fall was found to be unimodal and directly proportional to the age of plantation. No detectable amount of litter fall was found during the first six months of growing trees in the plantation. The peak period of litter fall coincided with the dryr periods of the year when evapotranspiration and temperature were on the rise. The present estimate of litter production 5.19 t ha^{-1} in a three-year-old plantation was higher compared to previous report of 4.32 t ha^{-1} from a coastal district of Tamil Nadu, India (Rajendran, 2001). It reveals that intensive management may help in higher litter production and nutrient recycling. The litter accumulation in 3.5-year-old *C. equisetifolia* plantation in Puerto Rico was much higher 16.2 t ha^{-1} year $^{-1}$ (Lugo et al., 1990) when compared to this estimation. Similarly, 3-year-old *C. glauca* recorded the highest litter of 848 g cm^{-2} compared to temperate and sub-tropical forests in Australia (Clarke and Allaway, 1996).

6. Nutrient Return through Litter

Nutrient concentration in fallen litter of *C. equisetifolia* is shown in Table 4. Carbon content of litter fall was 2.06 t ha^{-1} in 3-year-old plantation followed by that of 2- and 1-year-old plantations. Nutrient return through litter was in the order of Ca>N>K>Mg>Na>P>Fe>Zn>Cu>Cr in all age series.

Carbon content of litter fall was higher in 3-year-old plantation than in 2-year-old. Similar order was also reported in *C. equisetifolia* plantation at Coimbatore, Tamil Nadu, India (Rajendran and Devaraj, 2004). Nutrient cycling was high in a 3-year-old *C. equisetifolia* plantation and it may be due to more litter production and nutrient concentration (Fig. 2). Similarly, litter nutrient content in kg ha^{-1} varied from 100 to 256 for N, 11 to 18 for K, from 45 to 150 for Ca and 13 to 29 for Mg (Wang et al., 1991). Litter production was 0.86 kg $tree^{-1}$ for trees planted on farmland and nutrient content (N, P, K, Ca and Mg) of 2- year-old trees were 4.87, 0.22, 5.02, 4.59 and 0.52 g $tree^{-1}$ respectively as reported by Rajendran and Devaraj (2004).

Fig. 2. Nutrent cycling of Casuarina equisetifolia (t ha^{-1}) (Age-3years; density-10,000 trees ha^{-1})

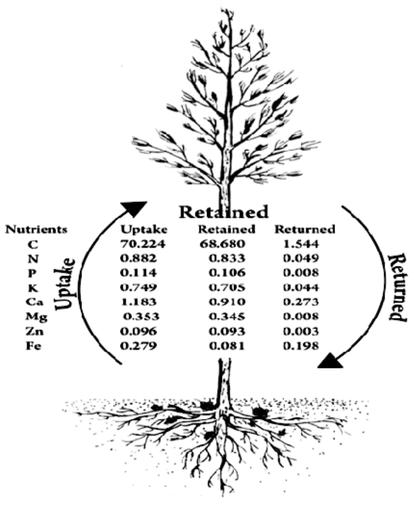

Table 4. Litter production, carbon content, and nutrient return through litter of 1, 2 and 3 year old *Casuarina equisetifolia* plantation in farm forestry.

Age (year)	Litter production (t ha^{-1})	C (t ha^{-1})	Nutrient return through litter (kg ha^{-1})									
			N	P	K	Ca	Mg	Fe	Zn	Cr	Na	Cu
1	0.64	0.21	10.12	1.16	2.44	11.82	1.97	0.15	0.02	0.01	1.60	0.02
2	4.69	1.69	75.78	8.95	17.10	90.22	15.32	1.21	0.23	0.07	12.23	0.19
3	5.19	2.06	87.85	9.49	18.88	99.16	18.01	1.41	0.31	0.08	13.85	0.29

7 Litter Decomposition

A linear regression was developed between mass loss of litter and decomposition period (days) and it showed significant and positive correlation ($r > 0.90$, $P \leq 0.05$). Relative litter decomposition rate was considered monthly and the value of annual decomposition constant was 1.83. Decomposition was faster during rainy season in November. The linear regression was developed between weight loss of litter and independent climatic variables (temperature, relative humidity and rainfall) with carbon of Casuarina litter showing a significantly positive correlation. Higher values for decomposition rate were recorded during rainy season.

7.1 Litter decomposing microorganisms

In the present study, seven dominant fungal species isolated and identified were *Alternaria alternate, Aspergillus niger, Penicilium* sp., *Rhizopus nigricans*, *Trichoderma viride, Curvularia lunata* and *Curvularia eragrostidis*. Two dominant bacterial species were isolated and identified, viz., *Pseudomonas fluorescens* and *Azospirillum brasilense.* In addition, basidiocarp of *Pleurotus florida* was predominantly found in litter accumulated in Casuarina plantation.

7.2 Litter decomposing macroorganisms

Populations of macroorganisms capable of decomposing fallen litter of Casuarina were: *Eudrilus eugeniae* in count of 3 per each 30 cm^2, lancetooth molusca (*Haplotrema vancouverense*) in an average of 2 m^{-2} and termites (*Odontotermes obesus* Rambur.) which were found in abundance.

The rate of biomass accumulation determines the nutrient demand of the site. In the case of *C. equisetifolia* plantation in farm forestry very high biomass accumulation in a short rotation period leads to high level of nutrient removal. However, Casuarinas are highly efficient in both litter production and nutrient uptake as indicated by the enormous increase in element concentration. In arid and semiarid lands, Casuarina litter adds good levels of all nutrients and minimizing water loss through evaporation.

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Casuarina equisetifolia based Agroforestry Systems for High Economic Returns for the Farming Communities in Tamil Nadu, India

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Key words: agroforestry species, growth, net annual return, annual agricultural crop

Abstract

Casuarina equisetifolia has emerged as an important fast growing species highly suitable for agroforestry, plantation forestry and industrial plantation. Due to its multipurpose nature, farmers, especially from the coastal areas in Tamil Nadu, India show interest in Casuarina cultivation for high economic returns. This species grows in a wide range of soil and climatic conditions and has a stable market for pulpwood, pole, fuel wood and props. The present study was conducted for evaluating Casuarina based agroforestry system in comparison with other fast growing trees viz. *Eucalyptus, Melia dubia,* and *Gmelina arborea* for growth and economic returns. Casuarina was intercropped during the first year with various annual crops like cotton (*Gossypium hirsutum*), groundnut (*Arachis hypogea*) and black gram (*Vigna mungo*). The economic return from Casuarina agroforestry systems was worked out and compared with the other tree-based systems along with the traditional agriculture. The results showed that Casuarina with groundnut-based agroforestry system registered the highest net annual return of USD 948 ha^{-1} yr^{-1} followed by Casuarina with black gram (USD 853 ha^{-1} yr^{-1}) and Casuarina with cotton (USD 796 h^{-1} yr^{-1}) and the traditional agriculture, only rice cultivation (USD 752 ha^{-1} yr^{-1}). Casuarina based agroforestry system also recorded higher return compared to the other fast growing tree based agroforestry system within a short rotation period of 3-4 years. Further, this paper discusses the growth pattern of Casuarina based agroforestry system, reasons for adoption of tree farming, marketing strategies and economic analysis in detail.

1 Introduction

Casuarina equisetifolia is an important multipurpose tree, widely grown in tropical and subtropical countries for a wide range of end-uses like fuel wood, charcoal making, props and pulp wood. Due to its multipurpose utilization and higher productivity, it is widely planted in the inland and coastal areas of the peninsular India. Agroforestry is gaining importance as a land use practice in different parts of India with the recent emphasis on sustainable agriculture. Tree-based land use practices could accompany both tangible and intangible benefits securing the interests of both local and global communities (IRS, 2005).

In the State of Tamil Nadu, *C. equisetifolia* is mainly grown in the districts of Cuddalore, Nagapattinam, Villupuram, Kancheepuram, Tiruvallur, Pudukottai, Tiruchirappalli, Thanjavur and Ramanathapuram covering different soil types in both coastal and inland areas. The most widely adopted spacing is 1x1 m with a rotation period of 3 to 5 years depending on irrigation facilities, market price fluctuation and farmers' need (Buvaneswaran et al., 2010). In Tamil Nadu, wood yield from four year old *C. equisetifolia* plantations is 60 to 80 t ha^{-1} under dry conditions and 80 to 120 t ha^{-1} under irrigated conditions (Venkatesan and Srimathi, 1989).

Agrisilviculture is an important system practiced by the farming communities in which fast growing and multipurpose trees remain compatible with annual crops and provide maximum economic returns in a sustained manner. The productivity of crops under agrisilviculture system gets affected due to competition for light, water and nutrients between trees and annual crops (Kareemulla et al., 2003). Planting multipurpose trees is an integral part of agroforestry for supplying products like wood, small timber, fodder and fruits, and importantly reduce the risk of crop failure in deficit rainfall years and act as a 'bio-insurance' to the farming communities. Tree species in agroforestry systems not only retard moisture evaporation but also improve soil properties. In a changing scenario of decreasing availability of agricultural land, degradation of soil and water resources, the new approaches in farming systems have made rapid strides in the last two decades.

Deliberate introduction of multipurpose trees like Casuarina in the existing farming systems and adoption of agroforestry approach is one of the viable options to achieve sustainability while optimizing productivity. Keeping in view of the above facts, the present study was undertaken to assess the productivity and economics of the Casuarina-based agroforestry system and compare it with the other fast growing tree species.

Table 1. Growth parameters of *Casuarina equisetifolia* in agroforestry system

Age (Years)	Casuarina with Black gram		Casuarina with cotton		Casuarina with groundnut		Pure plantation	
	Ht (m)	GBH (cm)	Ht (m)	GBH (cm)	Ht (m)	GBH (cm)	Ht (m)	GBH (cm)
1	4.0	5.0	3.5	4.5	4.2	5.2	3.5	4.0
2	8.5	13.5	8.5	13.0	9.4	14.5	8.0	12.0
3	12.6	26.5	12.5	24.0	13.7	28.5	11.5	24.0

2 Materials and Methods

2.1 Study area

The study zone included the Districts of Kancheepuram Vellore, Cuddalore and Villupuram. The total geographical area of the zone is 31,194 km^2. The area under cultivation is 50.5 percent of the total area of cultivation. The normal annual rainfall is 1109 mm. Major rivers are Polar, Ponniar, Cheiyear, Vellar, Thenpennai, Manimuthar and Komugi. Major dams used for irrigation are Mettur, Sathur, Veedur, Komugi, Manimuthar and Wellington. Irrigation is practiced through canal, well, lakes and dams. The major crops are paddy, maize, millets, groundnut, sugarcane and cashew nut. Kancheepuram District which is known as "Lake District" and also where the popular "Madhurandhagam Lake" present comes under this zone.

2.2 Establishment of agroforestry models

Casuarina-based agroforestry model was laid out in wide alleys at a spacing of 2 m x 1 m. Control plots were also established (crops without trees) for comparison and monitoring purpose. Land was ploughed and watering channels were made 8 m apart and 30x30x30 cm pits were dug in the channels at 2 m distance for establishment of casuarina based model. The density of casuarina was 5000 trees ha^{-1}. Before planting of casuarina, annual crops were sown at proper espacement. The experiment was conducted for a period of three years.

2.3 Observations made

After the establishment of field trial, periodical observation on growth parameters of tree component (including girth and height of the trees) and yield of annuals was recorded for calculation of the economics of the agroforestry systems. The sole crop yield was also recorded for comparison.

3 Results and Discussion

3.1 Reason for adopting tree cultivation

Studies were conducted on socioeconomic aspects of tree cultivation in farmlands in Tamil Nadu and the study revealed that, among the reasons for taking up tree cultivation, higher returns in a short rotation period compared to agriculture was cited as the major reason and ranked first with greater mean score of 54.33 under 'Garrett Scoring Technique' (Garrett and Woodworth, 1969). Low risk involved in tree cultivation ranked second and minimal care ranked third.

3.2 Growth attributes

Growth attributes of casuarina in the experimental plot up to three years' age in different agroforestry systems are provided in Table 1. It was observed that trees exhibited better growth under agroforestry system when compared to other planting configurations (block plantation). The girth and height of the trees increased steadily as age increased and attained a girth at breast height of 28.5 cm and height of 13.7 m at the end of third year. Irrespective of the agricultural crop, casuarina under agroforestry system registered more girth compared to sole crop.

3.3 Yield and economics of the Casuarina based agroforestry system

From the established agroforestry system, the yield has been worked out for pure Casuarina, pure agriculture and tree-crop combination based agroforestry systems for the period of three years. In the above model, intercropping activities have been carried out and the yield has been assessed. The economic productivity of the model was worked out and the average annual net income has been determined for Casuarina with different agricultural crops. Casuarina with groundnut based agroforestry system registered the highest net annual return of USD 1,326 ha^{-1} yr^{-1} followed by Casuarina with black gram (USD 1243 $ha^{-1}yr^{-1}$) and Casuarina with cotton (USD 1142 $ha^{-1}yr^{-1}$) and the traditional agriculture, only rice cultivation (USD 1021 $ha^{-1}yr^{-1}$).

When compared to agroforestry systems involving other fast growing trees like Eucalyptus, Melia and Gmelina, Casuarina-based agroforestry system registered higher economic returns in a short rotation period. It can be further enhanced if the potential of clonal plantation with uniform growth and high yield is utilized in agroforestry systems as is being practiced in China. In China's Hainan province, a single clone Bao-9 comprised over 95% of *C. equisetifolia* plantation during the year 2000 (Zhong et al., 2010).

4 Conclusion

Agroforestry models developed in farmlands with casuarina as the tree component and annuals like groundnut, black gram and cotton, as intercrops in a alley

Table 2. Economic analysis of different *Casuarina equisetifolia* based agroforestry systems (values in USD).

Particulars	Casuarina with Black gram	Casuarina with cotton	Casuarina with groundnut	Pure plantation
Cost of cultivation of tree component including maintenance (ha^{-1})	261.2	380.6	306.0	216.4
Cost of cultivation of annual crops for 3 years (ha^{-1})	196.3	187.5	216.4	NA
Benefit from tree components	2089.6	2089.6	2089.6	2686.6
Benefit from annual crops	925.4	865.7	1276.1	NA
Net income	2557.5	2387.2	2843.3	2470.1
Average annual net income	852.5	795.7	947.8	823.4

NA-Not applicable

cropping pattern for a short rotation period of 3 years yielded around 40 tonnes per ha resulted in higher economic returns.

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Evaluation and Biochemical Characterization of Casuarina Clones Resistant to the Bark Feeder *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae)

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Abstract

Casuarina equisetifolia L. is highly susceptible to the bark feeding caterpillar *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae). Girdling by the larvae make the stem weak and consequently the poles break at the point of infestation. Clones selected from provenances that shown tolerance/resistance to *I. quadrinotata* were raised in a bark feeder prone area to assess the growth and pest incidence. A few provenances and landraces of *C. equisetifolia* showed better growth, compared to local seed sources and were observed to be free from the pest infestations. *C. junghuhniana* seed from a seedling seed orchard was the fastest growing and also had no infestation. Attempt has been made to correlate the levels of bark feeder resistance in clones/provenances of Casuarina to the qualitative and quantitative variations in the chemicals present in the bark, besides the volatile profiles interfering with the behavioural modulations of the bark feeder.

1 Introduction

Casuarina equisetifolia L. is an important multipurpose tree species mainly grown for fuel wood, pulpwood and poles. It is also preferred for environmental services like windbreak and shelterbelt in many countries. In India, Casuarina is extensively planted by farmers as a short rotation commercial crop to supply pulpwood to paper industries. Insect pests, native as well as invasive, infesting tree species leading to outbreaks in plantation areas have become a regular event (Sasidharan, 2004). Casuarina is highly susceptible to bark feeding caterpillar *Indarbela quadrinotata* Walker (Lepidoptera: Metarbelidae). Girdling by the larvae makes the stem weak and consequently the poles break at the point of infestation resulting in reduced wood production. Although various chemical control measures are available to manage the bark eating caterpillar, its large scale application is neither eco-friendly nor economically viable. In this context, host plant resistance can be considered as an important tool for managing the bark eating caterpillar to minimize economic loss to farmers and industries.

Because of the concealed nature of the pest, the usual control measures are not successful in the plantations. Identification of bark feeder resistant/tolerant provenances warrants detailed investigation into the mechanism of host plant resistance against the pest. With this knowledge, tolerant varieties could be developed either through conventional tree breeding or biotechnological approaches. Therefore the present study was conducted to assess the incidence of the bark feeder, evaluate the impact of its attack on the growth of clones and to understand the chemical basis of host plant's resistance to the pest.

2 Materials and Methods

2.1 Establishment of clonal trial

Ten clones from provenance and landrace seedlots of *C. equisetifolia* showing no or low infestation of the pest were selected in the international provenance trial located at Puducherry (Balu et al., 2001; Table 1). They were propagated by rooting shoot cuttings and a clonal trial was established at Sirkali, Nagapattinam District, Tamil Nadu (11.2390° N, 79.7360° E) which is a hot spot area for bark feeder incidence. The 10 clones and one seedlot each of *C. equisetifolia* and *C. junghuhniana* were planted in a randomized block design with 12 replications and 3 trees of each accession per replication. Eight replications were planted at a spacing of 1 x 1 m and the remaining at 1 x 2 m. Height and girth were recorded at ages 6 months, one year and four years. Assessment of the clones for pest tolerance was made from second to fourth year after planting.

2.2 Analysis of biochemical parameters

Biochemical analysis of bark and wood of three year old trees sampled from three randomly chosen branches was carried out for phenols (Malik and Singh, 1980), tannin (Robert, 1971), Lipids (Floch et al., 1957), phenolic acids (Harbone, 1964) and free fatty acids (Dey and Harbone, 1993). The powdered material of the three branches were mixed together and used for chemical estimation.

2.3 Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA), and the mean differences were compared by Duncan's Multiple Range Test. All analyses were performed using SPSS for Windows (16.0) statistical software package

Table 1. Casuarina clones and seedlots studied for resistance to bark feeder infestation

Accession Number	CSIRO seedlot number	Provenance	Country
Casuarina equisetifolia			
1	18376	Sabah	Malaysia
2	18141	Robinson Island	Kenya
3	18153	Ela Beach	PNG
4	18161	Sarawak	Malaysia
5	15958	Queensland	Australia
6	18127	Ha Thinh	Vietnam
7	18013	Chandipur	India
8	18154	Panay Island	Philippines
9	16166	Northern Territory	Australia
10	18272	Viti Levu	Fiji
11	--	Local source	India
C. junghuhniana			
12	IFGTB	Seedling Seed Orchard	India

3 Results

3.1 Growth and pest incidence in clonal trial

The local seed source of *C. equisetifolia* showed the maximum infestation of 28 to 32% across the three years of observation followed by 15% in the clone of Sarawak provenance from Malaysia. Only 2 to 4% trees of clones of Philippines and Fiji origin showed insect damage. All other accessions including the *C. junghuhniana* seedlot remained pest-free throughout the study period (Table 2). Interestingly, clone of Chandipur, India (accession 7) showed no infestation whereas the other local seedlot was heavily infested. Similarly the accession 1 of Sabah provenance, Malaysia remained unaffected whereas Sarawak accession 4 from the same country showed moderate infestation. Growth performance of *C. junghuhniana* was significantly better than all accessions of C. *equisetifolia.* At the end of fourth year, *C. junghuhniana* seedlings, PNG, Kenya, NT Australia and Sabah, Malaysia were showing good growth in 1x1m spacing whereas *C. junghuhniana* seedling, Sabah, Malaysia, QL Australia, PNG and Kenya were performing better in 2 x1m spacing.

3.2 Analysis of biochemical characters

3.2.1 Moisture

The moisture content of the wood ranged from 38% in Queensland Australia to a maximum of 42% in Philippines. Moisture content in the bark was higher than that of the wood in all the clones. The bark of local variety *C. equisetifolia* recorded a moisture content of 50.6%, while *C. junghuhniana* showed 55.3% (Table 3).

3.2.2 Tannin

A comparison of the total tannin content in the wood and bark of Casuarina clones showed that the bark has much higher content of tannins (Table 3). The clone Chandipur recorded the highest tannin content (391.3 mg g^{-1} dry weight) followed by Kenya and Queensland, Australia with 374.8 and 350.6 mg g^{-1} dry weight of tannin respectively in the bark. The tannin content in the bark of local Casuarina variety was the lowest at 57.8 mg g^{-1} dry weight whereas *C. junghuhniana* bark showed 89.4 mg g^{-1} dry weight. The total tannin content in the wood ranged from 9.27 mg g^{-1} dry weight (Malaysia) to a maximum of 18.99 mg g^{-1} dry weight (Philippines).

