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## Message from the Coordinator WP 2.08.02

I am glad to meet you through this issue of NFT News with the wish that it will continue to be the main channel of communication among the members of our Working Party. I have taken over from Dr Antoine Kalinganire as the Coordinator of the Working Party along with Dr Zhong Chonglu and Dr Claudine Franche as Deputy Coordinators. Antoine stepped down at the Fifth International Casuarina Workshop held at Chennai, India in February 2014 after serving the Working Party for two terms since 2004. He took over from Mr Khongsak Pinyopusarerk, the first Coordinator and founding Editor, and sustained this Newsletter under challenging circumstances till recently. On behalf of all the members I thank Antoine and his Deputies, Mr Viton Luangviriyasaeng and Dr Ousman Diagne for taking care of the Working Party and the Newsletter.

Mr Khongsak Pinyopusarerk was instrumental in forming the Working Party during the 1995 IUFRO Congress held at Tampere, Finland by merging the former Casuarina and Nitrogen Fixing Trees Working Parties. He served as the first Coordinator of the Working Party and founded the NFT News in 1998. His continuous guidance and working partnership with members in many countries during the past two decades has had a significant impact on the improvement and cultivation of nitrogen fixing trees particularly in India and China, the largest Casuarina-growing countries and in Vietnam with the largest Acacia plantations. Khongsak continues to serve on the Editorial Team of the NFT News and work with several members of the Working Party towards a better utilization of genetic resources of NFT species in many countries.

NFTs have become more important than ever to secure livelihood peoples' and to preserve soil and environmental health in the era of climate change, unsustainable agricultural production and growing needs for wood and wood products worldwide. There is an increased interest to integrate NFTs in plantations of other trees and in agricultural systems to increase productivity of the land and to prevent over usage of chemical fertilizer. Apart from the traditional uses like wood and fodder, some of the NFTs are also regarded as potential source of biofuels. This issue of the Newsletter carries articles

on these areas and also on advanced molecular biological research to understand the nitrogen-fixing mechanism in actinorhizal plants.

There are unlimited opportunities to advance research in NFTs for enhancing the quality and extent of services and products obtained from them. Two decades of systematic genetic improvement programmes have increased the productivity of Acacia, Casuarina and Leucaena species, the most extensively planted NFTs in the tropics. We need to turn our research focus towards the understanding of effects of these high-input and short-rotation crops on the overall soil fertility, thus allowing sustainability of the current productivity levels. With site-appropriate planting material and silvicultural techniques both productivity and nutrient balance can be optimized to get the twin benefits of carbon sequestration and biological nitrogen fixation. The combined strength of the Working Party will be capable of addressing these challenges in the near future.

I seek your support for the timely publication of future issues NFT News. Please stay connected with the network by sending us your feedback on the current issue and contributions for the future issues. I extend a warm welcome to the new members of the Working Party and look forward to their experience - sharing on NFTs with the other members of the group.

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## Host-species dependent physiological characteristics and two-way transfer of nitrogen between rosewood *Dalbergia odorifera* and its hemiparasite *Santalum album*

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### Introduction

*Santalum album* L. (Indian sandalwood) has been over-exploited for its aromatic heartwood and root, which have cosmetic, religious and medicinal significance. Over the last two decades, several large-scale plantations of *S. album* have been established to meet future market requirements in Australia and China. Experiments on both pot and field plantations have shown that the growth performance of *S. album* is greatly enhanced by its successful attachment to suitable hosts, particularly to N<sub>2</sub>-fixing species (e.g., *Acacia*, *Casuarina* and *Sesbania*). Nevertheless, these hosts have a relatively low market value and the timely screening of suitable high-value host trees for *S. album* plantations is needed.

*Dalbergia odorifera* T. Chen, one of the most precious rosewoods in the world with diverse medicinal and commercial values, has been successfully planted with *S. album* in the Jianfengling Arboretum of Hainan Island, China (18° 42' N, 108° 49' E), since 1989. Nevertheless, a further understanding of the physiological interactions between *S. album* and *D. odorifera* is needed.

### Materials and methods

In a pot study we first tested effects of 1-year-old N<sub>2</sub>-fixing (*Acacia confusa*, *Dalbergia odorifera*) and non-N<sub>2</sub>-fixing (*Bischofia polycarpa*, *Dracontomelon duperreranum*) on ecophysiological characteristics and nitrogen (N) nutrition of *S. album*. We then examined the role of N<sub>2</sub>-fixation in two-way N-transfers between 7-month-old *Bradyrhizobium elkanii* nodulated *D. odorifera* and its hemiparasitic *S. album*. With four potted-pairings, <sup>15</sup>N were externally labelled to host or hemiparasite and the host either nodulated or grown on combined inorganic-N.