Table 2. Growth (4th year; mean ±SE) and extent of bark feeder infestation in the clonal trial of Casuarina

Accession	2 x 1m spacing		1 x 1m spacing		Infestation (%)*		
	Height (m)	GBH (cm)	Height (m)	GBH (cm)	2 years	3 years	4 years
Sabah, Malaysia	11.4±0.91	20.6±2.15	9.3±0.79	15.3±1.91	0	0	0
Kenya	10.5±0.89	18.3±1.87	9.3±0.81	14.8±1.25	0	0	0
PNG	10.4±0.95	17.4±1.14	10.2±0.89	19.8±1.98	0	0	0
Sarawak, Malaysia	9.7±0.91	15.4±2.10	6.2±0.30	11.1±0.91	15	16	15
QLD, Australia	11.9±0.97	19.6±1.68	7.3±0.35	12.6±0.99	0	0	0
Vietnam	9.7±0.87	17.6±1.59	7.4±0.30	12.6±1.20	0	0	0
Chandipur, India	9.0±0.88	15.2±2.32	7.8±0.31	13.5±1.15	0	0	0
Philippines	10.70±0.94	19.1±2.11	8.1±0.39	11.4±0.97	4	2	0
NT, Australia	10.1±0.87	15.7±1.95	10.1±0.82	16.2±2.10	0	0	0
Fiji	10.2±0.86	17.7±1.55	8.1±0.34	14.3±1.88	2	0	0
CE seedlot	10.9±0.88	18.3±1.98	8.6±0.40	14.5±1.16	35	28	32
CJ seedlot	10.8±0.81	17.7±1.65	10.2±0.85	20.7±2.23	0	0	0

*Data for both the spacing

Table 3. Biochemical parameters for wood and bark tissues of Casuarina clone and seedlot accessions

Accession	Wood density (kg m^{-3})	Moisture (%)		Tannin (mg g^{-1} dry wt)		Phenol (mg g^{-1} dry wt)		Lipids (mg g^{-1} dry wt)	
		Wood	Bark	Wood	Bark	Wood	Bark	Wood	Bark
Sabah, Malaysia	631	41.5	52.6	9.27	311.1	1.81	1.69	2.8	32
Kenya	604	41.6	51.2	7.38	374.8	0.47	1.94	3.2	52.4
PNG	643	40.2	43.1	13.14	310.1	0.93	29.62	26	28
Sarawak, Malaysia	606	42.2	50.1	9.275	212.5	0.50	1.55	11.6	13.6
QLD, Australia	696	38.0	55.3	8.62	350.6	0.18	1.60	1.2	10
Vietnam	626	41.1	54.1	6.91	344.1	1.06	1.70	5.2	4.8
Chandipur, India	643	34.5	49.5	7.38	391.3	1.76	1.66	4.8	24
Philippines	647	42.3	53.4	18.99	321.5	0.36	29.68	20.0	34
NT, Australia	697	40.1	52.2	7.86	248.8	0.18	1.48	2	7.2
Fiji	642	43.3	49.5	12.10	289.4	0.26	1.53	24	6
CE seedlot	602	40.8	50.6	12.57	57.8	0.78	6.58	24	30
CJ seedlot	681	42.0	55.3	7.86	89.4	3.34	1.71	1.6	6.4

3.2.3 Phenol

There was no variation between the phenol content of wood and bark in all clones, except Philippines and PNG clones where the bark phenol content (29.68 and 29.62 mg g^{-1} dry weight respectively) was many times more than that of the wood (0.36 and 0.93 mg g^{-1} dry weight respectively). Phenol content in wood ranged from a minimum of 0.18 mg g^{-1} dry weight (Queensland Australia) to a maximum of 1.94 mg g^{-1} dry weight (Kenya). Local Casuarina variety recorded a maximum of 6.58 mg g^{-1} dry weight of phenol in the bark whereas *C. junghuhniana* recorded 3.34 mg g^{-1} dry weight of phenol in the wood (Table 3).

3.2.4 Total lipid

The total lipid content was higher in the bark tissues than in the wood in all the clones, except the clone Fiji where the bark tissues recorded four times lesser (6 mg g^{-1} dry weight) than the lipid content in wood (24 mg g^{-1} dry weight). Total lipid content in the bark of Kenya clone recorded the highest value of 52.4 mg g^{-1} dry weight, followed by the bark of Philippines (34 mg g^{-1} dry weight) and Malaysia (32 mg g^{-1} dry weight). The local Casuarina variety recorded 30 mg g^{-1} dry weight of lipids in the bark and *C. junghuhniana* recorded 6.4 mg g^{-1} dry weight. In the case of Vietnam, the lipid content of bark and wood was comparable (Table 3).

3.2.5 Phenolic acids

TLC separation and UV characterization of bark and wood tissues of Casuarina for phenolic acids revealed that Salicylic acid and Protocatechuic acid were the two phenolic acids present in almost all the clones. The wood tissues of Malaysia and the bark tissues of Vietnam contained the phenolic acid Resorcinol. Vanillic acid was identified from the wood tissues of Kenya and NT, Australia, whereas it was identified in the bark of Sarawak and Fiji. The Philippines clone showed only Gentisic acid in the bark. In *C. junghuhniana* the bark tissues showed the presence of Phloroglucinol and Pyrogallol Gentisic acid in addition to Salicylic acid and Protocatechuic acid. Salicylic acid was the only phenolic acid identified in the bark of local Casuarina (Table 4).

3.2.6 Free fatty acids

The major free fatty acid components identified in the bark tissue were Linoleic acid, Oleic acid and Palmitic acid. The Kenya clone and Sarawak clone showed the presence of Myristic acid in addition to Palmitic, Oleic and Linoleic acid. *C. junghuhniana* bark tissue recorded a rich profile of fatty acids like Lauric acid, Stearic acid and Linolenic acid in addition to Palmitic, Oleic, Myristic and Linoleic acids. Palmitic, Oleic, and Linoleic were identified in the bark of local Casuarina (Table 4).

4 Discussion

The Casuarina trial laid out at a hot spot area for bark feeder incidence helped to compare not only the variation in pest infestation but also difference in growth rate of trees during the four years of growing period. *C. junghuhniana* performed better than *C. equisetifolia* in terms of growth as reported earlier (Nicodemus et al., 2005; 2015). In the present study, NT Australia and PNG clones of *C. equisetifoli*a performed better and are resistant to bark feeder attack (Balu et al., 2005; Sasidharan et al., 2001). The bark feeder incidence is usually observed in two year old Casuarina trees. The performance of local variety of *C. equisetifolia* used by farmers in plantations has below average growth and is highly susceptible to bark feeder and blister bark disease. The bark feeder preferred the local variety of Casuarina and the clone Sarawak under field conditions. Host preference estimated on the basis of feeding behavior such as orientation, amount of bark fed

Table 4. Analysis of phenolic acids in wood and bark tissues of Casuarina

Accession	Solvent I*		Solvent II*		Free fatty acid fraction
	Wood	Bark	Wood	Bark	Wood
Sabah, Malaysia	Resorcinol	Salicylic acid	Resorcinol	---	Myristic
Kenya	Protocatechuic Vanillic acid	Salicylic acid	---	Salicylic	Palmitic, Oleic, Myristic, Linoleic
PNG	Protocatechuic	---	---	Protocatechuic	
Sarawak, Malaysia	Protocatechuic	Protocatechuic	---	Vanillic	Oleic, Myristic, Linoleic
QLD, Australia	Protocatechuic	Protocatechuic	---	Salicylic	—
Vietnam	Protocatechuic	Resorcinol	Salicylic	Resorcinol	Myristic Linoleic
Chandipur, India	---	Salicylic acid	---	Salicylic	Linoleic
Philippines	---	---	---	Gentisic	—
NT, Australia	Vanillic acid Protocatechuic	---	---	---	Linoleic
Fiji	Salicylic acid	Vanillic acid Protocatechuic	---	—	—
CE seedlot	---	Salicylic acid	---	---	Palmitic, Oleic, Linoleic
CJ seedlot	---	Phloroglucinol Pyrogallol Salicylic acid Protocatechuic	---	Gentisic	Bark Palmitic, Lauric Oleic, Stearic, Linolenic, Myristic, Linoleic

*Solvent I: Ethyl acetate: Benzene, 9:11 Solvent II: Benzene: Methanol: Acetic acid, 8:45:4

under no choice situation and long term effects involving duration of post embryonic development enabled to identify variation in pest incidence (Jacob et al., 2013) probably due to the variations in the biochemical nature of Casuarina bark. Similar avoidance by *Hyblaea puera* towards leaves of teak clone was reported by Jacob et al. (2000). The effect of such a variation of food on the insect may be either due to the difference in the amount of food ingested or due to balance between nutritional and non nutritional value of the host (Johansson, 1964). The interplay of nutritive and non nutritive factors tends to influence the host preference of bark feeder on Casuarina clones as in acridids (Ananthakrishnan et al., 1985).

I. quadrinotata has a wide host range and can infest fruit trees and ornamental plants besides forest tree species (Patel and Patel, 2008). Varietal performance of *I. quadrinotata* on Guava and Citrus (Sandhu et al., 1979), Ber (Mann and Bindra, 1977) and Emblica (Chowdhary et al., 2005) has been reported. Variation in the incidence of bark feeding caterpillar on Casuarina provenances has been observed in the present study wherein through field screening, provenances like Kenya, Australia, Vietnam were free from the incidence of bark feeder, whereas Sarawak was found to be susceptible both during the early and late stages of growth (Balu et al., 2001). Similar examples of clones/provenances/varieties/land races of tree species exhibiting variation in pest incidence are available for species like Birch, *Betula pendula* (Prittinen et al., 2003), *Populs deltoides* (Singh, 2000), eucalypts (Farrow et al., 1994; Soria and Borralho, 1997) and teak (Jacob et al., 2000).

Variations in the nutritional quality between plant species or even within a plant can influence the food preference, growth rate, development time and hence the biotic potential of insect (Slansky, 1982). High level of tannin was recorded in the bark of Casuarina clones like Kenya. Oak leaf tannins influenced the feeding by oak moth Operophtera brumata (Feeny, 1970). Thus there seems to be grounds for supposing that high levels of tannins in Casuarina clones have a negative role as far as food preference and growth of bark feeder is concerned. Tannins are widespread in the plant kingdom and their levels are known to be highly variable in different poplar species and in different genotypes of the same species (Greenway et al., 1991). The total phenol content showed a higher trend in the bark of Casuarina clones like Philippines and PNG in the present study. Bark feeder preferred seed sources containing lesser phenol and tannin and higher levels of protein, nitrogen and lipid in Casuarina bark (Sasidharan et al., 2005). With reference to the stem borer *Anoplophora chinensison* on different *Casuarina* species highly resistant strains had high phenolic content (Hua Zhang et al., 2008). Presence of Salicylic acid, a hydroxy benzoic acid, an essential component imparting resistance to plants produced by the Shikimate acid pathway were identified in clones like Malayasia, Kenya, Chandipur Australia and *C. junghuhniana* . Hydroxy benzoic acid is known to play an important role in insect plant and pathogen interaction. Phenols and phenolic acids showed varying distribution among the different cultivars of Chickpea and red gram and the degree of resistance evident from the diversity of developmental pattern of *Heliothis armigera* (Ananthakrishnan et al., 1990). Similarly phenolic acids Resorcinol and Phloroglucinol identified in clone Vietnam and *C. junghuhniana* showed chronic effects on growth and in food consumption by *H. armigera.* The total lipid content in the Casuarina bark was higher than that recorded in the wood. In clones like Malaysia, Kenya, Philippines, PNG, Chandipur and the local variety, significantly higher content of lipids was recorded. Except for cholesterol and some amounts of fatty acids, insects have no major requirement for lipids. Presence of fatty acids like Myristic, Palmitic, Oleic, Lauric, Linolenic, and Linoleic acids were identified in the Casuarina bark. *Locusta migratoria* was found to be more sensitive to the absence of fatty acid in the diet than *Schistocerca gregaria* (Dadd, 1963). Meanwhile Linolenic acid in mulberry was shown to stimulate feeding activity in *Bombyx mori*. Linolenic and linoleic acids are phagostimulants for the fire ants *Solenopsiss aevissima* (David, 1983).

Damage from *I. quadrinotata* can be responsible for widespread economic loss in Casuarina plantation and can also increase the risk of infestation by fungal pathogens like blister bark (Klepzig et al., 1997). Since *C. junghuhniana* is growing faster than *C. equisetifolia*, the genetic variation existing in this species for resistance to bark feeding caterpillar needs to be investigated. The NT Australia and PNG clones may be deployed in plantations to be taken up in areas known to have high level of incidence of *I. quadrinotata* like that of the present location. The intermediate and poor performing clones in terms of growth observed in Sirkali may be suitable to other areas for cultivation. Hence they may be conserved in germplasm banks for testing and utilization in future. They could also be used as parents for breeding pest resistant varieties for future. Germplasm which show some inherent resistance to bark feeder infestation would provide considerable benefits for plantation forestry and agroforestry by maximizing survival of trees and enhancing productivity, especially in pest prone areas. The study also identified the interplay of chemicals in the bark of Casuarina clones in imparting resistance to the bark feeding pest. Identifying and evaluating the relative contributions of multiple factors in Casuarina clones that impart resistance to bark feeder will help to select a number of key chemical markers with which selection to bark feeder resistance without reducing the biomass productivity can be made and monitored through breeding. From the results it appears that resistance to bark feeder in Casuarina is due to multiple factors. Accessions like Kenya, PNG, Philippines, *C. junghuhniana,* NT Australia, QLD Australia and Fiji with bark feeder resistance may possess different ratios of allelochemical compounds. It may be possible to increase resistance by crossing clones where each contributes genes for biosynthesis of different allelochemicals. Research on interactions of trees with insect pests is advancing rapidly as a result of the implementation of genomics approaches (Lawrence et al., 2006) and is of critical importance for a fundamental understanding of the defense dynamics in trees like Casuarina for their further development and sustainability in plantation forestry with application for biomass productivity.

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Insect Pests of *Casuarina equisetifolia* in Tamil Nadu, India and their Eco-friendly Management

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Abstract

Over 60 species of insects have been reported to feed on live *C. equisetifolia* in India of which we have enumerated about 40 species from Tamil Nadu State. These include 3 species of stem borers, 2 species of bark feeders, 23 species of needle feeders, 11 species of sap suckers and 1 species of seed feeder. Among these, the bark eating caterpillar, *Indarbela quadrinotata* attained serious proportions in adult trees while the bagworm, *Cryptothelia crameri*- a needle feeding pest was found to affect seedlings. The bark eating caterpillar is reckoned as the most serious pest of this tree in Tamil Nadu and its infestation caused 2 to 5 percent mortality of trees in grown up plantations. Further, the pest attack resulted in 6.35 percent loss in diameter increment and 7.33 percent loss in height increment per annum. Since the bark eating caterpillar remains in well fortified borer holes, it has only a very few natural enemies. However, the pest can be controlled through the entomopathogenic fungus, *Beauveria bassiana* in an eco-friendly manner. A few seed sources of *C. equisetifolia* exhibit high level of tolerance to *I. quadrinotata* which offers scope for selection and breeding for pest resistance.

1 Introduction

Casuarina equisetifolia is an evergreen tree, native of Australia, Pacific islands and South-East Asia. It is a tree of multiple utilities, the wood is used as fuel, scaffold and pulpwood; its root association with *Frankia* helps to enrich soil nitrogen. Because of the adaptabilities to different eco–climatic and edaphic zones, *C. equisetifolia* gained importance in afforestation programmes in a variety of habitats ranging from deserts, coastal sand dunes, mine dumps and other places, where soil nutrients and water are scarce (Kondas, 1981).

C. equisetifolia was introduced to India – at Karwar in 1868 and Orissa in 1916. Though *C. equisetifolia* is almost free from major insect pest problems in its native land, this species is encountered by several severe problems in countries where it has been introduced (Mathew, 1994). Over 60 species of insects have been reported to feed on live Casuarina in India (Beeson, 1941; Bhasin et al., 1958; Browne, 1968; Ahmad, 1992; Subramanian et al., 1992). Since Casuarina plantations raised mainly by smallholding farmers are scattered, the impact of various insect pests on this tree crop has not been fully realized and no comprehensive study has been undertaken to manage the pests. The present study was undertaken to identify the key pests affecting this tree species in Tamil Nadu, a major Casuarina growing State in India, and to understand its impact on growth of the plants and to develop eco-friendly management practices to contain the pest problem.

2 Materials and Methods

2.1 Survey and evaluation of pests

The *C. equisetifolia* plantations in Tamil Nadu mainly fall under four agro-climatic zones viz. the North-eastern zone (AC Zone-I), Cauvery delta zone (AC Zone-IV) Southern zone (AC Zone-V) and High rainfall zone (AC Zone-VI). Altogether, 181 plantations in these zones were enumerated and 27 selected randomly covering different agro-climatic zones, age classes and satisfying the condition of Probability Proportional to Size (PPS) of plantations (Table 1). Details of nurseries were also collected from different offices of the Forest Department in the State. In order to generate data on the nature and extent of damage by insects which feed on *C. equisetifolia*, the selected plantations and nurseries were visited at monthly intervals from 2008 to 2012. Based on the extent of insect damage, the intensity of infestation was qualitatively assessed and recorded as major, moderate and minor. Climatological data were collected from the local weather stations and the influence of various parameters on the infestation of the bark eating caterpillar was analyzed.

2.2 Identification of natural enemies

The Casuarina nurseries and plantations raised in different agro-climatic zones were periodically visited and the natural enemies occurring on important pest species were collected. Subsequently, they were identified with the help of taxonomists on different groups.

Table 1. Agro-climatic zone wise details of *Casuarina equisetifolia* plantations surveyed and selected for studying pest infestation

Name of Zone	No. of plantations with ≥ 50% plant survival	No. of plantations selected for study
North-eastern zone (ACZ-I)	46	5
Cauvery delta zone (ACZ-IV)	30	6
Southern zone (ACZ-V)	100	11
High rainfall zone (ACZ-VI)	5	5
Total	181	27

2.3 Assessment of impact of bark eating caterpillar infestation on tree growth

The growth parameters of trees were monitored during 2009 to 2012 in protected (insecticide treated) and unprotected (allowed for natural infestation of the bark eating caterpillar) plots laid out in a 2006 *Casuarina equisetifolia* plantation raised by the Tamil Nadu Forest Department at T. S. Pettai, in Cuddalore Forest Division (Lat.: 11° 40' N; Long.: 79° 75' E) to get an idea about the impact of the pest attack on growth of the trees. This area has an altitude of 7 m above sea level, mean annual rainfall of 824 mm, humidity of 85% and mean maximum and minimum temperature of 33.69 °C and 25.03 °C respectively. The methodology developed by Varma et al. (1998) for studying the impact of defoliation caused by *Hyblaea puera* on the growth of teak with some modifications (i.e. in the present case, the protected and unprotected plots have been replicated) was adopted.

Eight plots of 25x25m size were laid out randomly in the plantation and the trees in each plot were numbered. Four of these plots were earmarked as 'Protected Plots', in which periodical application of insecticides to the borer holes and surrounding areas was done to control the infestation of the bark eating caterpillar. The remaining four plots were earmarked as 'Unprotected Plots', which were allowed to be infested by the pest naturally. Since the bark eating caterpillar infestation generally occurs after three years' age, diameter growth was recorded from 2009 onwards at 6 monthly intervals to determine the loss in diameter increment of trees due to infestation. The height of the trees was estimated using a regression equation: log ht. = -1.410 + 0.871 log dbh (adjusted R^2 = 0.87; F = 1217.747, $p < 0.001$), worked out based on the diameter-height relationship for *C. equisetifolia* (Sasidharan, 2004). The loss in height increment of trees was also worked out. The intensity of infestation of the pest was recorded at half yearly intervals.

2.4 Evaluation of botanicals and entomopathogens

The entomopathogenic fungus, *Beauveria bassiana* was isolated from the naturally infected bark eating caterpillar larvae and pure culture developed on Potato Dextrose Agar (PDA) and Sabouraud Dextrose Agar (SDA) medium. Another entomopathogenic fungus, *Metarhizium anisopliae* was obtained from National Bureau of Agriculturally Important Insects (NBAII), Bengaluru for the experiments. The nucleus cultures of the fungal pathogens were again cultured in the laboratory on SDA medium. The full grown fungal culture along with the medium was crushed in sterile distilled water and filtered to prepare a stock solution. The spore counting was done using a standard haemocytometer. These stock solutions were diluted with sterile distilled water and different spore concentrations were made for the experiments, as per the methodology followed by Swaran and Varma (2003).

The oils of *Jatropha curcas* and *Hydnocarpus pentandra* were extracted with petroleum ether, from ripe seeds in the laboratory. The Neem oil and *Pongamia pinnata* oil were obtained from the local market and used for the experiments. Known volumes of the oils were diluted with sterile distilled water to make the required concentration of the oil formulation. A few drops of Teepol were also added to the formulation to act as dispersing agent. In the case of *Hydnocarpus pentandra,* known volume of the seed oil was added to a commercial adjuvant (Tween-20) to make the required concentration.

Ripe fruits of *Melia azedarach* (Persian lilac) were collected from avenue trees in Coimbatore and de-pulped to get the seeds. Seed kernels were soaked in distilled water overnight, crushed and filtered. A few drops of Teepol were also added to the extract to act as dispersing agent.

Some of the products like Formulation 'A' (Containing methanol extract of plants viz. *Aristolochia bracteata, Justicia adhatoda, Melia dubia, Murraya koenigii* and *Vitex negundo*) and Formulation 'B' (Containing methanol extract of plants viz. *Aristolochia bracteata, Justicia adhatoda, Melia dubia, Murraya koenigii* and *Vitex negundo* + oil of *Hydnocarpus pentandra* in the ratio of 1:1) developed by Bioprospecting Division of IFGTB were also used against the bark eating caterpillar in the prescribed concentrations.

The effective concentrations of botanicals and the entomopathogens were determined through laboratory experiments and their field efficacy was studied between 2011 and 2013 in *C. equisetifolia* plantations located at T. S. Pettai, Cuddalore Forest Division, Tamil Nadu. Trees infested with bark eating caterpillars were identified, the borer holes marked and the frass materials over the holes

Table 2. List of insects recorded on *Casuarina equisetifolia* in Tamil Nadu, India

Insects (order, family and species)	Locality of occurrence	Parts affected / mode of attack	Remarks
Coleopotera			
Bostrychidae			
Sinoxylon sp.	Coimbatore	Bores into stem and branches	Minor
Buprestidae			
Psiloptera sp.	Neyveli	Gnaws bark of young shoots and needles	Minor
Cerambycidae			
Batocera rufomaculata De Geer	Ramanathapuram	Bores into stem	Minor
Niphonamalaccensis Breuning	Mahadanapuram	Feeds on bark of stem and young branches	Minor
Curculionidae			
Myllocerus discolor Bohem.	Coimbatore	Feeds on needles	Minor
M. improvidus H.L.Andrews	Neyveli	Feeds on needles	Minor
M. undecimpustulatus Faust.	Mahadanapuram, Pirappanvalasai and Coimbatore	Feeds on needles	Minor
M. viridanus Fab.	AC Zone 1, 4 and 5	Feeds on needles	Minor
Myllocerus sp.	Coimbatore	Feeds on needles	Minor
Mylabridae			
Mylabris pustulatus Thunb.	Neyveli	Feeds on young needles and flowers	Minor
Mylabris sp.	Pudukkottai	Feeds on needles	Minor
Scarabaeidae			
Oxycetonia versicolor Fab.	Neyveli	Feeds on needles	Minor
Hemiptera			
Cicadidae			
Platyplura hampsoni Dist.	Rameswaram	Makes incision in branches for egg laying	Minor
Eurybrachys tomentosa Fab.	Coimbatore	Sucks sap from young shoots	Minor
Margarodidae			
Icerya purchasi Maskell	Pudupattinam, Mahadanapuram, Rameswaram and Coimbatore	Sucks sap from needles and young branches	Moderate
Membracidae			
Otinotus oneratus Walker	AC Zones 1, 4 and 5	Sucks sap from young portion of stem / branches	Minor
Oxyrachis tarandus Fab.	AC Zones 1, 4 and 5	Sucks sap from young portion of stem / branches	Minor
Pentatomidae			
Chrysocoris purpureus Westwood	Mahadanapuram	Sucks sap from young portion of stem / branches	Minor
Chrysocoris stocherus L.	Pudukkottai	Sucks sap from young portion of stem / branches	Minor
Halys dentatus Fab.	Coimbatore	Sucks sap from young portion of stem / branches	Minor
Nezara viridula L.	Puduppattinam and Coimbatore	Sucks sap from young portion of stem / branches	Minor
Pseudococcidae			
Ferrisia virgata Cockerall	Coimbatore	Sucks sap from needles	Minor
Nipaecoccus vastator (Maskell)	Puduppattinam	Sucks sap from needles	Moderate
Hymenoptera			

Table 2. Contd.

Table 2. List of insects recorded on *Casuarina equisetifolia* in Tamil Nadu, India

Insects (order, family and species)	Locality of occurrence	Parts affected / mode of attack	Remarks
Torymidae			
Bootanelleus orientatlis Mathur and Hussey	Coimbatore	Feeds on seeds	Minor
Isoptera			
Termitidae			
Odontotermes obesus Rambur	Tirupporur and Mahadanapuram	Feeds on outer bark	Minor
Lepidoptera			
Cosmopterigidae			
Microlepidopteran needle miner	Rameswaram, Kanyakumari and Killai	Mines into needles	Minor
Ascotis selenaria Denis, ssp. i*mparata* Walker	Coimbatore	Feeds on needles	Minor
Metarbelidae			
Indarbela quadrinotata Walker	AC Zones 1, 4 and 5	Bores into stem and feeds on bark	Major
Lasiocampidae			
Metanastria hyrtaca Cramer	Neyveli	Feeds on needles	Minor
Taragama siva Lefebvre.	Neyveli	Feeds on needles	Minor
Lymantridae			
Dasychira mendosa Heubner	Coimbatore	Feeds on needles	Minor
Lymantria detersa Walker	Coimbatore	Feeds on needles	Minor
Psychidae			
*Chalioides*sp., nr. *vitrea* Hampson	Killai	Feeds on needles	Minor
Cryptothelia crameri (Westwood.)	AC Zones 1, 4 and 5	Feeds on needles	Moderate
Orthoptera			
Acrididae			
Orthacris maindroni Bolivar	Coimbatore	Feeds on needles	Minor
O. ruficornis Bolivar	AC Zones 1,4 and 5	Feeds on needles	Minor
Oxya fuscovittata (Marschall)	Puduppattinam	Feeds on needles	Minor
O. nitidula (Walker)	Puduppattinam	Feeds on needles	Minor
Xenocatantops sp.	Puduppattinam	Feeds on needles	Minor
Pygomorphidae			
Neorthacris auticaps Singh and Kevan	Mahadanapuram	Feeds on needles	Minor

Abbreviation: AC Zones: Agro-climatic zones

removed. The borer holes were again examined on the next day to ascertain availability of the caterpillars inside the borer hole. The caterpillar has the habit of closing the borer hole with frass material and silken thread, when it is removed. Three milliliters of the plant extract/ oil formulation/ spore suspension was applied into each borer hole and the surrounding bark, with a pipette after removing the frass cover (Sasidharan and Varma, 2007). The bark eating caterpillar has the habit of coming out of the borer hole at the time of death. Hence the larval mortality was assessed by counting the number of dead larvae found outside the borer hole. The results were subjected to Z-test and significance determined.

3 Results

3.1 Survey and evaluation of insectpests

In the present study, 40 species of insects were found infesting *C. equisetifolia* in Tamil Nadu. They include stem borers (3), bark feeders (2), needle feeders (23), sap suckers (11) and a seed feeder (Table 2). They belonged to the orders, Coleoptera (12 species), Hemiptera (11 species), Hymenoptera (1species), Isoptera (1 species), Lepidoptera (9 species) and Orthoptera (6 species). Among these, only four species viz.*Indarbela quadrinotata*, *Icerya purchasi*, *Nipaecoccus vastator*, and *Cryptothelia crameri,* are of concern in Tamil Nadu State and the others are of no major economic consequence. Out of these four species, the bark eating caterpillar, *I.quadrinotata* was found to be the key pest of *C. equisetifoila* in Tamil Nadu. The intensity of infestation of this insect across different Agro-climatic zones of the State is given in Table 3**.** In general, wide variation in the infestation intensity was noticed within and between the Agro-climatic zones. The maximum infestation (39.8%) was recorded in North-eastern zone, followed by Cauvery Delta Zone (33%). The Southern Zone had very low infestation (2.6%), while the plantations raised in the High Rainfall Zone were not attacked by the pest.

3.2 Influence of climatological factors on infestation of bark eating caterpillar

Analysis of correlation between climatological factors and infestation of the bark eating caterpillar has shown that, the pest attack has significant positive correlation with the mean minimum temperature. The mean maximum temperature and mean rainfall showed a negative correlation with the percentage of infestation, while mean humidity exhibited a positive correlation. However, these correlations were not found to be significant (Table -4).

3.3 Natural enemies recorded from Casuarina nurseries and plantations

The bark-eating caterpillar, *Indarbela quadrinotata* was found to have not many natural enemies, probably owing to its peculiar hiding habit. The only bio-control agent detected was the fungal pathogen, *Beauveria bassiana*, which infected the caterpillars after summer showers,

Table 3. Intensity of infestation by *Indarbela quadrinotata* on *Casuarina equisetifolia* in different agro-climatic zones of Tamil Nadu, India

AC Zone	S.No.	Name of plantation	Age(year)	Infestation (%)	Mean Infestation (%)
North Eastern Zone (ACZ-I)	1	2006 Singarathoppu	4	0	39.78
	2	2006 Sonan Kuppam	4	83.3	
	3	2006 Sothikuppam	4	0.5	
	4	2005 Alawandar Bit 1&2	5	39.1	
	5	2006 Alawandar	4	76	
Cauvery Delta Zone (ACZ-IV)	6	2006 Puthupettai Bit I&II	4	61.14	33.00
	7	2006 T.S.Pettai	4	51.65	
	8	2006 Vadakku Pichavaram	4	27.16	
	9	2006 Vellingarayanpettai	4	45.54	
	10	2008 Puduppattinum TCPL	2	5.55	
	11	2005 Madavamedu	5	40	
Southern Zone (ACZ-V)	12	2004 Rajamadam	6	0	2.66
	13	2001 Rajamadam bit 1	9	0	
	14	2005 Rajamadam Agniyar	5	0	
	15	2006 Mumbalaipattinum	4	0	
	16	2001 Perungalur west bit 7	9	3.78	
	17	2003 Perungalur west	7	21.95	
	18	2002 Veerangudi	8	3.34	
	19	2008 Perungalur	2	0.2	
	20	2005 Senthilveethi	5	0	
	21	2005 Tharuvaikulam	5	0	
	22	2005 Vellapatti	5	0	
High Rainfall Zone (ACZ-VI)	23	2006 Pannaiyur	4	0	0
	24	2006 Periyakadu	4	0	
	25	2006 Veerabahupathy	4	0	
	26	2005 Mugilangudi bit-1	5	0	
	27	2005 Ilandayadi bit 2	5	0	

Table 4. Correlation of climatological factors and bark eating caterpillar infestation

AC-Zone	% Infestation	Mean max. temp (°C)	Mean min. temp (°C)	Mean rainfall (mm)	Mean humidity (%)
I	39.78	33.79	24.54	1054	77.15
IV	33	33.25	24.84	950	84.88
V	2.66	33.70	24.00	776	79.27
VI	0.00	33.95	23.78	1469	72.44
Correlation co-efficient (r)		-0.50	0.91*	-0.27	0.52

*Significant at 1% level

resulting in large scale mortality of the pest larvae. The common predators found in Casuarina ecosystem were spiders and praying mantids, many species of which were found feeding on larvae of lepidopteran insects and beetles. The coccid, *Nipaeccoccus vastator* was commonly devoured by the coccinellid beetle, *Micraspis* sp. in nurseries of *C. equisetifolia*, at Pudupattinam.

3.4 Impact of infestation of bark eating caterpillar on tree growth

The loss in diameter and height increments of Casuarina trees recorded from the protected and unprotected plots is shown in Table 5**.** The loss in diameter ranged from 4.07 percent to 14.29 percent whereas it was 5.72 percent to 15.65 percent for height. The overall annual loss in diameter and height was found to be 6.35 percent and 7.33 percent respectively and it significantly correlated with the pest infestation (Table 6).

3.5 Field evaluation of entomopathogens and botanicals

The naturally occurring entomopathogenic fungus, *B. bassiana* at $5x10^7$ spores ml^{-1} was found to cause the highest larval mortality of 93.3 percent followed by *Melia azedarach* seed kernel extract (66.6%), *Pongamia pinnata* seed oil (63.3%), Formulation-A (60%), *Hydnocarpus pentandra* seed oil (56.6 %), Neem oil (50%) and Formulation 'B' (46.6%). Jatropha oil at 5 percent concentration was able to cause only moderate level of larval mortality (33.3%), while the fungus, *Beauveria bronginartii* at a concentration of $5x10^7$ spores ml^{-1} resulted in very low larval mortality of 13.3 percent (Table 7). There was no larval mortality in the trees maintained as control.

4 Discussion

Majority of the pests recorded on *C. equisetifolia*, from Tamil Nadu are polyphagous, i.e. they feed on several host plants, as in the case of many other exotic trees introduced to India. Except the bark eating caterpillar, *I. quadrinotata*, all other pests cause either moderate or minor damage and hence they are not of serious concern at present. Due to changes happening in the ecosystem it is likely that a few of the occasional and minor pests may adapt and attain serious pests status. Nair (2001) reported that, both indigenous and exotic forest trees are equally prone to insect infestation, contrary to the belief that, exotic species are attacked more in places of introduction. The pest situation in *C. equisetifolia* also testifies this fact.

The present study indicated that, the bark eating caterpillar, *I. quadrinotata* has only one natural enemy, i.e. *Beauveria bassiana* similar to many pests which have concealed habits. Since the bark eating caterpillar lives in well-fortified borer hole during the day time and comes out through tunnels made of its excreta, bark particles and silken thread to feed the bark only during night time, it escapes the attention of the parasites, which otherwise act as biocontrol agents on lepidopterans.

Correlation analysis of the infestation level of different agro-climatic zones with the climatological factors revealed that, the pest attack has significant positive correlation with mean minimum temperature of the area. Weather has both direct and indirect effects on

Table 5. Diameter and height increment loss due pest infestation in protected and unprotected plots

Duration (month)	DBH (cm)		Height (m)		Loss in increment (%)	
	Protected plots	Unprotected plots	Protected plots	Unprotected plots	DBH	Height
6	4.00	3.43	5.43	4.58	14.29	15.65
12	6.91	6.63	9.09	5.57	4.07	5.72
18	9.18	8.21	12.11	10.52	10.57	13.13
24	11.56	10.09	15.07	12.86	12.70	14.66

Table 6. Reduction in growth increment vis-à-vis bark eating caterpillar infestation

Duration (month)	Infestation of bark eating caterpillar (%)	Mean DBH increment loss (cm)	Mean height increment loss (m)
6	36.61	0.57	0.85
12	37.56	0.28	0.52
18	39.64	0.97	1.59
24	40.3	1.47	2.21
r	0.87*	0.90*	

*Correlation co-efficient significant at 1% level

phytophagous forest insect populations (Martinat, 1987). Azmy et al. (1978) found the population build up of wood borers like *Zeuzera pyrina*, *Bostrychopsis reichei* and *Phonapte frontalis* was influenced by temperature regime.

Reports on the impact of a pest on tree growth are not many. Quantification of growth loss due to insect pest is essential to justify the need for controlling the pest population. The bark eating caterpillar infestation caused 6.35 percent reduction in diameter increment and 7.33 percent in height increment per annum. The feeding of the caterpillar on the bark of the trees also makes room for entry of wound pathogens and severely affected plants succumb to the combined attack of the pest and pathogens. On the basis of studies carried out in IFGTB, in different parts of Tamil Nadu State, it has been shown that the bark eating caterpillar causes 3 to 5 per cent mortality of trees annually, which can result in further economic loss.

Nair et al. (1996) found that 44 percent of the potential volume growth in *Tectona grandis* remained unrealized due to the infestation of the defoliator, *Hyblaea puera.* Applenton et al. (1997) observed that the Psyllids – *Psylla acacia* and *P. uncatoides* were capable of significantly reducing the growth rates of *Acacia melanoxylon*. Armour et al. (2003) found that the defoliation of Scot Pine, *Pinus sylvestris* by the larvae of the moth, *Bupalus piniaria* reduced the radial and volume increments substantially. Defoliation and resultant growth loss in Sitka Spruce, following repeated attack by the Green Spruce Aphid, *Elatobium abietinum* was noticed by Straw et al. (2005). In the present case, the damage caused by *I.quadrinotata* is on the stem, while feeding on the outer and inner bark and when the larva makes shelter tunnel by boring into it. These activities weaken the trees in several ways, thus affecting the pace of growth.

Considering the cost effectiveness and eco-friendly nature, the biological control agents and botanical pesticides have large scope for field application. In this context, the white muscardine fungus, *Beauveria bassiana* is highly promising in the management of the bark eating caterpillar. It has over 700 recorded host species (Moore and Prior, 1993) and occurs on several pests of agricultural and horticultural crops and a few major pests of forest trees in India. It was recorded on the teak leaf skeletonizer, *E. machaeralis* (Patil and Thontadarya, 1981). *I. quadrinotata* (Arshad and Hafiz, 1983), *Ailanthus* webworm, *Atteva fabriciella* (Ali et al., 1991) and the teak stem borer, *Sahyadrassus malabaricus* (Ali and Sudheendrakumar, 1991). Hence *B. bassiana* can be considered as a potential biocontrol agent against the bark eating caterpillar. Though a closely allied species, *Beauveria bronginartii* was also field tested in the presence case, it's performance was not found to be as effective as *B. bassiana.*

The *Pongamia pinnata* seed oil (29% of the seed) contains Karanjin (1.25%) and Pongamol (0.35%) as the major constituents (Mathur et al., 1990). The antifeedant, repellent or deterrent effect of Pungam seed oil on some of the pest species, especially the storage pests have been reported (Khaire et al., 1993; Kachare et al., 1994). Several authors also found direct insecticidal effect of Pungam seed oil on some pests (Kulat et al.,1997; Hussain et al., 1996). The outcome of the present study is also in agreement with the above findings and has revealed the potential of Pungam seed oil in managing the bark eating caterpillar.

Various extracts from *Jatropha curcas* seeds and leaves showed molluscicidal, insecticidal and fungicidal properties and Phorbol esters have been detected as one of the toxic principles responsible for these biological activities. Henning (2009) considered Jatropha oil as a stronger insecticide than Neem extract. The Jatropha oil was also capable of exercising fairly good control of the bark eating caterpillar in the field condition.

The extract from the fruit of *Melia azedarach* was found to be effective on a wide range of insects (Saxena et al., 1984; Schmidt et al., 1998; Senthilnathan and Sehoon, 2006). Different parts of Neem tree, *Azadirachta indica* such as leaves, seed kernel and seed oil are traditionally used in India for the management of several agricultural pests. The bioactivity of Neem is due to some of the active principles, mainly Azadirachtin (and other complex limonoides) present in the plant parts / products (Schumutterer, 1990; Liang et al., 2003). Therefore, these botanicals are also considered to be promising in the management of the bark eating caterpillar.

The antimicrobial activities of the oil of *Hydnocarpus pentadra* (Chaulmoogra oil) are well known in India, from time immemorial as this oil is used in Ayurveda and other traditional systems of medicines against many diseases, including leprosy. Prophylactic treatment with oil cakes of

Table 7. Field evaluation of botanicals and entomopathogens against bark eating caterpillar in *Casuarina equisetifolia*

Botanicals/ entomo pathogen/ concen tration	Day-wise larval mortality										Total mortality	No. of larvae used for testing	Percent mortality*
	1	2	3	4	5	6	7	8	9	10			
Neem oil (5%)	6	4	1	3	0	1	0	0	0	0	15	30	50[a]
Pongamia pinnata seed oil (5%)	9	3	2	3	0	2	0	0	0	0	19	30	63.33[ad]
Jatropha oil (5%)	6	1	1	0	0	0	2	0	0	0	10	30	33.33[ade]
Formulation-A (1%)	13	2	1	0	0	0	2	0	0	0	18	30	60[a]
Formulation-B (1%)	11	1	2	0	0	0	0	0	0	0	14	30	46.67[ad]
Hydnocarpus pentandra seed oil (10,000 ppm)	0	0	2	0	0	3	4	0	6	2	17	30	56.66[ad]
Melia azedarach seed kernel extract (5%)	0	3	2	6	0	0	2	3	2	2	20	30	66.66[adf]
Beauveria bronginartii 5x10^7) spores/ml)	1	0	0	0	0	0	2	0	1	0	4	30	13.33[be]
Beauveria bassiana (5x10^7) spores/ml)	1	0	0	0	0	12	8	6	1	0	28	30	93.33[c]
Control	0	0	0	0	0	0	0	0	0	0	0	30	0

* Mean value superscripted with the same letters are not significant at 5% level by DMRT.

Neem and *Hydnocarpus pentandra* has also provided good results against Rhinoceros beetle (*Oryctes rhinoceros*) (GISD, 2014). The oil has deterrent properties and is used against ants and other insects (Ghosh, 2000). The oil consists mostly of the cyclic 18 and 16 carbon fatty acids known as Chaulmoogric and hydnocarpic acids (Thormar, 2011).

Our earlier studies have revealed that, some of the *C. equisetifolia* provenances and landrace seedlots exhibiting remarkable tolerance against the bark eating caterpillar. They include highly tolerant seed sources like Kilifi and Robinson Island, (Kenya), Queensland (Australia) and moderately tolerant ones like Wangetti Beach and Northern Territory (Australia), Mamor and Montazah (Egypt), Akalan (Philippines), Vanua Levu (Fiji), Watamu, Malindi and Gede (Kenya) (Balu et al., 2001; Sasidharan et al., 2005 & 2007). Therefore, there is also much scope for selection and breeding of *C. equisetifolia* for bark eating caterpillar tolerant varieties, which can be planted in areas that are highly prone for outbreak of the pest.

5 Conclusion

The bark eating caterpillar, *I. quadrinotata* is the most serious pest of *C. equisitifolia* in Tamil Nadu and its infestation results in loss of growth in trees. Other insects associated with *C. equisetifolia* are not of serious concern at present. Biocontrol through the entomopathogenic fungus, *Beauveria bassiana* is the most effective eco-friendly management practice to contain the pest. Further, there is ample scope for developing an integrated pest management approach through exploitation of host plant resistance, application of eco-friendly botanical pesticides and biocontrol agents like entomopathogenic fungi and need based dependence on safe insecticides, only in case of emergencies.

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Improving Casuarina Productivity through Farm Forestry Programme by International Paper-APPM Limited, India

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Key words: Andhra Pradesh, clone, farm forestry, productivity, pulp yield

Abstract

The International Paper – APPM Limited has been implementing Casuarina-based farm forestry programme in the State of Andhra Pradesh in India since 1986. The major objective of this programme is to improve the productivity of Casuarina plantations leading to increased farm income and securing pulpwood raw material availability. More than 1.28 billion seedlings were produced from 50 nurseries and supplied to farmers at a subsidized cost till 2013. These seedlings were planted in around 188,000 hectares by more than 50,000 farmers bringing better economic returns compared to the seedlings produced from other sources. Clonal forestry has been introduced in the State through large scale planting of the Casuarina natural hybrid clone which has increased wood production more than three-fold compared to the unimproved seedling plantations. As part of the initiative to further improve the quality of planting material, the Company has established clonal seed orchards which have started producing seed crop. Field experiments have been taken up to optimize the stocking in plantations to suite the type of planting material used. Results from these initiatives and their industrial and socio-economic impact are discussed in this paper.

1 Introduction

With 8% annual consumption growth and almost no fresh capacity additions, India's supply deficit for paper is likely to widen in the coming years. Indian Paper Industry's annual output was 11 million tonnes in 2011-12 against the operating capacity of 12.75 million tonnes, whereas the consumption was 11.23 million tonnes, leaving a deficit of 0.23 million tonnes. The deficit is expected to be around 2 million tonnes in 2016 (Rajesh and Jamly, 2013). In India, major paper mills use hard woods for pulp and papermaking. Short supply of fibre raw material from the local market resulted in reduced production and increased competition among different industries. Therefore sustainable supply of pulpwood is the key concern which is mainly addressed through plantations developed under social and farm forestry programmes.

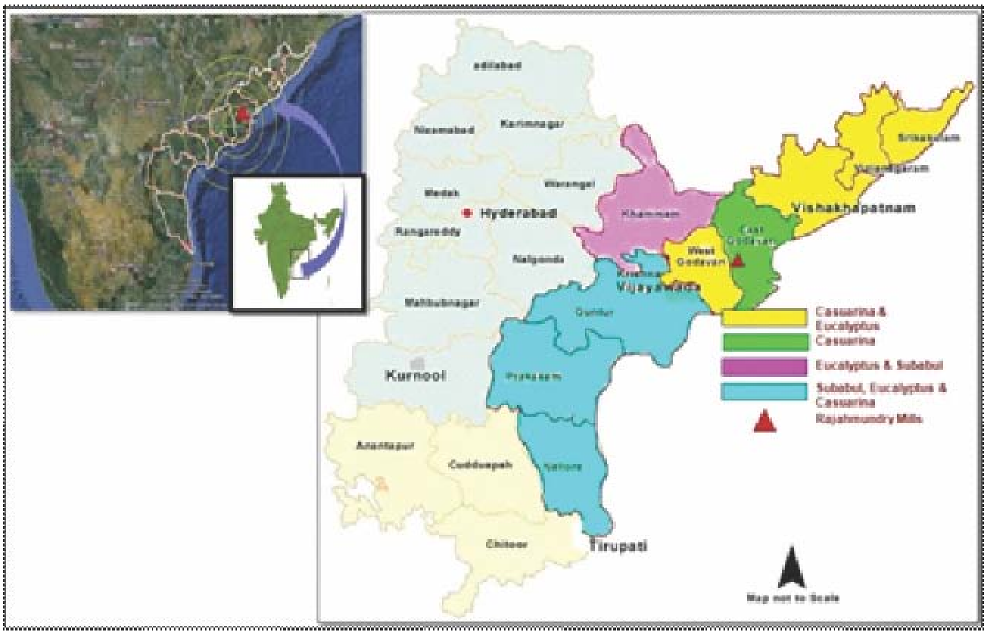
International Paper-APPM Limited (IPAPPM; formerly known as The Andhra Pradesh Paper Mills Limited), a group company of International Paper in India, is a leading manufacturer of pulp and paper products catering to both domestic and foreign markets. IPAPPM produces 240,000 metric tonnes of writing, printing and copier papers annually from its two manufacturing units in the State of Andhra Pradesh. The entire pulpwood requirement of IPAPPM is sourced from the farm forestry plantations in the State of Andhra Pradesh. The total geographical area of the State of Andhra Pradesh is 27.5 million ha, with a potential to practice farm forestry in about 38% of this area. With the third longest coastline in the country (972 km), Andhra Pradesh has extensive Casuarina plantations and its wood constitutes about 60% of hard wood raw material requirement of IPAPPM. The Company is closely working with the farming communities for nearly three decades to adopt new planting material and cultivation techniques to improve plantation productivity and raise their farm income.

Casuarina equisetifolia is widely grown in Andhra Pradesh mainly for pulpwood, poles and fuel wood in short rotations of 3 to 4 years. It is preferred by farmers for its high adaptability and fast growth under different edaphic conditions and requiring minimal investment in terms of labour and other resources. *C. junghuhniana* has recently been introduced to farmers and its cultivation area is steadily increasing. Farm forestry involving Casuarina has been a main stay in sustaining the livelihoods of agrarian communities (Jain and Mohan, 2011). This paper describes the various components of IPAPPM's farm forestry programme and the benefits derived from them.

2 Farm Forestry Programme

Started in 1986, the farm forestry programme was mainly implemented in the marginal and degraded lands in the coastal districts of Andhra Pradesh with low productivity (Fig. 1). The project area lies between 12° 41' and 22° N latitude and 77° and 84° 40' E longitude. Prior to launching the programme, socio-economic status of the farmers was studied carefully and appropriate strategies were adopted so that both the Company and the farmers are benefitted from the programme. The major activities taken up under the farm forestry programme are detailed below.

Fig. 1. Map showing the areas of IP-APPM's farm forestry programmes undertaken in Andhra Pradesh, India.



2.1 Production and supply of high quality planting material

2.1.1 Seedling material

In India, Casuarina is traditionally grown in high-density plantations (ca. 10,000 trees ha^{-1}). Such a practice requires a large number of seedlings to cover the planting area. The cost of seedlings should also be affordable to the generally resource-poor farmers. Both these needs are achieved through production of bare-root seedlings. Three-month old seedlings grown in sand beds are removed from nursery and directly field-planted. Although container-raised seedlings grow better than bare-rooted ones in the field, their cost is considered prohibitive by the farmers. In order to combine the benefits of both the means of seedling production, a new method called treated bare-root seedlings was developed. The root system of these seedlings is treated with a mud paste added with a gum to stick to the roots. This prevents moisture loss and pathogenic infection while transportation from nursery to field (Jain and Mohan, 2001). It also kept the seedling cost low and dispenses with the use of polythene bags.

Table1. The extent and beneficiaries of farm forestry programme of IP-APPM between 1989 and 2013

District	Planted area (ha)	Number of seedlings (millions)	Number of beneficiaries	Human working days generated (millions)
Srikakulam	8814.21	61.40	2374	4.41
Vijayanagaram	2366.01	17.07	394	1.18
Visakhaptnam	81111.39	592.19	24877	40.56
East Godavari	33086.46	240.85	14524	16.54
West Godavari	10441.67	110.61	3666	5.22
Krishna Guntur	18232.40	107.85	2613	9.12
Prakasam	29300.12	129.06	4890	14.65
Nellore	3259.44	23.92	302	1.63
Khammam	1040.66	1.52	100	0.52
Total	187652.35	1284.47	53740	93.83

Table 2. Wood production and estimated environmental impacts of farm forestry programme of IPAPPM (data in millions)

District	Wood yield (MT)	Fuel wood generation (MT)	Fodder generation (MT)	Nitrogen fixation (MT)	Carbon sequestration (MT)	Organic manure (MT)	Rain water fixation in soil (m^3)
Srikakulam	0.77	0.111	0.035	0.003	0.389	0.074	669.303
Vijayanagaram	0.21	0.030	0.009	0.001	0.104	0.020	179.662
Visakhaptnam	7.10	1.026	0.318	0.024	3.580	0.684	6159.160
East Godavari	2.90	0.419	0.130	0.010	1.460	0.279	2512.407
West Godavari	0.91	0.132	0.041	0.003	0.461	0.088	792.884
Krishna Guntur	1.60	0.231	0.071	0.005	0.805	0.154	1384.470
Prakasam	2.56	0.371	0.115	0.009	1.293	0.247	2224.893
Nellore	0.29	0.041	0.013	0.001	0.144	0.027	247.504
Khammam	0.09	0.013	0.004	0.000	0.046	0.009	79.022
Total	16.42	2.374	0.736	0.056	8.283	1.583	14249.304

2.1.2 Clonal seed orchard

In order to use genetically improved seeds for raising seedlings, IPAPPM developed clonal seed orchards of *C. equisetifolia* with technical cooperation from the Institute of Forest Genetics and Tree Breeding which supplied selected superior clones for developing the orchards. Two orchards were established in Jangalpalem and Palacharla during the year 2008 and 2009 respectively. These orchards have 16 female and 15 male clones (roughly 1:1 ratio) but the number of female trees (480 per ha) is kept twice that of male trees (2:1 ratio). Each female clone has around 30 trees whereas each male clone has 16 trees. In addition a pollen dilution zone was created around the orchard by planting guard rows of male clones. A wide spacing of 3 metres between trees was adopted to facilitate mechanized ground management and maximizing pollen movement. Two rows of female trees are alternated with a row of male trees so that every row of female trees has a male row on one side. While only one female clone is planted in each row, one tree of each of all male clones is planted in male rows so that adjacent female trees receive pollen from all male cones.

2.1.3 Clonal forestry

Till a decade ago almost the entire Casuarina plantations in India was raised through seed-derived planting material only. In the recent years, a male hybrid clone of *C. junghuhniana* x *C. equisetifolia* has been successfully deployed in clonal plantations. IPAPPM has established 5 clonal propagation centers in nine coastal districts of Andhra Pradesh. To meet cuttings requirements for mass propagation, hedges have been developed and maintained in clonal hedge garden with proper nutrient and irrigation management. The hedge plants were maintained below 1 m in height to produce healthy and juvenile cuttings and to minimize aging factor. At present, around 10 ha of Casuarina hybrid clonal hedge garden is maintained to meet cuttings requirement for clonal propagation. Experiments have been undertaken whether clonal plants can be produced without containers as in the case of treated bare-root seedlings. Rooting of up to four cuttings in a single container was also attempted to reduce the cost of clonal plants.

2.2 Optimizing planting density

Field experiments were conducted to determine the optimal spacing to be adopted between trees in plantations so that the potential of land and the tree crop is fully realized. Any reduction in the stocking of plantations will also result in reduced cost for seedlings and cultivation needs. Rectangular spacing trial with 1 x 1 m, 1.5 x 1 m, 2 x 1m, 3 x 1m, 2.5 x 2 m and 3 x 2 m was established in 2012.

Table 3. Production of Casuarina clonal plants (in millions) and rooting performance in IPAPPM

Name of CPC	2012			2013		
	Cuttings Charged	Cuttings Rooted	Rooting (%)	Cuttings Charged	Cuttings Rooted	Rooting (%)
Divancheruvu	164.39	71.3	43.37	169.7	113.11	66.65
Devipuram	89.9	20.17	22.44	77.25	43.37	56.14
Theeda	NA	NA	NA	80.3	41.28	51.41
Ongole	68.21	17.65	25.88	75.47	35.3	46.77
Total	322.5	109.12	33.84	402.72	233.06	57.87

Table 4. Proximate analysis of Casuarina wood

Parameter	Casuarina Hybrid	*Casuarina equisetifolia*
Chip Packing Density, BD basis (kg m^{-3})	256	306
Cooking Chemical (White liquor)	17.25	17.75
Sulphidity of W.L (%)	17.9	18.3
Anthraquinone (%)	0.05	0.05
Cooking temperature (°C)	165	165
Kappa Number	16.0	15.6
Total yield (%)	46.7	46.5
Unbleached Pulp Evaluation (Valley Beater)		
Burst factor	37.2	35.5
Breaking Length (m)	6315	6210
Tear Factor	71	66

Another trial involving Nelder's wheel shape design was conducted to evaluate the variation in growth among four sources of Casuarina planting material viz., Casuarina hybrid clone, *Casuarina junghuhniana* SSO seedlings, *C. equisetifolia* CSO seedlings and *C. equisetifolia* seedlings from local source under different levels of stocking.

3 Major Output from Farm Forestry Programme

3.1 Distribution of quality planting stock and benefits

IPAPPM has produced more than 1.28 billion seedlings from its 50 field nurseries and supplied them at a subsidized cost to farmers between 1986 and 2013. These seedlings were planted in around 188,000 hectares by more than 50,000 farmers bringing better economic returns compared to the locally available seedlings (Table 1). Planting, tending and harvesting of these plantations resulted in many other benefits like employment to rural labour force, securing pulpwood raw material for the industry, fuel wood availability in the rural areas, soil amelioration through biological nitrogen fixation, carbon sequestration and rainwater harvesting. Estimates of these indirect benefits are given in Table 2. Through optimizing cutting production from hedges and rooting environment, the rooting percentage was enhanced to about 58% in 2013 from 34% in the previous year (Table 3). Further, the production cost of planting material has been kept low through novel approaches like usage of sand medium for rooting cuttings and adoption of 'treated bare-root seedlings' and the benefits are passed on to the farmers.

3.2. Contributions of clonal seed orchards

IPAPPM's two-year old clonal seed orchards of *Casuarina equisetifolia* have started producing seeds but the production is low and is expected to increase in the next few years. Although all the 25 female clones have started flowering, seed production has been highly variable among them. They are grouped into high, medium and low fruit-bearing groups and observations on seed production and germination for each of them are being observed. About two thirds of the female clones were found to be high fruit bearers and the rest either medium or low fruit producers. Once the CSO reaches its full production, its seeds will be deployed in the field nurseries of IPAPPM for seedling production.

3.4 Productivity and wood quality improvement

Data recorded from plantations harvested showed a strong effect of seedling quality on wood yield. Conventional bare rooted seedlings produced 50 tonnes per ha whereas polybag-raised seedlings recorded 75 tonnes per ha in a four-year rotation period. Treated bare-root seedlings yielded 80 tonnes per ha and the clonal plantations

Table 5. Survival and growth of two year old Casuarina hybrid clone under various spacing tested in a rectangular design.

Spacing (m)	Trees planted	Survival (%)	Height (m)	DBH (cm)	Volume (m^3)
3 x 2	315	92.38	5.81	4.77	0.0019
2.5 x 2	432	95.60	5.16	4.32	0.0015
3 x 1	732	88.80	4.85	4.08	0.0014
2 x 1	876	92.92	4.33	3.39	0.0009
1.5 x 1	876	92.35	3.87	3.06	0.0008
1 x 1	876	92.69	3.74	2.85	0.0007

Table 6. Two years growth of Casuarina accessions under different spacing tested through Nelder's wheel type design

Accession	Stocking ha^{-1}	Height (m)	DBH (cm)	Single tree volume (m^3)	Volume ha^{-1} (m^3)
Casuarina hybrid	2039	3.9	1.63	0.00027	0.55
clone	7965	4.1	1.80	0.00035	2.76
	5413	4.1	2.03	0.00045	2.42
	3707	12.2	1.60	0.00082	3.03
	2522	4.1	1.99	0.00042	1.06
	1720	4.0	1.79	0.00034	0.58
Casuarina equisetifolia	2039	6.1	2.83	0.00128	2.60
CSO Seedlings	7965	6.2	3.31	0.00179	14.29
	5413	6.8	2.96	0.00155	8.41
	3707	6.4	2.47	0.00102	3.79
	2522	6.9	3.09	0.00173	4.35
	1720	5.7	2.39	0.00085	1.46
Casuarina junhguhniana	2039	6.8	4.42	0.00348	7.09
SSO seedlings	7965	7.0	3.84	0.00269	21.46
	5413	8.6	4.67	0.00494	26.75
	3707	8.1	4.68	0.00468	17.34
	2522	6.8	4.64	0.00386	9.74
	1720	6.3	5.39	0.00482	8.29
Casuarina equisetifolia	2039	4.3	2.23	0.00055	1.13
local seedlings	7965	4.8	2.59	0.00084	6.70
	5413	5.1	2.87	0.00109	5.91
	3707	5.1	4.77	0.00306	11.34
	2522	5.6	2.93	0.00126	3.17
	1720	5.3	3.18	0.00140	2.40

produced the maximum of 165 tonnes per ha in a similar rotation age. In case of Casuarina, the pulp yield to wood ratio is generally 1:4. The Casuarina hybrid clone which yielded maximum pulpwood was tested for its pulping characters in comparison with wood of *C. equisetifolia*. It was found that wood from four year old trees of Casuarina hybrid yielded higher pulp compared to that of *C. equisetifolia* and also possessed favourable values for burst factor, breaking length and tear factor (Table 4). This confirms that by planting the fast-growing hybrid clone both wood production and pulp yield per unit area is increased benefitting both the farmer and the industry.

3.5 Optimizing planting material and spacing to improve productivity

Among the six spacings adopted in the rectangular design, the Casuarina hybrid clone showed the maximum growth with 3 x 2 m spacing followed by 2.5 x 2 m and 3 x 1 m spacing (Table 5). At the age of 2 years, 5.8 m height and 4.77 cm DBH was recorded with 3 x 2 m spacing while 2.5 x 2 m spacing showed 5.2 m height and 4.32 cm DBH. Traditionally Casuarina is cultivated under high density plantings in south India but the present study reveals that genetically improved planting material like clones may require more spacing to express their full potential. Casuarina hybrid clone also showed the best performance when grown under low stocking (3700 trees ha^{-1}) when tested in the Nelder wheel type design (Table 6). Two years growth recorded in this trial showed that spacing requirement for the best growth differed among the various accessions of planting material used in the test. Casuarina hybrid clone and the local seed source of *C. equisetifolia* recorded the best growth under wide spacing (3707 trees ha^{-1}) while the SSO seedlot of *C. junghuhniana* required moderate stocking (5413 trees ha^{-1}) to put up the best growth. Interestingly the CSO seedlot of *C. equisetifolia* performed better under narrow spacing (7965 trees ha^{-1}). Among the four accessions tested in the spacing trial, the SSO seedlot of *C. junghuhniana* recorded substantially better growth than the others as reported earlier (Nicodemus et al., 2005; 2015). These experiments will be continued up to the full rotation age and the ideal spacing for different types of planting material like clone and seed orchard-derived seedlings will be determined.

3.7 Long-term tree improvement programme

A new collaborative Casuarina improvement programme has been initiated with participation of IFGTB and major paper industries coordinated by IPAPPM. The main objective of this research is to bring new genetic resources to broaden the existing genetic base in India. More than 30 provenance and landrace seedlots obtained from the Australian Tree Seed Centre, CSIRO, Australia have been deployed in multilocation testing. These trials will be sources of new selections for clonal forestry and seed orchard development in the future.

4 Conclusion

Casuarina productivity has been substantially increased in the State of Andhra Pradesh through introduction high quality planting material and efficient cultural practices by the IPAPPM. This has in turn helped in securing the raw material availability for the Company and also improved the socio-economic conditions of the resource-poor farmers of the State. New initiatives like seed orchard development, optimizing plantation density, introduction of new genetic resources will further enhance the productivity of Casuarina benefitting both farmers and the industry.

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Improving Pulpwood Production and Farmers' Livelihood through Captive Plantations in Degraded Lands: Initiatives of The West Coast Paper Mills Limited and Society for Afforestation, Research and Allied Works

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Key words: afforestation, Casuarina, captive plantation, degraded lands, Eucalyptus, sustainability, wood

Abstract

The present paper highlights the economic, environmental and social contributions of pulpwood plantations developed by The West Coast Paper Mills Limited (WCPM) and its implementing arm, Society For Afforestation, Research and Allied Works (SARA). These plantations are mainly developed in marginal and degraded lands under rain-fed condition which mostly remain uncultivated at present. Farmers enter into an agreement with the Society for a period of five years for establishment and maintenance of plantations at the Society's cost. WCPM provided buy-back-guarantee for the pulpwood produced at a predetermined price. Genetically superior planting material like tested clones of Acacia, Casuarina, Eucalyptus and Leucaena are produced in modern nurseries and supplied to farmers at a subsidized cost. Farmers are provided with technical guidance and choice of species and cultivation methods and encouraged to adopt agroforestry in their plantations. More than 21,000 ha of plantations have been established since 2006 and harvesting of the earliest raised plantations has yielded around 100,000 tonnes of pulpwood. The yield from the clonal plantations on degraded land under rain-fed conditions is 60 to 70 tonnes per hectare in a five year rotation which is considerably higher than the conventional seedling raised plantations. The captive plantations have also provided environmental benefits like rehabilitation of saline land, restoration of water bodies, reduced soil erosion and carbon sequestration and social benefits like generation of employment opportunities to landless agricultural workers preventing their migration to urban areas.

1 Introduction

The Indian paper industry has been facing a tough challenge of meeting its raw material needs in a cost-effective and sustainable manner. Since industries do not own large land area in India, they actively work with farmers to grow trees in their land that will eventually produce the wood for industries. These industrial plantations developed by paper industries in farmers' land have proved to be successful in benefitting both the industries and farmers in the last decade. This paper presents the initiatives and achievements of The West Coast Paper Mills Limited (WCPM) through its implementing arm namely Society for Afforesation, Research and Allied works (SARA) in the areas of plantation development and livelihood improvement.

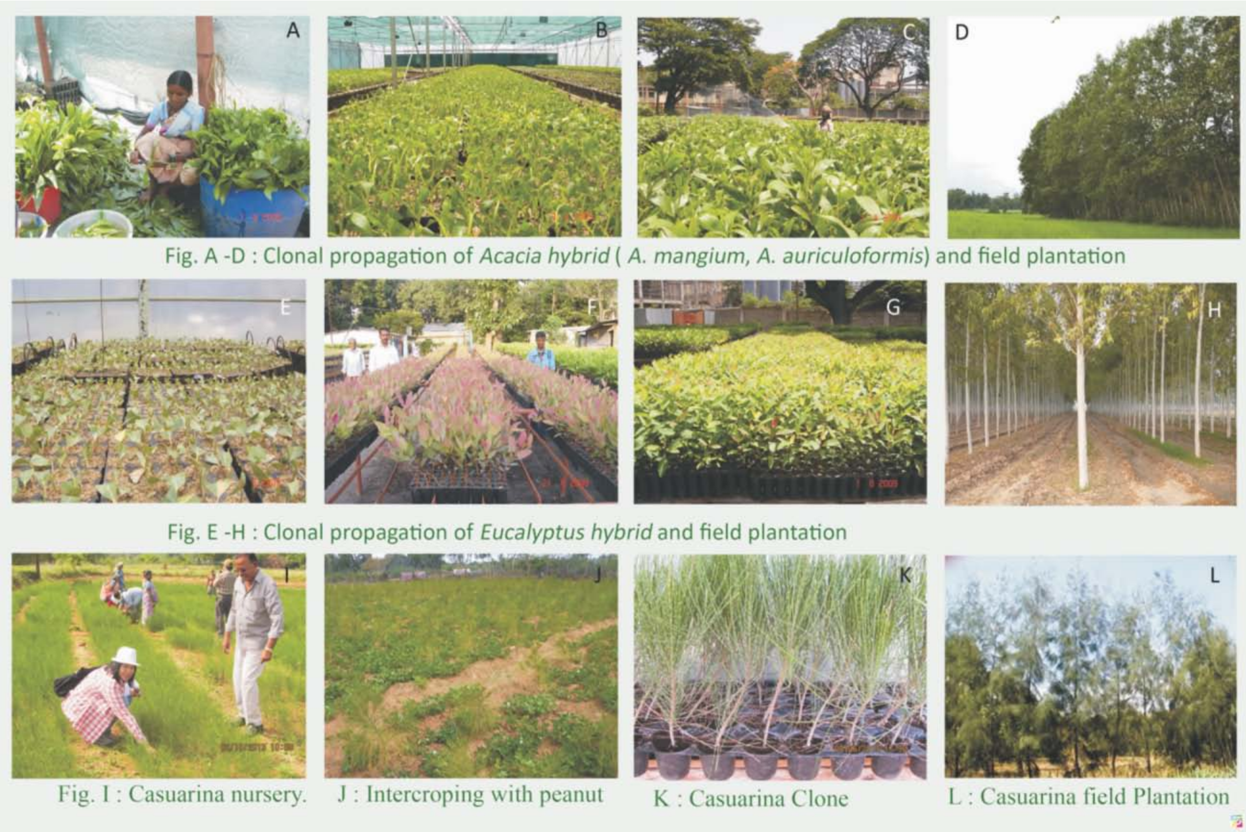
1.1 WCPM-SARA Initiative

The WCPM is a major wood-based paper manufacturing industry in India with an annual production of 320,000 MT of paper and paper products. It is located on the bank of river Kali at Dandeli, Karnataka State. The Company is engaged in plantation activities for nearly three decades under various forestry programmes. Since 2006, WCPM has been promoting plantations of pulpwood species in its core area of operation to maintain adequate supplies of pulpwood at competitive prices. The Company strives to procure its raw material requirement within a radius of 300

Table 1. The extent and locations of pulpwood plantations established by WCPM-SARA

Name of Section	District and State	Plantation area (ha)
Ram Nagar	Uttar Kannada-Karnataka	1055
Khanapur	Belguam-Karnataka	5182
Chennamana Kittur	Dharwad-Karnataka	2676
Koppal	Koppal-Karnataka	2495
Malgi	Sirsi-Karnataka	1835
Chandgad	Kolhapur- Maharashtra	2235
Radhanagri	Kolhapur- Maharashtra	2601
Kuluwalli	Dharwad-Karnataka	1025
Nellore	Gudur-Andhra Pradesh	2277
	Total	21381

Fig. 1. Clonal propagation and plantations of Acacia, Casuarina and Eucalyptus.



km from its Mill through promoting pulpwood plantations in the degraded and fallow lands of farmers.

Since the main raw material for WCPM is wood, the Company has developed its own Controlled Wood Policy followed since 2010. As per this Policy, the Company is fully committed to procure the raw material from known sources. To achieve this objective, it has formed a society called Society for Afforestation, Research and Allied Works (SARA). SARA is a non-governmental, non-profitable organization, registered under the Karnataka Societies Registration Act 1960 during 2001 and is supported and financed by WCPM. The Society has eminent foresters, researchers, technologists, agriculturists, financial experts and progressive farmers as members. During the last eight years, the society has covered more than 21,000 ha of degraded lands located in about 1900 villages of Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu States.

2 Captive Plantation Programme

2.1 Contract for Farming

SARA is actively engaged in establishing captive plantations through its scheme called Contract For Farming (CFF) under which the entire plantation activities are being done on farmers' degraded, barren, fallow land to promote plantations. SARA has so far covered around 8133 farmers as members under the society who have entered into an agreement for CFF for a period of five years. The Society meets all expenses related to plantation establishment and maintenance during the agreement period and WCPM procures the wood at a predetermined price. Thus the farmer is freed from investing in raising plantation and also provided with buy back guarantee for the wood produced. Most of the plantations were raised in various districts of Maharashtra, Karnataka and Andhra Pradesh States and their details are provided in Table 1.

2.2 FSC–FM Certification for "Well Managed Group Plantation"

All the plantations established by the Society since 2011 are duly certified by FSC-FM (Forest Stewardship Council-Forest Management) through a scientific certification system. SARA has been complying with the standards and principles set by FSC for sustainability in terms of economic, environmental and social requirements since 2011 and is among the pioneers in India having this certificate for "Well Managed Group plantations". It has developed various policies from time to time to comply with the following objectives of FSC:

- Promoting the responsible management of forest worldwide.
- Environmentally appropriate: Protecting and maintaining natural communities and high conservative value forests.

Table 2. Location and annual production targets of various nurseries of WCPM-SARA

Location and State	Species	Annual production (million plants)	Remarks
Dandeli-Karnataka	Eucalyptus, Acacia, Casuarina, Leucaena	5-6	Clonal and seedlings
Khanapur-Karnataka	Eucalyptus	0.5-1	Seedlings
Chandgad-Maharashtra	Eucalyptus, Acacia	0.5-1	Seedlings
Koppal-Karnataka	Leucaena	1	Seedlings
Gudur-Andhra Pradesh	Eucalyptus	2-3	Clonal
Cuddalore-Tamil Nadu	Casuarina	8-10	Clonal and seedlings

- Socially beneficial: Respecting the rights of worker communities and indigenous people.
- Economically viable: Building markets, adopting best value and creating equitable access to benefits.

2.3 Production of quality planting material through hi-tech nursery

SARA is also actively engaged in producing high quality planting material like superior clones of Eucalyptus, Acacia, Casuarina and Leucaena. It has set up a hi-tech clonal nursery in Dandeli spread in an area of 3000 m^2 with five mist chambers capable of producing 5 to 6 million clonal plants in a year (Fig. 1). Eucalyptus is the largest propagated species followed by Casuarina, Acacia, and Leucaena. Two Casuarina nurseries have been established at Pattanur and Katrampakkam in Tamil Nadu State with a production capacity of 8 to 10 million seedling and clonal plants. The Company is also running many satellite nurseries in various locations in Karnataka, Maharashtra and Tamil Nadu States to produce plants for its distribution programme (Table 2). Seedlings are distributed to farmers at a subsidized price to promote plantations and to make the high quality plants affordable to farmers.

2.4 Harvesting of pulpwood after maturity

The rotation period for harvest under the Contract For Farming programme of SARA is five years. First rotation crop of plantations established during 2006, 2007, and 2008 has been harvested and farmers were paid for their pulpwood received from their field. All arrangements for efficient harvesting of the wood are made by SARA. Harvesting of wood provides employment to the local communities resulting in their livelihood support and increase in living standards. In the case of coppicing crops like Eucalyptus and Leucaena, farmers enter into an agreement with SARA for maintenance and harvesting of coppice crop. This arrangement helps in continuous maintenance of plantations and cash flow to farmers and raw material availability to the Company.

2.5 Promotion of agroforestry models through pulpwood species

Practicing agroforestry in the farm forestry plantations helps in realizing the full potential of the land for the farmer which results in additional income. It also helps SARA to minimize maintenance activities like weeding. Farmers are encouraged to grow crops like maize, chilly, peanut and tomato as intercrops in their plantations. Intercropping of Eucalyptus with agriculture crop is widely accepted and well documented (Sharma et al., 2009). It is also commonly planted along the edges and bunds of agricultural fields and along with agricultural crops (Tejwani, 1994). Eucalyptus is cultivated throughout India and widely regarded as suitable for manufacturing of high quality paper (Srinivas, 2009). Other species like Acacia, Casuarina and Leucaena are commonly grown with intercrops in the southern part of India. Traditional rain-fed crops like sorghum and maize are cultivated with scattered trees whereas high stocking is maintained when irrigation is available for short-term crops like Casuarina and Leucaena. Poles of three to four year old trees of these crops are also widely used for housing in rural areas of south India providing additional market and high price for the wood (Rajendra and Mohan, 2014).

3 Social, Environmental and Economical Benefits

Cultivation of pulpwood plantations provides assured farm income to farmers who are facing hardships in agriculture like monsoon failure, reduced yield due to pest attack and fluctuating prices apart from contributing to industrial production and environmental benefits (Bhojvaid, 2006). It also helps in conserving the natural forests or High Conservative Value Forests (HCVF) by reducing pressure on these valuable resources for wood. While undertaking its plantation activities, SARA is providing free fodder to the livestock of farmers. It grows various types of fodder grass such as *Stylosanthes scabra* and *S. hamata* in the plantation area. Income from agricultural crops grown in the plantations during the first year after planting provides additional income to the farmers resulting in improved living standard. SARA plantations have also provided significant environmental benefits to society which include rehabilitation of saline land, restoration of water bodies, reduced soil erosion and carbon sequestration. The social benefits include generation of employment opportunities to landless agricultural workers and thereby preventing their migration to urban areas. It is estimated that the captive plantation activities of SARA provide around 784 person days of work per hectare during the rotation period. The Society also takes up activities under Corporate Social

Responsibility for the WCPM which include distribution of stationary items in village schools, establishing bore wells for drinking water, avenue plantation in village schools and public roads and repair of village roads for local communities.

3.1 Benefits to paper industry

The chief concern of any paper industry in India is to sustain the raw material supply in the near future. At present the shortage is so acute that a few industries including WCPM are importing wood from ASEAN countries. Captive plantation activities of SARA have ensured availability of raw material within a short distance from the Company. So far around 100,000 metric tonnes of pulpwood have reached the Mill from the SARA plantations. The yield from the clonal plantations on degraded land under rain-fed conditions is 60 to 70 tonnes per hectare in a five year rotation which is considerably higher than the conventional seedling raised plantations as widely reported for Eucalyptus clonal plantation in India (Lal and Kulkarni, 1992).

4 Conclusion

The captive plantation programme of WCPM-SARA has benefitted farmers to raise profitable pulpwood plantations in their marginal and degraded lands and supported the industry with locally produced raw material. Practice of agroforestry and use of genetically superior planting material has helped in maximizing land productivity and farm income apart from contributing to social and environmental benefits like employment generation and soil amelioration.

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Protective Role Played by Casuarina Plantations during Tsunami and Cyclone *Thane*: A Case Study

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Key words: biowall, coastal area, mangrove, multipurpose trees, shelterbelt

Abstract

Shelterbelt plantations of Casuarina helped in reducing damage caused by Tsunami waves and cyclone in the coastal areas of Tamil Nadu and Puducherry. Individual trees of different species also saved human lives by providing shelter during the tidal waves. New shelterbelt plantations involving Casuarina and other multipurpose tree species were established as biowalls to protect the human habitats in the coastal areas and as a source of fuel wood, minor timber and oil seeds. Plantations of mangrove species were also established in the backwaters to strengthen the defense provided by this unique vegetation during Tsunami. Involving local people particularly women helped in successful implementation of shelterbelt creation and maintenance.

1 Introduction

Regarded as one of the deadliest natural disasters in recorded history, Tsunami that struck the coasts of Tamil Nadu and Puducherry in India on 26 December 2004 caused huge loss of lives and property. More than 8000 people died and the survivors in affected areas lost almost everything they owned. Massive rehabilitation efforts were taken up by Government, Non-Governmental and international organizations to help the affected people to begin a new life. Increasing tree cover in the coast and creating a protective barrier with tree plantations between the sea and the human dwellings were among the post-Tsunami environmental improvement activities. This paper discusses the role of trees in protecting human life and property in the coastal areas and the composition and effectiveness of a barrier of trees between the sea and human habitats.

2 Post-Tsunami Vegetation Assessment

Over a hundred years ago, the entire east coast of southern India had continuous vegetation in the form of Dry Evergreen Forests. Due to the clearing for human settlements and other developmental activities, a major part of this unique vegetation has been lost exposing the habitats to the open sea. The damage caused by Tsunami was the greatest in areas where there was no vegetation between the sea and human habitats and minimal where the original forests were retained in places like Point Calimer. The damage caused by Tsunami was minimized by the standing vegetation, which reduced the force of giant waves. Even the sparsely occurring trees played an important role in protecting the life of people. Many Tsunami survivors narrated that they could survive only because they could hold on to the branches of the trees till the waves receded.

An assessment was carried out to record the ability of different tree species to withstand and survive the effect of Tsunami in the coastal areas. Among the trees that survived, Casuarina was the most effective in protecting the human habitats during Tsunami. The status different tree species after the Tsunami is given in Table 1.

3 Casuarina Plantations as Biowalls

Many areas in the coastal region had protective walls built between the sea and human habitats. But most of them have been damaged due to the action of moisture and salt-laden wind and high water table in these areas. Although barriers formed by heaping boulders were effective, such material was not available in many locations. Casuarina is among the very few trees that can withstand the salt spray and establish well in shelterbelt conditions. During Tsunami, the force of the waves was reduced in areas wherever shelterbelts of Casuarina were available. As a result there was increased interest in raising more plantations of Casuarina as 'biowalls'.

The biowall was created with the main objective of giving protection to the coastal communities. They are also designed in such a manner that it serves as a physical barrier but also provide produce and services to the local communities. They were established 50 meters away from the High tide line so that it is not affected by incursion of salt water repeatedly. Trees were planted in a narrow spacing of one metre from the high tide line for the next 50 meters in a criss cross manner to act as the first line defense against wind and wave action. Behind the Casuarinas, Coconut trees were planted for a width of 25 meters followed by a mixed species plantation involving *Thespesia populnea, Pongamia pinnata* and *Calophylllum inophyllum* for the next 25 metres. One or two rows of bamboos complete the plantings for biowall establishment. A cost-effective fencing for the young plantations was erected by planting poles of *Lannea coramandelica* which readily root and establish as posts for the fencing and thorny branches of bamboo were used as fence material attached to these

Table 1. Effect of Tsunami on the coastal vegetation in Karaikal and Puducherry, India

Common name	Scientific name	Status after Tsunami
Coconut	*Cocos nucifera*	Not affected
Palm	*Borassus flabellifer*	Affected
Casuarina	*Casuarina equisetifolia*	Not affected
Prosopis	*Prosopis juliflora*	Dried after Tsunami
Odian	*Lannea coramandelica*	Not affected
Pungan	*Pongamia pinnata*	Not affected
Punnai	*Calophyllum inophyllum*	Not affected
Poovarasu	*Thespesia populnea*	Not affected
Neem	*Azadirachta indica*	Dried after Tsunami
Mangrove	*Avicenia officinalis, Rhizophora* species	Not affected
Pandanus	*Pandanus* species	Not affected

poles. The combination of the species used in biowalls has the potential to meet food, biodiesel, secondary timber and fuel wood needs of the local population in addition to protecting their habitats from natural calamities. These plantations effectively reduced the damage caused by the cyclone, *Thane* which struck the coastal area during 2012 with wind speed of 80 to 120 km per hour.

4 Establishment of Mangrove Plantations

During the Tsunami, mangrove vegetation in the backwaters of Karaikal region helped in reducing the damage caused by the waves in the nearby areas. They also prevented drifting away of fishermen's boats by the waves. As part of post-Tsunami environmental improvement programme, mangrove plantations were established in the backwaters in many areas in Karaikal and Puducherry region. Seedlings of *Avicenia* and *Rhizophora* species were raised in polybags for about 6 months to reach a height of 60 cm and used for raising plantations. Mangrove seedlings were planted in the mudflats of backwaters by making trenches following a fish bone model. The mangroves seedlings were planted in such a way that they become submerged by during high tide to prevent drying.

5 Participation of Local People in Plantation Development

Since the Tsunami affected the traditional livelihood opportunities of coastal people, it was essential to instill confidence by providing alternate source of employment to them. This was best achieved by involving them in the shelterbelt development programme. All nursery, planting and maintenance activities were undertaken involving the local people particularly women. The creation of biowall provided employment opportunities to them during most difficult post-Tsunami period. They were explained about the importance of the plantations and benefits they woud receive from the trees in future so that they involve themselves in protection of these plantations. Small-scale decentralized nurseries were established close to the planting area and village settlement. Women were trained in nursery and plantation techniques. Irrigation ponds were dug up to provide protective watering to the plants immediately after the planting and also to meet the requirements of the villagers.

6 Conclusions

Shelterbelt plantations involving Casuarina as a major component and other multipurpose species play an important role in protecting the human dwellings in the coastal areas and also meet the requirement of fuel wood, minor timber and other needs of the local people. These plantations and other natural vegetation in the coastal region have to be protected to prevent damage to the existing landscape including sand dunes which offer natural protection to the people. Involvement of local people in the plantation development and maintenance activities is the key to long term management of these assets.

Evaluating the Role of *Casuarina equisetifolia* Plantation as Carbon Sink

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Key words: *Casuarina equisetifolia,* growth, biomass, productivity, carbon accumulation, farm forestry

Abstract

A field study was conducted to assess the growth, productivity and carbon accumulation in a farm forestry plantation of *Casuarina equisetifolia* in Pudukkottai, Tamil Nadu, India. DBH-based regression models were used to estimate biomass and carbon sequestration of this plantation. At the end of the rotation period, i.e. third year, the height recorded was 13.4 m, DBH 6.54 cm and volume 0.037cm^3. The total biomass produced by each tree ranged from 15.3 to 24.3 kg with a mean of 20.13 kg. Carbon content was 7.16 kg tree^{-1} in the above-ground biomass and 1.29 kg tree^{-1} for below ground biomass. Total biomass carbon content ranged from 5.96 to 11.06 kg tree^{-1} with a mean of 8.45 kg tree^{-1}. In general, biomass allocation and sequestered carbon was found to be the maximum in the stem followed by needle and branches and twigs. The regression models used in the study are based on the easy-to-measure trait, diameter at breast height and hence will be helpful in assessing and carbon accumulation in Casuarina plantations.

1 Introduction

Agroforestry and farm forestry are widely considered as potential ways of improving environmental and socioeconomic sustainability (Alavalapati and Nair, 2001). Farm forestry systems can play an important role in carbon mitigation programmes through carbon sequestration and can reduce the pressure on existing natural forests by providing fuel, fodder, timber and wood products directly to the farmers. On the other hand it may provide many indirect environmental benefits such as soil and water conservation, biodiversity conservation and soil nutrients enrichment (Singh and Lodhiyal, 2009). Trees promote sequestration of carbon into soil and plant biomass. Therefore, tree-based land use practices could be viable alternatives to store atmospheric carbon dioxide due to their cost effectiveness, high potential of carbon uptake and associated environmental as well as social benefits (Costa, 1996).

Casuarina equisetifolia is a fast growing multipurpose actinorhizal tree. It is well suited to sandy soil, resistant to saline spray and has the ability to grow in high density planting. It is proved to be useful as wind breaks and shelter belts to arrest the fury of cyclonic winds along the coast and to control soil erosion, It is also used as firewood, pole and pulp wood. In south India, it is cultivated in short rotations of 3 to 4 years under a high stocking of around 10,000 trees per hectare. Farmers prefer Casuarina for their agro and farm forestry plantations mainly for higher profitability than other annual crops and the minimal cultivation requirements.

Understanding the overall agro-ecosystem productivity and carbon sequestration potential can help in the effective management of ecosystem and mitigate the global warming. In an ecosystem, carbon is stored in different pools: above-ground live biomass (primary store), below-ground live tissue, necromass (which consists of plant litter usually on the soil surface or in the soil). The present study was carried out to estimate the growth, biomass and carbon sequestration of three year old *Casuarina equisetifolia* trees in a farm forestry plantation and to develop allometric regression models for non-destructive estimation of biomass and carbon sequestration.

2 Materials and Methods

2.1 Study area

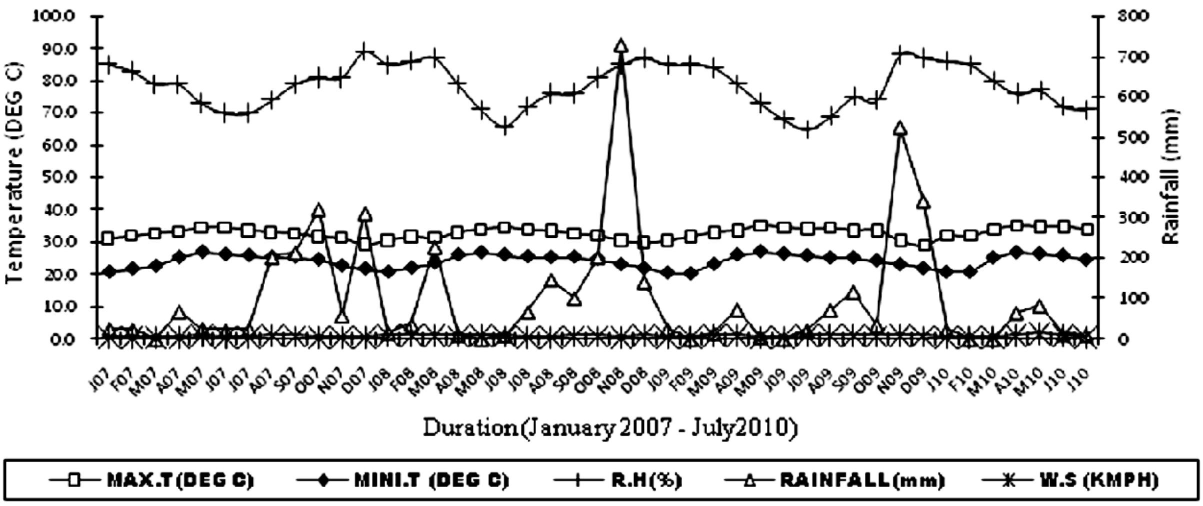
This study was conducted in the farm land at Vaithikovil in Pudukottai district located at 10° 23' N latitude and 78° 49' E longitude at an elevation of 179 m above msl. The maximum temperature at this site ranges from 24 to 38 °C and the annual precipitation is between 650 and 700 mm

Table 1. Biomass and carbon content (kg tree^{-1}) in 3 year old *Casuarina equisetifolia* trees under farm forestry

Tree Components	Stem	Bark	Branch & Twig	Needle	Fruit	Total AGB	Root	Root Nodule	Total BGB	Total Biomass
Biomass	9.64 (0.223)	1.19 (0.089)	2.13 (0.014)	3.73 (0.137)	0.25 (0.012)	16.95 (0.49)	2.90 (0.133)	0.26 (0.014)	3.17 (0.137)	20.12 (0.618)
Biomass carbon	4.12 (0.131)	0.49 (0.037)	0.85 (0.046)	1.59 (0.067)	0.09 (0.004)	7.161 (0.269)	1.18 (0.079)	0.11 (0.005)	1.29 (0.079)	8.45 (0.336)

Note: AGB - above ground biomass; BGB - below ground biomass. Values given in parenthesis are standard errors.

Fig.1. Meteorological details of the study site at Pudukkottai, Tamil Nadu



(Fig. 1). The soil type was loamy sand (sand 77.6%: silt 12.4%: clay 10%) with a pH of 8.5. Organic content of the soil was 1.58%. The nitrogen, phosphorus, potassium, calcium and magnesium contents were 0.47, 0.09, 0.38, 0.60 and 0.26 % respectively.

2.2 Biometric observations

2.2.1 Estimation of growth

The girth at breast height (1.37 m) and the total height were measured with a measuring tape. The individual tree volume was estimated as Volume= $(G/4)^2 \times H$; where G is the girth of tree at breast height and H is the tree height (Ravichandran et al., 2003)

2.2.2 Estimation of biomass

Random sampling method was adopted to estimate the biomass. Randomly selected 18 trees were harvested every year. Biomass components of tree (i.e., bole wood, bark, branch, twig, needle, fruit, root and root nodule) were separated and fresh weight was measured. Roots were excavated from 1.5 m^3 soil volume. For each component, 100 g of fresh samples were oven-dried at 80 °C for dry weight estimation and nutrient analysis.

2.2.3 Estimation of predicted biomass

The above-ground biomass and carbon sequestration were estimated by using measurements of total height and DBH. The data were subjected to regression analysis (Montero and Montagnini, 2005; Rana et al., 2001). The following regression models were tried: ln y = a + b ln (d) where, y = biomass or dry weight (kg $tree^{-1}$) of the component; d = diameter at breast height (DBH) in cm; H = tree height in m; a = intercept; b = slope or regression coefficient. The mean diameter was used in the regression equation of different components to get an estimate of mean biomass.

2.3 Carbon analysis

Oven-dried plant samples were powdered with the help of grinding machine and samples were analyzed. The carbon content (percent) in plant components (needles, twigs, branches, bark, bole, fruit and root) were estimated on CHNS analyzer (Vario III).

2.4 Statistical analysis

The data were analyzed by SYSTAT-10 to determine the statistical significance for differences in growth, biomass and carbon sequestration.

3 Results

3.1 Growth

At the end of the rotation period, i.e. third year, the height recorded was 13.4 m, DBH 6.54 cm and volume 0.037cm^3.

Table 2. Allometric models used to estimate total biomass (kg $tree^{-1}$) in a three year old C*asuarina equisetifolia* f arm foresrtry plantation

Tree components	Regression equation	r^2
Stem	ln (biomass) = 1.344 + 0.492 ln (DBH)	0.920
Bark	ln (biomass) = -3.129 +1.745 ln (DBH)	0.900
Branch & twig	ln (biomass) = -1.145 +1.011 ln (DBH)	0.904
Needle	ln (biomass) = 0.331 +0.526 ln (DBH)	0.931
Root	ln (biomass) = -0.794 +0.989 ln (DBH)	0.938

Table 3. Allometric models used to estimate total biomass carbon (kg tree^{-1}) in a three year old C*asuarina equisetifolia* farm foresrtry plantation

Tree components	Regression equation	r^2
Stem	ln (C) = 0.198+ 0.649 ln (DBH)	0.908
Bark	ln (C) = -3.993+1.733 ln (DBH)	0.913
Branch & twig	ln (C) = -2.152+1.056 ln (DBH)	0.904
Needle	ln (C) = -1.230+0.902 ln (DBH)	0.907
Root	ln (C) = -2.412+1.366 ln (DBH)	0.901

3.2 Tree biomass and carbon content

The above ground biomass consisted 57% of stem, 22% needle, 13% branches and twigs, 7% bark and 1% fruit in three year old trees. The below ground biomass had 96% root biomass and the remaining accounted by root nodules.

3.3 Predicted biomass

The predicted biomass on plantation basis was derived through a set of regressions between easily measurable parameters (DBH and Height) and dry biomass of different tree components (stem, bark, needle, branches and twigs and roots). The relationship between DBH and dry mass of all tree components showed a positive correlation (Table 2). These models were evaluated to predict tree biomass and the equations with the best fit were selected. The total biomass produced by each tree ranged from 15.3 to 24.3 kg with a mean of 20.13 kg (Table 1). The r^2 values ranged from 0.9 to 0.938 for the regression models.

3.4 Predicted biomass carbon

Carbon content was predicted through the allometric regression model, ln (C) = a+b ln (DBH) where C is the total carbon content in kg and DBH is the diameter at breast height in cm. The r^2 values ranged from 0.901 to 0.913 (Table 3). Carbon content was 7.16 kg tree^{-1} in the above-ground biomass and 1.29 kg tree^{-1} for below ground biomass. Total biomass carbon content ranged from 5.96 to 11.06 kg tree^{-1} with a mean of 8.45 kg tree^{-1} (Table 1). All the tree parameters measured and predicted showed significantly positive correlation (Table 4).

4 Discussion

4.1 Growth

The growth of Casuarina depends on light, moisture and available nutrients. The nutrient availability in the rhizosphere may be influenced by the tripartite association of Casuarina roots with *Frankia* and arbuscular mycorrizha (Reddell, 1990; Rajendran and Devaraj, 2004). Growth recorded in the present study for three year old trees (13.4 m height and 6.5 cm DBH) compares favourably with earlier growth reports for *C. equisetifolia* grown in different parts of the world. The height of 1, 2 and 3 years old trees was 1.6, 2.2, and 2.5 m respectively in the Philippines (Halos, 1983) and it was 2.05 m height and 3.6 cm diameter after two years under arid conditions in Egypt (El-Lakany, 1983). The growth improvement may be attributed to the genetically improved planting material used and systematic and effective cultivation practices. Increased growth was observed with height ranging from 9.8 to 11.9 m and diameter 5.3 to 7.4 cm in two year old trees when systematic cultivation methods and application of biofertilizers were adopted (Rajendran and Devaraj, 2004).

4.2 Tree biomass

Results from the presence study indicate that fast-growing species like *C. equisetifolia* accumulate biomass and carbon rapidly during the initial stage of their life span. This period could be 3 years in the well managed farm forestry plantations. This shift in the accumulation of biomass and carbon may be related to intensive silviculture management such as superior genetic materials, appropriate spacing, regular watering, weeding and manuring. In the present study, biomass allocation and carbon in tissue types decreased in the order: Stem>needle>branch and twig>root>bark> fruit. Allometric equations have been developed for estimating biomass or volume for many tropical fast growing trees including *Acacia mangium* (Halenda, 1989), *Eucalyptus saligna*, *E. grandis, E. robusta* and *E. urophylla* (Schubert et al., 1988; Dudley and Fownes, 1992), *Leucaena leucocephala* (MacDicken and Brewbaker, 1988) and *Casuarina equisetifolia* (Dudley and Fownes, 1992). Biomass estimation equations generally vary with species, age, bole shape and/or bole wood density. Variation in these characteristics result from one or more of the causes like genetic differences between populations, environmental variability among sites and crowding of trees that affect tree shapes (Campbell et al., 1985). Therefore, biomass regression equations, generalized for a geographic region rather than a single age-class population, have been developed to minimize errors in estimated biomass that result from such variability in sampled trees (Pastor et al., 1983/1984). In seven year old *C. equisetifolia*, the above-ground biomass showed stand average using DBH based regression model (141 t ha^{-1}) and d^2H based model (142 t ha^{-1}) and percentage contribution of the above-ground biomass, bole wood accounted for 61.9%; branch 20.6%; twig 7.3%; leaf 5.7% and bark 4.5% and belowground biomass 17.5% (Rana et al., 2001).

Table 4. Correlation values for tree growth, biomass and carbon of *Casuarina equisetifolia* plantation under farm forestry

Characters	DBH	Height	Volume	Total biomass
DBH	1.000			
Height	0.862*	1.000		
Volume	0.979**	0.928**	1.000	
Total biomass	0.966**	0.877**	0.952*	1.000
Carbon	0.972**	0.851*	0.961**	0.967**

*Significant at 0.05 level (2-tailed).
*Signicfiant at 0.01 level (2-tailed).

4.3 Tree Carbon

The present study showed a total biomass carbon of 8.45 kg $tree^{-1}$ in three year old trees which was distributed in the order of stem>branch and twig>needle>root>bark. An earlier study reported, carbon sequestration potential of *C. equisetifolia* bole as 16.7 MT ha^{-1} $year^{-1}$ of C (Alfisols) and 4.7 MT ha^{-1} $year^{-1}$ of C recorded in Vertisols in Cuddalore District, Tamil Nadu, India (Ravi et al., 2010). In East Timor, *C. equisetifolia* (≥30 years) contained up to 200 Mg C ha^{-1} in above ground biomass (Lasco and Cardinoza, 2007). Four year old Casuarina plantation stored 48.2 Mg ha^{-1} carbon in the above-ground biomass and 10.5 Mg ha^{-1} in roots (Parotta, 1999). In Casuarina, the total carbon pool of revegetated stands was larger than that of rehabilitated stands and increased from 80.97 tonnes C ha^{-1} in the 8 year-old stand to 211.32 tonnes C ha^{-1} in the 16 years old stand and 158.17 tonnes C ha^{-1} in 19 year old stand (Ntshotsho, 2006). Regression models developed from a narrow range of diameters may be more accurate at predicting carbon stock for a population of trees in that range than a model developed from a wider range of diameters (Losi et al., 2003; Brown, 1986).

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Silviculture and Agroforestry of Casuarina in Tamil Nadu, India

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Casuarinaceae includes eighty species of trees and shrubs, distributed mainly in Australia and Asia. Casuarina trees are adaptable to various environmental conditions. *Casuarina equisetifolia* was introduced in India during nineteenth century to meet the requirements of rural people for fuel, fodder, poles, and shelter belts. Various research trials for standardization of cost of production, watering regime, yield per unit area, nursery technology, silviculture operations like espacement, thinning, rotation, growth rate, were conducted under different programmes by the Research Wing of Tamil Nadu Forest Department. Some important studies are comparison of growth between *Casuarina equisetifolia* and *C. junghuhniana*, effect of single and double sprigs planted in a pit, introduction trials, espacement trials, best season for planting, studies on agroforestry models, on-farm trials, suitability of Casuarina for water logged areas and shelterbelt models. Seeds were collected from genetically superior trees and distributed to the farmers and the forest department. Based on the experience gained from the previous studies, the current focus is on shortening of rotation, reducing cost of production, identifying the genetically superior trees, multiplication of clones and studies on post-harvest technology.

Effect of Mycorrhizal Fungi and *Frankia* on Growth of Casuarinas

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Casuarinas are symbiotic plants associated with mycorrhizal fungi and *Frankia* which can help plants uptake nutrients to improve growth and increase tolerance under abiotic stress. Research on these aspects were carried out from 1993 to 2012 in southern China with particular reference to the growth of *Casuarina equisetifolia, C. Junghuhniana* and *Allocasurina littoralis* inoculated with a group of ectomycorrhizal, endomycorrhizal fungal isolates and *Frankia* strains in nursery, glasshouse and field conditions. There is a strong interaction between mycorrhizal fungi or *Frankia* and tree genotype (provenance, family or clone). Right combinations of mycorrhizal fungal or *Frankia* isolate and tree genotype were screened for *C. equisetifolia* and *C. Junghuhniana*. Only some mycorrhizal species and isolates could improve the growth of Casuarinas, thus selection of effective mycorrhizal fungi is necessary. Mycorrhizal or *Frankia* inoculation can improve nutrient uptake of seedlings, and increase tolerance to abiotic stress and survival. Inoculum application should follow the principle “suitable mycorrhizal fungal or *Frankia* isolates must match suitable site and tree species”, based on more than 20 years of research experience.

Biosignalling Molecules and Biomass Documentation of *Frankia*-inoculated *Casuarina equisetifolia*

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Hopanoids are intuitive secondary biosignalling molecules of *Frankia* which is a nitrogen fixing actinomycete symbiont inducing root nodules in a diverse group of about 200 species. *Casuarina equisetifolia* is an economically important tree nodulated by *Frankia*. Some of the bioactive secondary molecules were found to be more important for establishing the root-associated growth of *Frankia* due to the presence of a mixture of mono- and sesqui-terpenoids, and fatty acid derivatives. Over 56 compounds were detected from different day culture fractions of *Frankia* using GC-MS-MS, and a total of 19, 23 and 14 components were characterized and identified in 15, 25 and 30th day cultures respectively. Some of the derivatives retained at 15th day were reported to produce vesicles in roots of *C. equisetifolia*. The analysis indicated variations in the presence of biotransformed signaling molecules, especially the hexa decanoic acid, phthalic acid and their derivatives at different stages of its growth period. The compounds, such as isoterpinolene, 2, 4, Phenol-bis (1, 1-dimethyl ethyl) and 1- Dotriocantanol produced in later stages of *Frankia* growth period tend to restrict the oxygen disturbances in the nitrogenous activity and thereby enhancing nodulation and nitrogen fixation in *C. equisetifolia*. The ureides, allantoin and allantoic acid, represent major fractions of the soluble nitrogen pool of nodulated *C. equisetifolia* throughout vegetative and reproductive growth. Allantoin content was profoundly high for the *C. equisetifolia* seedlings treated with 25th day *Frankia* culture as compared to 15th and 30th day cultures. Biosignalling compounds identified from *Frankia* culture were found to increase biomass of Casuarina, especially the plant height, nodule number and nodule weight. In the present study, it was found that the *Frankia* produced a complex of bioactive compounds, like hoponoids and terpenoids at different stages during its growth period. Their role in plant-pathogen and plant-insect interactions is being studied to determine their potential in pest control.

Symbiotic Signaling and Nodulation Ability of six *Frankia* Strains Isolated from Nodules of Casuarinaceae Trees in Egypt

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The objective of the project that is currently developed between Canal Suez University in Ismailia in Egypt and IRD Montpellier in France, is to study a collection of Egyptian *Frankia* strains for their potential to produce symbiotic factors contributing to an optimal dialogue with the plant root system. To achieve this goal, transgenic *Casuarina glauca* plants that express the reporter gene ßglucuroniase (gus) under the control of the symbiotic promoter CgCCaMK have been obtained. CgCCaMK encodes a calcium/calmodulin-dependent kinase that is essential for *Frankia* root hair infection and for its intracellular progression through the actinorhizal nodule. In transgenic *C. glauca*, the ProCgCCaMK-gus construct is induced in lateral roots during the perception stage of the reference strain *Frankia* CcI3, and responds to *Frankia* CcI3 supernatants containing the still unknown Root Hair Deforming Factor(s). Inoculations of transgenic gus-plants were performed with six different *Frankia* strains isolated from actinorhizal nodules in Egypt. A kinetic of nodulation was also established for all strains and the number of nodules were counted two months following *Frankia* inoculation. Two strains, named 15(6) and Manef 4, were found to induce a strong reporter gene activity during the early stages of the symbiotic process. The largest number of nodules (more than 60 nodules per plant) was obtained with the strain 15(6). From these data, *Frankia* strains that will be the most appropriate for degraded areas in Egypt are expected to be identified. This work was supported by a bilateral grant PHC IMHOTEP 27467SA (2012-2013).

Identification of Saline Tolerant Clones of *Casuarina equisetifolia* using Biochemical Markers

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Soil salinity has become a major factor limiting crop productivity worldwide, especially in arid and semi arid regions. *Casuarina equisetifolia*, being a multipurpose species with farmer- friendly attributes and industrial demand, is preferred by farmers for cultivation it in all types of soil. In this context identification clones suitable for different soil conditions, especially saline soils is essential to expand Casuarina cultivation. A study was undertaken to identify biochemical parameters as markers for screening clones of *C. equisetifolia* for saline tolerance at nursery stage. Salinity induction experiments were carried out with 25 clones using Hoagland solution as growth medium. Clones were classified into 4 groups based on survival after 3 months namely, Tolerant (withstands up to 250mM), Moderately tolerant (up to 200 mM), Low tolerant (up to 150 mM) and Sensitive (up to 100 mM). Biochemical analysis for soluble proteins, phenols, proline, peroxidase, ascorbic acid, chlorophyll A, chlorophyll B, total chlorophyll, anthocyanin, anthocyanin:chlorophyll ratio, root and shoot sodium and root and shoot potassium ions were conducted before and after saline induction. In addition physiological parameters such as membrane injury index, relative water content, chlorophyll stability index and morphometric parameters were studied. ANOVA showed that except root sodium, collar diameter, membrane injury index and relative water content, all other parameters significantly differed among clones. Canonical Discrimination Analysis to identify the highly discriminating parameters for grouping the clones showed proline, protein and chlorophyll A, phenol and anthocyanin and chlorophyll B as discriminating parameters, however, Chlorophyll A and B were found to be non-consistent. An increasing trend for proline and decreasing trends for protein and phenol with increasing saline concentration were observed. Anthocyanin showed an initial increase on saline stress followed by a decline. Threshold levels for the biochemical parameters were identified to classify the saline tolerant and sensitive clones. On validation of the results on a new set of clones, proline successfully grouped the clones to the extent of 50%, protein to the level of 60% and both Phenol and Phenol + Protein to the scale of 80%. This study shows that biochemical markers could be used for screening *C. equisetifolia* clones at nursery stage for saline tolerance for further field testing and breeding experiments.

Anatomical Variation in Tissue Characteristics between Juvenile and Adult Materials as well as Male, Female and Monoecious Trees of *Casuarina equisetifolia*

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The transition from juvenile to adult phase has been explained as a change in the reproductive competence but it is also marked by species-specific changes in a variety of vegetative traits including leaf shape, leaf anatomy, adventitious root production, disease resistance and a number of secondary compounds. Results from the early attempts to identify sex of the plants based on morphological and physiological parameters have not been consistent. In recent years, there have been serious studies on the genetic basis of sex determination and early sex identification using molecular / biochemical markers. In the present study phylloclad cuttings from four positions viz. lower (juvenile) to upper (mature) parts of nine year old male, female and monoecious trees were investigated for nine anatomical characters (pith diameter, thickness of the phloem tissue, number of xylem vessels per unit area, diameter and area of the xylem vessels, roundness, aspect ratio and fullness ratio of the xylem vessels) using an image analyser. Significant differences were observed between the juvenile and adult tissues. Pith diameter and thickness of phloem tissue varied among the stem cuttings obtained from the four positions. A decreasing trend was observed from position 1 to position 4 for pith diameter and a reverse trend was observed for the thickness of phloem tissue. Similar was the trend for diameter of the remaining area (excluding pith and phloem), which was observed to decrease from position 4 to position 1. None of the other parameters examined in this study could be used to distinguish juvenile and adult materials at the anatomical level. Pith diameter, diameter of the area excluding pith and phloem, diameter, area, roundness and aspect ratio of the xylem vessels varied significantly among the male, female and monoecious trees. Pith diameter was found to be maximum for monoecious trees followed by female and male trees. A reverse trend was noticed for diameter of the remaining area. Average diameter and area of the xylem vessels were higher for the male trees than the female and monoecious individuals. With reference to the shape of the xylem vessels, male trees exhibited better roundness than the female and monoecious trees.

Assessment of Variability in Nitrogen, Chlorophyll and Free Proline Content in Half-sib Progenies of *Casuarina* Species in Peninsular India

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The objective of this study was to investigate total nitrogen, chlorophyll "a", "b" and "total" content at in 12 months old progeny of five species of *Casuarina* planted at Gangavati, Karnataka State, India during 1999. The total nitrogen content was significantly higher in *C. equisetifolia* (26.3%) followed by *C. glauca* (23.7%) and lower in both *C. obesa* (19.9%) and *C. cristata* (20.9%). *C. equisetifolia* had maximum root nodulation and nitrogen fixing ability. *C. glauca* and *C. cunninghamiana* recorded similar total nitrogen content. Further the study revealed that total chlorophyll (0.40 µg g^{-1}) and chlorophyll "b" (0.11µg g^{-1}) content were significantly higher in *C. equisetifolia* and lower in both *C. obesa* and *C. cristata*. Similarly, chlorophyll "a" content was higher in *C. cunninghamiana* (0.09 µg g^{-1}) and lower in both species of *C. equisetifolia* and *C. obesa*. Chlorophyll has a precise role in the photosynthesis activities, and has a profound effect on growth and biomass production in plants. *C. cunninghamiana* (28.9 µg g^{-1}) followed by *C. glauca* (26.04 µg g^{-1}) and *C. equisetifolia* (25.1 µg g^{-1}) had higher free proline content indicating tolerance to drought and saline conditions when compared to *C. cristata* (15.9 µg g^{-1}) and *C. obesa* (16.1 µg g^{-1}).

Descriptors and DUS Testing Guidelines for Casuarinas

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Domestication and breeding of crops lead to releasing of high yielding varieties for commercial cultivation. These new varieties have to be suitably described for easy identification and to protect the intellectual property rights of the breeders and institutions. In order to protect the intellectual rights of the breeders, Government of India enacted the “Protection of Plant Varieties and Farmer’s Rights Act 2001". All the new and existing agricultural and tree varieties can be protected under this Act. They will be subjected to a test of Distinctiveness, Uniformity and Stability (DUS) using mainly morphological descriptors before their registration for IPR protection. The Institute of Forest Genetics and Tree Breeding has developed DUS testing procedures for Eucalyptus and Casuarinas and these guidelines have been published in the Plant Variety Journal of India in February, 2013. A total of 34 descriptors have been developed to discriminate varieties and clones of *Casuarina equisetifolia*, *C. junghuhniana* and their hybrids. They consist of bark, branchlet, crown, branch, sexual system, stamen and cone characters. The DUS tests are normally conducted at two locations and observed in two flowering seasons. One out of nine plants can be an off-type. The descriptor-based discrimination of clones and varieties will help in efficient field identification of varieties and by protecting the rights of breeders and farmers increase investments in breeding and varietal development programmes.

Variation in Rooting Response Among Clones and Rooting Methods in *Casuarina equisetifolia, C. junghuhniana* and their Hybrids

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Casuarina is widely grown as a commercial tree crop in farmlands of Southern India especially in the coastal region. In order to maximize wood production, clonal plantations are becoming popular among farmers and industries. A large amount of clonal planting material is needed to meet the demand. This task can be achieved through identification of clones and rooting methods which are amenable to simple and low-cost vegetative propagation. The present study investigated variation in response among clones of *Casuarina equisetifolia, C. junghuhniana* and their interspecific hybrids aged from one to four years under solid and water media for rooting. Softwood cuttings were treated with different concentrations of auxins (IBA and NAA) and planted either in soil or water culture cups. Results showed that the highest rooting percentage was obtained for cuttings collected from one year old trees treated with 300-500 mg L^{-1} IBA and NAA rooted in water medium. In general hybrid clones showed faster and better rooting than clones of parent species. They also responded well for rooting in water medium. The results of this study can be used to develop simple and cost-effective techniques for large scale production of Casuarina clones.

Growth Performance of Clonal and Seedling Accessions of Casuarina in Different Agro-climatic Regions of Tamil Nadu, India

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Casuarina is an important fast growing short rotation tree species used as raw material by wood-based paper industries in India. *Casuarina equisetifolia* is widely grown in the coastal areas of Tamil Nadu State and in recent years, *C. junghuhniana* Miq. is grown in the inland areas since it is moderately drought tolerant. The Tamil Nadu Newsprint and Papers Limited (TNPL) is producing around 6 million seedlings of *C. equisetifolia* and 1.5 million clonal plants mainly of the natural hybrid clone (TNFD CJ1) of *C. junghuhniana* x *C. equisetifolia* every year. In order to widen the genetic base of clonal plantations on-farm trials were conducted with TNFD Cj1 and a new clone of *C. junghuhniana* developed by the Institute of Forest Genetics and Tree Breeding (IFGTB CJ9), two seedlots of *C. equisetifolia* derived from seed orchard and unimproved sources in different agroclimatic regions in Tamil Nadu. Farmers involved in the farm forestry program were trained to follow Precision Silviculture Management (PSM) for spacing, pruning, drip irrigation and fertigation suitable for a given planting site. The biometric observations like total height and Diameter at Breast Height measured during 12, 24 and 36 months after planting were subjected for statistical analysis. The study revealed that growth performance of Casuarina IFGTB CJ 9 clonal plantation was significantly better than the widely planted clone and the seedling-raised plantations under the agroclimatic conditions of test locations.

Performance of Casuarina Hybrid *(C. junghuhniana* x *C. equisetifolia)* as the Fast Growing Tree for Bio-energy in Saline Soil

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The growth performance in term of biomass of Casuarina hybrid (*C. junghuhniana* x *C. equisetifoli*a) and other fast growing species (14 species in total) planted in saline soil with influence of tidal current of sea water was evaluated at Samutsakorn province, Thailand. Soil salinity of this site was measured in terms of the Electrical Conductivity (EC) which was observed to be moderate in salinity level (EC = 4.2-4.4 mS cm^{-1}). The trial was planted in Randomized Complete Block Design (RCBD). As the site was low-wet land, ridging as a soil preparation was done before planting. Harvesting was done during December 2013, when the trees reached 2 years age. After 2 years of planting, *Melaleuca leucadendron* presented the highest survival rate 100 %, followed *Acacia ampliceps* at 97.78%, while *A. dificilis* and *A. torulosa* could not survive in this type of soil. In term of growth as dbh, *A. ampliceps* and Casuarina hybrid showed significantly higher value than the others with 8.41 and 8.33 cm respectively. With reference to total height, Casuarina hybrid showed obviously the highest value with 7.9 m height, followed by *Eucalyptus camaldulensis* with 6.3 m height. Regarding the biomass yield performance, Casuarina hybrid provided the highest stem, leaves and total biomass which were 34.39, 21.05 and 66.72 t ha^{-1}, respectively followed by *A. ampliceps* with 21.68, 10.57, and 52.63 t ha^{-1} for stem, leaves and total biomass respectively. Results of this study indicated that Casuarina hybrid and *A. ampliceps* are two potential fast growing species in saline soil for energy plantations.

Quality Casuarina Planting Material - A Farmer's Dream

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Puducherry is known for Casuarina (*Savukku* in Tamil) cultivation and is considered the hub of Casuarina nurseries. The Puducherry and adjoining areas like Cuddalore, Tindivanum, Marakkanam, Veedur and other parts of Tamil Nadu State are largely planted with Casuarina. The populality of Casuarina is due to various factors like resistance to drought and water stagnation, prevalence of ready market and particularly it requires minimal labour and field maintenance requirements. The land area under cultivation of Casuarina is shrinking steadily due to activities like urbanisation and construction of highways encroaching the area suitable for cultivation. This necessitates that productivity need to be increased in the available land. To increase the productivity, quality seedlings are a prerequisite, as the proverb goes what you sow shall be reaped. However, availability of large quantity of quality planting materials is a matter of concern. The planting materials are supplied from the nurseries present in and around Puducherry but improved seeds are not available to farmers and also the seedling materials are not available at the right time. Awareness among farmers on the importance of quality seedling and the method of transportation and planting is low. Most of the seedlings are overgrown measuring more than 60 cm with poorly developed or lengthy coiled roots when planted. The bare-rooted seedlings are taken in bundles in two wheelers, or mini-truck to long distances in open conditions exposed to hot sun. Hence it is essential to train and create awareness on the importance of quality of seeds and seedlings and transportation method and improved planting techniques to farmers and nursery growers.

Role of Forestry Extension in Expanding Casuarina Cultivation Area in Tamil Nadu, India

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Casuarina is among the most important tree species cultivated in the coastal districts of Tamil Nadu State since 1951. Due to its multiple use and good economic returns, farmers also showed interest in cultivating Casuarina crop either as a pure crop or as inter crop in their farmlands in other parts of the State. Research works are being carried out on Casuarina cultivation by the Research Organization such as IFGTB, TNAU, Tamil Nadu SFRI and wood based industries like TNPL. Though many technological innovations are taking place by continuous research of various research organizations in the State, the transfer of these innovations is not happening to the expected level. Research findings emanated from these institutes are being transferred to the farmlands through 30 Forestry Extension Centres formed all over the State by the Forest Department of Tamil Nadu. These Forestry Extension Centres are involved in transferring information on Casuarina cultivation to farmers by organizing training programmes, laying demonstration plots in progressive farmers' land, publicity and propaganda and organizing awareness programme among the farming community. Thus, the Forestry Extension programme supports the expansion of Casuarina cultivation in Tamil Nadu State.

Standardization of Nutrient Management for Enhancing Productivity in *Casuarina* Species

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Casuarina is suitable for pulpwood and fuel wood. Casuarina species have been the farmers' favorite as they grow well in agrarian ecosystem. In the recent years, Casuarina wood is in great demand for paper making. With increase in paper consumption, many paper mills have expanded their production capacities and are now facing shortage of fibrous raw material. Under such situation a paradigm shift is essential from general silvicultural packages to site-specific management packages to harvest higher utilizable biomass at the shortest rotation period. To elucidate information on enhancing the productivity of Casuarina, a field experiment was conducted with Casuarina species viz., *C. equisetifolia* and *C. junghuhniana* from seed and clonal sources raised under five nutrient levels. Two weedings and one pruning were given up to 18 months after planting (MAP). The study was taken up at Agricultural Research Station, Bhavanisagar during 2010-2012. This area had a mean annual rainfall of 700 mm received in 50 rainy days. The soil type was vertisol of Irugur series which was low in N and P and high in K. seed raised plants of *C. equisetifolia* with 50% more fertilizer application (112.75:270:150 NPK Kg ha-1) recorded the maximum productivity over the soil test recommendation value and significantly recorded the maximum available N, P, K in the soil compared to all the other interactions. This interaction also recorded high N, P, K content in the needles of *C. equisetifolia*.

Impact of Casuarina Wind Breaks – A Case Study

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Agriculture is the mainstay of economy in the State of Tamil Nadu. The predominant crops grown are rice, sugarcane, banana, cotton, fruits and vegetables. Among the various crops, banana occupies 27 per cent share of production among the crops cultivated in Tamil Nadu. During the recent past, the banana growers faced serious problems of lodging and uprooting of banana stems due to heavy wind, hurricane and cyclone which resulted in considerable economic loss. Against this backdrop, Forest College and Research Institute has promoted a linear model wind break using *Casuarina equesitifolia* (MTP1) and *C. junghuhniana* Asur hybrid clone (MTP2) along and around banana plantations in Mettupalayam taluk of Coimbatore district. Casuarina is a multipurpose tree species amenable for agro and farm forestry system and also as wind break and shelter belts. The Casuarina based wind breaks established around banana plantations were investigated for its impact on productivity and economics. Economic impact of Casuarina grown as border crop in banana plantations was studied in the Pugulur and Jadayampalayam regions of Mettupalayam taluk in Coimbatore district. Partial budgeting technique was employed to show the effect of change(s) in crop enterprises. Hence, it was analysed through the partial budgeting technique which showed a positive net return of income in the banana cum Casuarina plantation compared with the pure banana crop. The results indicated that the sample farmers following this model gained higher net income through higher yield and also found that it prevented soil erosion in their fields as an added advantage besides improving soil fertility through nitrogen fixation.

Carbon Sequestration Potential, Calorific Value and Biomass Productivity of Five Casuarina Species in Dry Land Ecosystem of Peninsular India

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The objective of this study was to investigate the carbon sequestration, growth performance, biomass production, calorific value and merchantable volume of five *Casuarina* species under rain-fed conditions at Agricultural Research station in Gangavati, Karnataka State, India. The highest total biomass of 395 t ha^{-1}, merchantable stem volume of 125 m^3 ha^{-1} and calorific value of wood of 4884 kcal kg^{-1} were recorded for *C. equisetifolia* followed by *C. glauca* (308 t ha^{-1}, 102 m^3 $ha^{-1}$and 4562 kcal kg^{-1} respectively), *C. cunninghamiana* (275 t ha^{-1}, 88 m^3 ha^{-1} and 4707 kcal kg^{-1}), *C. cristata* (242 t ha^{-1}, 67 m^3 ha^{-1} and 4286 kcal kg^{-1}) and *C. obesa* (250 t ha^{-1}, 62 m^3 ha^{-1} and 4650 kcal kg^{-1}). The adaptability (survival 66.5 to 86.7 %) and its role in sequestering carbon that addresses the mitigation of climate change was highly significant among Casuarina species . The average total tree biomass obtained was 294 tonnes per hectare. Considering about 48 % of a biomass is carbon, the carbon sequestration and carbon dioxide sinks rate obtained during year for *C. equisetifolia* (189 t ha^{-1} and 695 ton ha^{-1} respectively) was higher than *C. glauca* (148 t ha^{-1} and 542 ton ha^{-1}), *C. cunninghamiana* (132 t ha^{-1} and 4840 t ha^{-1}), *C. obesa* and *C. cristata*. Biomass and carbon accumulation were relatively higher in *C. equisetifolia* followed by *C. cunninghamiana* and *C. glauca*.

Performance of Economically Important Medicinal Plants in Casuarina-based Agroforestry Systems in Tamil Nadu, India

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The present investigations were undertaken to study the performance of economically important medicinal plants in agroforestry systems with two species of *Casuarina* in Tamil Nadu, India. The soil fertility was found to be improved under shade conditions. Among the two tree species, the highest macro- and micronutrient presence was found in the soil of *Casuarina equisetifolia* followed by *C. junghuhniana*. The *Gloriosa superba* produced maximum seed yield in open field compared to agroforestry system at Jayangondam location. The colchicine content of *G. superba* seed extract was high in *C. junghuhniana* based agroforestry system at Jayangondam followed by Sivagangai. The maximum shoot length and root length of *Decalepis hamiltonii* were recorded in *C. equisetifolia* based agroforestry systems compared to *C. junghuhniana* based agroforestry systems and open field. The highest shoot length and root length of *Hemidesmus indicus* were observed in *C. equisetifolia* based agroforestry systems when compared to *C. junghuhniana* based agroforestry systems and open field. Among eight medicinal plants, *Decalepis hamiltonii, Gloriosa superba* and *Hemidesmus indicus* had shown good performance in Casuarina based agroforestry systems. Medicinal plants based agroforestry has the potential to provide additional income to the farmers besides enhancing environmental services.

Effect of Growth Promoting Treerich Biobooster on Casuarinas

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Effect of Treerich Biobooster, made of organic biocompost like vermicompost, decomposed coir pith, vermiculite, effluent waste, decomposed green manure along with Plant Growth Promoting Rhizobacteria (PGPRs), Pink Pigmented Facultative Methylotrophs (PPFM), arbuscular mycorrhizal fungi, and Frankia was evaluated for its effect on the seedling growth of *Casuarina equisetifolia* L. and *C. junghuhniana* Miq. Conventional soil potting mixture (sand, soil and farm manure) was used as control. Jartopha seed kernel cake and Aegle seed cake were also evaluated along with aforementioned biocomposts to develop a composite Treerich Biobooster. The inoculation of PGPRs, PPFM, AM fungi and Frankia significantly increased the germination percentage of Casuarinas by 40-43 percent compared to conventional nursery practice. Nutrients such as carbohydrates, protein, chlorophyll, Ca, K, N, P Mg, S and organic carbon were found to be significantly increased in the treated seedlings when compared to control. The growth performance was found to be significant in terms of shoot length, collar diameter, biomass yield and survivability from 30 to 60 days after treatment which reduced the use of chemical fertilizers. The combined effect of bioinoculants with organic biocompost significantly increased the germination percentage, survivability and biomass yield which are considered as performance indicators of quality planting material. This may be due to the ability of bioinoculants to mobilize the nutrients from the substrate into the plants in usable form. Application of seed kernel cakes drastically reduced the pest incidence. Hence, the organic biocompost product, Growth Promoting Treerich Biobooster is recommended as a potential potting media and an alternate to conventional soil, sand, FYM mixture for production of healthy quality planting stock of Casuarianas.

Resistance of Different *Casuarina equisetifolia* Clones to *Anoplophora chinensis* and *Lymantria xylina*

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Casuarinas are predominant species of the coastal shelter forest in southeastern China. *Anoplophora chinensis* and *Lymantria xylina* are serious insect pests which cause mortality in *Casuarina equisetifolia* shelter forests. Breeding resistant varieties is one of the effective methods to control insect damage and crop loss. In order to identify clones resistant to *A. chinensis* and *L. xylina*, 48 *C. equisetifolia* clones were screened in Huian of Fujian province during 2010-2012. Based on investigation and artificial inoculation in the plantation, the population density, damage rate and eclosion rate were assessed. The results showed that 3 clones, Hui13, Hui76 and Hui83 were strongly resistant to *A. chinensis*, with the average population density of larva less than 0.12 insect per plant, damage rate lower than 12 %, and eclosion rate was zero. After artificial inoculation, less than 20% of larvae could develop into adults on these clones. The 2 clones, Hui58 and Hui88 were sensitive to *A. Chinensis*, with the average population density of larva more than 1.0 insect per plant, damage rate over 66%, and eclosion rate over 35%. More than 60% of larvae could successfully develop to adults after artificial inoculation. Feeding selectivity, development characteristics and frass amount of *L. xylina* larvae were observed by rearing larvae with twig of different *C. equisetifolia* clones under indoor conditions. Results showed that 4 clones, Zhanjiang3, Hui83, Hui76 and GuangdongA8-2, were strongly resistant to *L. xylina*. The relative frequency feeding number of larvae on these 4 clones was less than 0.03, pupation rate of larva lower than 15-55 %, and mean weight of pupae less than 0.6 g per pupa. Frass weight of larva fed on the twig of these 4 clones was less than those of other clones. The 2 clones, Dongshan2 and Kangfeng, were susceptible to *L. xylina*. Relative feeding frequency number was over 0.08, pupation rate of the larvae 70%, mean pupa weight more than 0.66 g per pupa, and frass weight more than others.

Occurrence and Management of the Needle Feeder, *Lymantira ampla* Walker (Lepidoptera: Lymantridae) in Casuarina Plantations of Coastal Tamil Nadu, India

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Casuarina cultivation is an important source of livelihood in coastal areas of Tamil Nadu and Puducherry States of India. Though *Casuarina equisetifolia* is a hardy species generally free from major insect pest problems in India, infestation by the bark feeder, *Indarbela quadrinotata* and the needle feeder, *Lymantria ampla* is causing economic loss in the recent years. While the bark feeder is a polyphagous insect commonly found on *Casuarina* species both in inland and coastal areas, infestation by the needle feeder is restricted to the coastal districts, Villupuram and Cuddalore of Tamil Nadu and Puducherry. The attack occurs annually beginning normally in June /July and lasts up to March next year. Hairy caterpillars of the insect feed on young needles at the lower branches and as they grow move to the upper branches of the trees. Voracious feeding of needles puts the trees under stress and affects their growth. The incidence of pest attack was found severe during the low rainfall years and more prevalent in younger plantations aged between 6 months and 3 years than old plantations. Studies on the ecology of the pest conducted for a period of two years revealed that the pest remained in the plantations throughout the year except during April and May with a peak period of attack between September and November. The caterpillars complete the larval period and descend to the ground and pupate underneath the dry needles spread on the plantation floor. Gravid wingless female emerges out of the pupa, ascends the stem and lays the eggs. Male is winged, dark brown and less active during the day time and normally seen resting on the needles and branches. A species of pentatomid predator and three species of parasites (species of braconid, apanteles and dipteran) were found to be the natural enemies of this pest. Occurrence of a species of entomopathogenic fungus (Beauveria) and 12 isolates of entomopathogenic bacteria (*Bacillus thuringiensis*) were also recorded. Integrated methods of management of the pest involving cultural, mechanical, botanical and chemical measures were standardized and the effect of naturally occurring microbial pathogens like *B. thuringiensis* and entomopathogenic fungus against the pest was evaluated.

Agrobacterium as a Vector for the Genetic Transformation of Trees in the Family Casuarinaceae

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The combination of the proper tissue culture protocol with a suitable genetic transformation method is essential for tree species and recovery of transgenic trees and introduction of genes involved in important silvicultural traits. Organogenesis is the method of choice in species such as *Casuarina glauca* and *Allocasuarina verticillata*. It consists of plant regeneration through bud formation on excised epicotyl fragments grown on the proper tissue culture medium. Whereas both microprojectile bombardment and *Agrobacterium* were first tested in Casuarinaceae, *Agrobacterium tumefaciens*-vector system is currently employed for genetic transformation of epicotyl fragments excised from 45-day-old plantlets, and regeneration of stable transformed plants. The best agrobacterial strain is C58C1(pGV2260) carrying derivatives of the binary vector BIN19. Selection of transformed cells is achieved on kanamycin (50 to 100 mg L^{-1} depending upon the tree species). Large scale propagation of the transgenic plants can then be obtained by using cuttings of transformed shoots. Response of *A. rhizogenes* was also exploited to develop a rapid method to generate so called composite plants in *C. glauca*, in which transformed root system is grown on a non-transgenic aerial shoot. Generally nine months are necessary to obtain a rooted transformed *C. glauca* plant with *A. tumefaciens*, whereas the expression of chimeric genes in composite plants can be studied in less than four months . To ensure a commercial use of transgenic Casuarinaceae trees, the evaluation of potential risks resulting from their release into the environment need to be studied.

Incidence of Blister Bark Disease of Casuarina across Species and Locations in South India

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Blister bark or wilt disease caused by the fungus *Trichosporium vesiculosum* Butler is a threat to plantations and breeding populations of *Casuarina equisetifolia*. So far there has been no effective control measure reported for this disease and the infected tree invariably dies. The disease has not been reported in other species of *Casuairna* and a large variation in disease incidence exists within *C. equisetifolia*. In the present study, a species-provenance trial involving *C. equisetifolia, C. junghuhniana, C. Cunninghamiana* and *C. cristata*, two progeny trials each of *C. equisetifolia* and *C. junghuhniana* were surveyed for disease incidence. The extent of disease occurrence varied among species, provenances and families. Only *C. equisetifolia* was found to be affected by the disease and the other three species remained disease-free. Within *C. equisetifolia*, trees of Kenyan landrace remained unaffected while the Malaysian provenance showed 59% of disease incidence. Provenances from Papua New Guinea and Australia provenance were also found be least affected by the disease. Among the families of *C. equisetifolia*, disease incidence ranged from 0-80% in Karur, Tamil Nadu while the disease was not observed in the trial at Tirupati, Andhra Pradesh involving the same set of families. In the two progeny trials of *C. junghuhniana*, only the *C. equisetifolia* families included for comparison purpose were affected by the disease. These observations indicate that the within- and between-species variation for disease susceptibility can be utilized in selection and breeding programmes to develop seedlots and clones with disease resistance combined with other economically important traits for enhancing plantation productivity.

Semi-mechanized Felling and Debarking Operations in Farm Forestry Plantations of Casuarina

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The Tamil Nadu Newsprint and Papers Limited (TNPL) requires about 1800 MT of pulp wood per day. Traditionally, felling and debarking operations in plantations are carried out by using manual labour by the farmers and paper mills in India. But recent factors like increased demand for pulpwood and shortage of labour force has made manual harvesting of Casuarina plantations difficult. Currently TNPL requires a minimum of 2000 workers per day for felling and debarking operations. Using Casuarina wood with bark for papermaking results in higher chemical consumption for cooking and bleaching of pulp leading to increased pollution load in effluents discharge apart from increasing the cost of paper production. If debarked wood is used these problems can be managed and in addition the entry of the Non Process Elements (NPE) into the paper making process can be reduced particularly in the recovery cycle. To improve the quality, productivity and uniformity of output, removal of bark from the wood is necessary. To minimize the labour requirement for felling and debarking and to use more of debarked Casuarina pulpwood, TNPL carried out semi-mechanized felling operations in farm forestry plantation sites by using petrol power chainsaws and debarking at mill site with the help of rotor type debarker machines imported from China. The results indicated that the manpower requirement is reduced by about 52 per cent in mechanical harvesting and debarking compared to felling and debarking operations by manual labour and the cost of felling and debarking is less by about 20 per cent in semi mechanized harvesting. Based on the evaluation studies, TNPL is implementing semi mechanized felling at plantations and mechanized debarking at mill site.

IGBAAS- a Web Resource for Bioinformatic Analysis of Genes Conferring Abiotic Stress Tolerance

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Casuarinas are the preferred trees for planting along tropical coastlines due to their ability to function as a protective shield against wind and waves. They are also planted for reclamation of waste lands. Abiotic stresses like salt stress limit their growth. Several studies have reported existence of considerable variation in the ability of Casuarina to tolerate salt stress. Researchers have therefore, focused on understanding the physiological and molecular basis of salt tolerance in Casuarina, so that the trait could be bred into highly productive genotypes. Recent sequencing efforts by the Institut de Recherche pour le Developpement (IRD), France, have led to unraveling of the salt induced transcriptome in *Casuarina glauca.* With drastic drop in sequencing costs, similar efforts are proposed to be undertaken in salt tolerant clones of *C. equisetifolia*. Voluminous gene sequence data in Casuarina are thus being generated. To harness these sequence resources for development of molecular markers for salt tolerance, they need to be analyzed and classified according to gene function. The Institute of Forest Genetics and Tree Breeding, Coimbatore, has recently hosted the beta version of the database "In silico Gene Bank for Adaptation to Abiotic Stresses - IGBAAS" at http://igbaasifgtb. icfre.gov.in . IGBAAS presently comprises around 3000 gene sequences involved in abiotic stresses (salt, drought, freezing and heat) from different species. To facilitate researchers in the development of molecular markers for salt tolerance in Casuarina, we intend to incorporate selected transcriptome sequence data pertaining to salt tolerance in Casuarina, based on the user friendly functional classification available at IGBAAS. IGBAAS is searchable for nucleotide and protein sequences of genes, their degenerate primers, and related information from Wikigenes, and Pubmed. The users can also edit and update sequence information available in the database, and also upload new gene sequence information. The database has direct connectivity to bioinformatic tools that enable users to select sequences from the IGBAAS database, convert them to FASTA format, and perform bioinformatic analysis like getting multiple sequence alignment (ClustalW), BLAST, Primer-BLAST, siRNA design and reverse complement. The site also provides links to research groups working in abiotic stress tolerance. Advanced versions of IGBAAS that facilitate query for species cum biochemical-pathway specific gene information, in addition to updated gene sequence information from NGS data are envisaged.

Latest Technological Advancement in Casuarina Plantation Including Clonal Technology

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Seshasayee Papers and Boards Limited (SPB) is a large integrated pulp and paper mill in Tamil Nadu, India. The mill is consuming about 400,000 tonnes of wood (Casuarina and Eucalyptus) as raw material for its manufacturing process. The above quantity is being procured mainly from the Tamil Nadu Forest Plantation Corporation and from the farmers. The Company produces about 350 tonnes of quality papers per day, using wood and sugarcane fibre known as bagasse. In order to have a sustainable supply of wood-based raw material for the industry, the Company has started Contract Farming during 2005 to plant Eucalyptus and Casuarina and has covered about 21,000 ha so far. With unique scientific management and advanced technical know-how, great emphasis is being given for genetically superior planting material combined with improved silvicultural practices. Significant gain in the productivity of *Casuarina junghuhniana* has been achieved through clonal planting material collected from clonal hedge garden, adopting optimum space for planting, application of organic and inorganic fertilizers. Nearly two million clonal plants have been produced and supplied to farmers every year at subsidized cost by using low cost clonal propagation technology. More than 50 million Casuarina seedlings are also raised from seeds of Seedling Seed Orchards and supplied to the farming community ever year. The productivity of farm forestry clonal plantation of Casuarina has been enhanced to 125 to 150 metric tonnes from 60 to 75 metric tonnes per hectare through seed-raised plantation in 3-4 years rotation.

A Value Chain Approach for Improvement and Utilization of Casuarina in Tamil Nadu

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Casuarina is a preferred tree crop by farmers and wood based industries due to its fast growth, multiple utility, nitrogen fixing ability and amenability towards all types of agro and farm forestry systems. The species though an exotic has been improved both genetically and silviculturally but the availability of quality seeds and seedlings till the recent past was dismally modest. This coupled with unorganized supply chain with uncertain price fluctuation have created widening of gap between the producer and consumer. Against this backdrop, the Tamil Nadu Agricultural University (TNAU) has conceived and designed a value chain approach for the improvement and utilization of Casuarina in association with various stake holders. The value chain approach was attempted to auger the productivity through technological intervention by developing high yielding and short rotation clones like TNAU Casuarina MTP 1 and TNAU Casuarina MTP 2 which have the potential of yielding over 150 tonnes per ha in 36 months which benefitted both farmers and industries. Development of decentralized clonal production center has ensured availability of quality planting material of high yielding varieties at subsidized price. The research team also intervened through organizational value chain model by conceiving a Quad Partite Value Chain Model incorporating research institutes, farmers, wood based industries and financial institutions as stake holders. The intervention through price fixation and forecasting have ensured the periodic market prices to the farmers and helped them early and high returns. The high yielding clones have yielded an income of over Rs.700,000 (USD 10,770) per hectare in three years. The incorporation of agricultural crops like pulses, vegetables and hybrid tree model with other fast growing tree species have also yielded early returns which attracted over 25000 farmers practising Casuarina based industrial wood farming in about 36000 ha distributed in 29 districts of Tamil Nadu. The value addition of Casuarina needle into briquetting technology have offered additional revenue to the growers. The interventions made through technology, organizational and marketing and thereby assures a complete value chain model for further promotion of Casuarina based industrial wood farming.

Economic Analysis of Different Silvicultural Practices in *Casuarina* Species

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The potential of multipurpose tree species in enhancing the diversity, sustainability and productivity of marginal ecosystem has received increased attention in recent years. Besides this, choice of alternative tree species to meet raw material requirement of industries is the need of hour. Casuarina has been the farmers favorite as it fits well in agrarian ecosystem. Short gestation period, periodic returns in the form of pruned branches right from year two, ability to improve the soil fertility and ready marketability are the major attractions for this species. In addition cultivation has been steadily increased in private lands due its multiple utilities such as fuel wood, scaffolds, rafts for barricades and sheds and pulp wood. The energy content of the wood is high (4127 kcal), and even the woody cones are used for firing clay wares in kilns. Casuarina cultivation is encouraged by the Tamil Nadu Forest Department through schemes like Tree Cultivation in Private Lands and Emergency Tsunami Reconstruction Project. Casuarina wood is purchased from the farmers by the paper industries in Tamil Nadu, Tamil Nadu Newsprint and Papers Limited and Seshasayee Paper and Boards Limited. To elucidate the economic potential of Casuarina cultivation, a field experiment was conducted with two *Casuarina* species namely, *C. equisetifolia* and *C. junghuhniana* Miq from seed and clonal sources raised under five nutrient levels with a common irrigation schedule. Two weedings and one pruning were given up to 18 months after planting (MAP). The study was taken up at Agricultural Research Station, Bhavanisagar during 2010-2012. The economic analysis of the experiment on *Casuarina* species with different spacing, planting materials and different nutrient levels, revealed that wider spacing at 1.5 m x1.5 m with seed as the planting source in *C.equisetifolia* with 25% more fertilizer applications over the soil test recommendation value recorded the highest BCR value of 2.5 and 2.01 at 15 per cent and 30 per cent respectively and the IRR was 50.32.

Insurance for Casuarina Plantations – An Innovative Intervention in Industrial Agroforestry

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The State of Tamil Nadu in India is known for its long coastal zone wherein the three districts viz., Cuddalore, Chennai and Villupuram located along the coastal zone are of potential significance for development of Casuarina based pulpwood plantations. These three districts alone contribute over 60% of pulpwood supply in Tamil Nadu. These districts are frequently hit by natural calamities including heavy wind, Cyclone, Hurricane and Tsumani which resulted in large scale uprooting of many tree species causing huge economic loss to the farmers besides creating uncertainty in Casuarina based pulpwood supply chain. Occurrence of pest and diseases abd fire also cause serious damage to Casuarina plantations. All these biotic and abiotic damages cause huge economic loss to the tree growers. To protect the Casuarina plantations against natural calamities, Forest College has roped in United India Insurance, Chennai and developed a comprehensive "Tree Insurance Scheme" for Casuarina plantations. The premium rate for basic plan has been fixed at 1.25% of the input cost. The premium to be paid for one acre (about 0.4 ha) plantation would be Rs. 337 (first year) and Rs. 758 (third year) depending on the input cost of the plantations. This insurance protection would cover the perils like forest or bush fire, lightening, riot and strike, storm and cyclone, flood and inundation and also the damages due to wild animal attack. In the event of damage due to any of the above mentioned perils, the farmer would be settled with the input cost of respective trees. This comprehensive insurance plan for tree crops is a pioneering and timely intervention which will help to expand the Casuarina industrial wood plantations and will create self reliance in raw material security besides protecting the farmers' interest.

1 USD = Rs.65 (approx.)

Need for Development of Casuarina Based Marketing Information System in Tamil Nadu

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The practice of growing Casuarina based agroforestry systems in farm lands of Tamil Nadu is followed for more than three decades. But during last decade, the area under Casuarina has shown increasing and positive trend in Tamil Nadu. Nearly 64,000 ha of area is under Casuarina cultivation during 2011-2012. In the early years, farmers started growing Casuarina mainly for firewood purpose but now have multiple choices for selling their produce. In Tamil Nadu, most of the Casuarina wood is transacted through four important marketing channels. The length of the channels differs with the different purposes / uses of the final produce. Various uses of Casuarina namely firewood, pulpwood and poles and banana staking follow different channels. The intermediaries involved in each marketing channel also differ. Each purpose requires the role of different intermediaries, transport and grading facilities. The marketing efficiency of Casuarina also differs according to the purpose of sale. On the other side, there is no established market for this produce and there is no dissemination of market information to the Casuarina farmers and farmers depend on industry for marketing of their products and also for price information. They have to rely on intermediaries for getting information on market arrivals and price. There is no free flow of market information between buyers and sellers. Due to this asymmetric information farmers were not able to sell their produce at the highest price possible. The present study showed that the market for Casuarina wood is found to be imperfect. In this context, identification of major production and market centres of Casuarina and dissemination of market information such as quantity arrivals, requirement, price through various Information and Communication Tools and Techniques will help the farmers to get more remunerative prices for Casuarina.

Mapping of Casuarina Plantation in Ariyalur District, Tamil Nadu, India using Remote Sensing and Geographical Information System

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The wood-based paper industries in Tamil Nadu, India require nearly a million tonnes of pulpwood but the current production is far below the demand. To meet the demand for pulpwood, the wood-based paper industries encourage the farmers to raise Casuarina plantations. Since the economic returns from Casuarina plantations is high compared to the traditional agricultural crops, the extent of plantations in farmlands has been expanding rapidly in different districts of Tamil Nadu. In this context, spatially accurate and reliable information on the Casuarina plantations is needed for formulating strategic land use planning and understanding its consequences on ecosystem. Hence a study was initiated to develop a comprehensive methodology for mapping different Casuarina plantations in Ariyalur District of Tamil Nadu. For mapping purposes, we used the Resourcesat-2/ L-4FMX (5m resolution) procured from National Remote Sensing Centre, Hyderabad. The procured satellite image was geocorrected using Survey of India Topo Sheets. The identification of Training pixels and plots were done by using the plantation data collected with the help of GPS. Data on other crops and plantations were also collected during the field visit to increase the classification accuracy. Supervised classification was used to classify the image supplemented with visual analysis of the image and recode technique was used to reclassify the misclassified pixel with the help of Google Earth and field check. The results indicate that the data and processing techniques used offer a reliable approach for mapping Casuarina and other plantation crops.

An Approach for Identification of Casuarinas by Using Markov Random Field-based Method for Super-Resolution Mapping from Satellite Images

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Casuarinas help to maintain ecological and environmental stability and play a key role in subsistence economy. However, more information about Casuarinas is needed for their proper management and usage. In this study, we aimed to investigate Casuarinas at three locations i.e., Butler Bay, Jackson Creek and Ekiti Bay at Little Andaman Island; South Andaman in India by using Markov Random Field (MRF) based Super Resolution Mapping (SRM). In this study ASTER image with spatial resolution of 15 m is used for all experimental tests. Quality of SRM is compared with Maximum Likelihood Classification (MLC) classified map. The results of this study were validated using Google Earth data. Simulated Annealing (SA) parameters were tuned on real data and result is compared with study done on simulated data before. Method parameter of MRF based SRM was evaluated on ASTER data. Accuracy was assessed at fine and coarse resolution using kappa statistics and error measures. It is observed that SRM outperformed MLC in case of fine and coarse resolution. Experimental test was done on the ASTER data to study the optimal neighbourhood system size with respect to various Scale factor (S). It was found that resultant map of SRM was smoother than MLC. This study dealt with high resolution case. Moreover, MRF based SRM has successfully identified Casuarinas at S = 2 by using ASTER image with spatial resolution of 15 m. SRM result was successfully validated using Google Earth data.

A Study on the Gasification Performance of *Casuarina equisetifolia* Wood

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Demand for energy from all possible sources is growing manifold day by day. One of the chief sources of energy viz. harnessing fossil fuel is fraught with heavy load of pollutants to the ambient environment. Biomass is widely considered as a potential fuel and it is a renewable energy resource for the present and future. The wood of *Casuarina equisetifolia* possesses one of the highest calorie values yielding high quality fuel wood and is also an important livelihood source for rural people. A study was conducted at the Institute of Wood Science and Technology to analyze the gasification properties of Casuarina wood by using 1 Kw downdraft gasifier. The reactor temperature varied from 600-1143 °C. The quality of gas samples were analyzed through Gas Chromatography and heat value of the producer gas. Result showed that compared to wood wastes, Casuarina wood producing high amount of carbon monoxide and hydrogen gases.

Sociological Perspectives of Casuarina Cultivation in Livelihood Security of Marginal Farmers in Tamil Nadu, India

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Casuarina was introduced into India to meet the demand of firewood for steam locomotives during the late nineteenth century. It has been socially accepted as an important fuel wood species in the peninsular India. In Tamil Nadu State, it is regarded as a cash crop by farmers particularly in the coastal areas. It has high marketability as fuel wood, scaffolding in the construction industry and pulpwood for paper industry. Being a multipurpose species its services also include salt tolerance, drought resistance, ability to fix atmospheric nitrogen in symbiotic association with *Frankia* and sand dune stabilization. The stem form, crown and branch characteristics are highly suitable for shelterbelts and windbreaks in agroforestry systems. In the State of Tamil Nadu (Latitude: 11° 00' N, Longitude: 78° 00' E) the land holding is skewed towards small and marginal farmers. Even though the small and marginal farmers occupied a major share of the total land holdings, they were operating only about 55 per cent of the total area and the average size of holding is very meager at 0.37 hectare. However, Tamil Nadu is the one of the pioneering States in adopting Casuarina in the agroforestry systems, primarily to maximize the profits and to reduce the labour intensive agriculture crop. Rapid adoption of tree cultivation in the farm lands drastically restricted the farm employment opportunities of the rural poor and disadvantaged. The technically and economically viable tree growing innovations are remotely benefitted marginal farmers over the years. On the other hand the landless and "socially disadvantaged" marginal farmers as traditional farm laborers forced to leave the native villages in search of wage employment and to unfamiliar sub urban and urban areas, which involve many costs and risks. The subsistence oriented innovative technologies are required to be incorporated in the farm forestry programmes with special focus on household forestry schemes and also in common property management for the better social progress.

The Extent of Infestation by the seed pest, Bootanelleus orientalis (Mathur & Hussey) among family seedlots from breeding orchards of Casuarina equisetifolia and *C. junghuhniana*

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In India Casuarina is cultivated extensively in the coastal areas of the States of Tamil Nadu, Andhra Pradesh, Odisha, Maharashtra and Gujarat. Such extensive planting programmes require continuous supply of good quality seeds. While this demand is met through the seeds collected from the existing seed orchards and plantations, the need for improved seeds with higher productivity gaining importance among the Casuarina growers including the farmers. The quantity of improved seeds currently available from the limited area of seed orchards is inadequate to meet the demand. To bridge the gap between the demand and supply of superior seeds more seed orchards with improved germplasm are being established by the Institute of Forest Genetics and Tree Breeding and the State Forest departments. Community seed orchards with the improved seed sources are also established in public land to cater the need of local people. As the germination percentage of Casuarina seed is low, the end-users need to have seeds nearly 40% more than the actual requirement. Insect damage is cited one of the reasons for poor germination although the per cent damage caused by the insect is reported only 1-2% by the previous researchers. In the present study an attempt was made to identify and assess the extent of damage caused by the pest on 120 seed lots of *C. equisetifolia* and *C. junghuhniana* collected from seed orchards located in Tamil Nadu and Puducherry. The insect was identified as *Bootanelleus orientalis* (Mathur & Hussey) belonging to the family Torimidae under the order Hymenoptera. The per cent of damage caused by the pest was observed to be varying among the seedlots and the locations. While the damage level ranged from 1 to 86% the highest per cent 85.3 was recorded with the seedlot *C. equisetifolia* of Karunya 139 (1) . In the case of *C. junghuhniana* the highest per cent was observed to be 1 with the seed lot CJ 3. This variation has implications for the seed orchard management, seed collection and processing to improve the seed quality of Casuarina.

List of Participants

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