### Results

Photosynthetic rates, shoot, root and haustoria biomass, N and total amino acid were significantly greater in paired *S. album* grown with N<sub>2</sub>-fixing hosts (*D. odorifera* the best). Foliage and root <sup>15</sup>N of *S. album* were significantly lower when grown with N<sub>2</sub>-fixing than with non-N<sub>2</sub>-fixing hosts. Haustoria of *S. album* attached on *D. odorifera* roots and N<sub>2</sub>-fixation supplied 41- 44% of total N in *D. odorifera*. Biomass, N and <sup>15</sup>N

were significantly greater in both nodulated *D. odorifera* and *S. album* grown with paired nodulated *D. odorifera*. Significantly higher plant <sup>15</sup>N-recovery was in N-donor *D. odorifera* (68-72%) than in N-donor *S. album* (42-44%) irrespective of nodulation. N-transfer to *S. album* was significantly greater (27.8-67.8 mg/plant) than to *D. odorifera* (2.0-8.9 mg/plant) and 2.4-4.5 times greater in nodulated than in non-nodulated pairs. Irrespective of nodulation, *S. album* was the N sink plant. Amounts of two-way N-transfer were increased by the presence of effective nodules, resulting in greater net N-transfers (22.6 mg/plant) from host *D. odorifera* to hemiparasite *S. album*.



Figure 1. A mixed plantation of *Dalbergia odorifera* (dark green foliage) and *Santalum album* (light green foliage) in Southern China

## Conclusions

The results on the physiology of the root hemiparasite *S. album* presented in this study collectively provide an insight into the complex interactions between the parasite and the host during the early growth stages of *S. album*. A range of questions remain concerning the heartwood development of both *S. album* and its suitable host *D. odorifera*. Further research in this area is needed to allow the development of a superior methodology for the concurrent plantation of these two valuable timber species.

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## *Casuarina equisetifolia* : A multipurpose nitrogen fixing tree in Senegal

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## Introduction

Land degradation has become a major concern in Senegal with serious environmental and economic consequences. Coupled with climate change events it adversely affects agricultural yield. The main causes of land degradation in Senegal are salinity, drought and soil erosion. Nearly half of the 3.8 million ha of arable land is affected by salinity. Drought due to deficit rainfall increases salinization and together they adversely affect soil fertility and productivity. Soil erosion also causes loss of soil nutrients through land waves in arid and semi-arid areas.

In Senegal, reversing causes of land degradation is a national priority and different strategies are being promoted including the use of nitrogen-fixing trees to improve soil fertility. Generally these trees are pioneer species, highly adapted to harsh environments such as drought and salinity (Sayed, 2011). Members of the Casuarinaceae family are widely used in land rehabilitation for their ability to improve soil fertility through the symbiotic relationship with nitrogen-fixing bacteria, *Frankia* and /or mycorrhizal fungi (Diagne *et al.*, 2013). These microorganisms improve nutrient uptake and increase plant performance in stressed environments (Smith and Read, 2008).

In Senegal, *Casuarina equisetifolia* has been established in Niayes region along the western coast between Dakar and St Louis. This fast-growing tree plays a major role in the rehabilitation of salinity-affected lands in Senegal. Particularly in Niayes region, *C. equisetifolia* plantation plays an important socio-economic role and this review discusses

the many uses of *C. equisetifolia* and its importance in improving soil fertility in Senegal.

## *Casuarina equisetifolia* - a multipurpose tree in Niayes region

*Casuarina equisetifolia* plantations in Niayes region act as windbreaks, stabilize sand dunes and improve soil fertility. Niayes is the largest vegetable-producing region in Senegal fulfilling needs of Dakar City. In this region, farmers are able to grow vegetable crops only with the protection provided by *Casuarina* plantations (Fig. 1). Litter fall and biological nitrogen fixation from *Casuarina* trees improve soil fertility and thereby increasing vegetable production. Sustaining and increasing vegetable production in this region depends on the services to be provided by *Casuarina* trees. New plantation techniques need to be developed to replace the existing *Casuarina* plantations that are more than 60 years old to



Figure 1. *Casuarina equisetifolia* plantation with onion crop in Niayes region, Senegal (Photo: N. Diagne)

derive better environmental services from them for enhancing vegetable production.

*Casuarina equisetifolia* produces a large quantity of leaf litter. Plantations between 6 and 34 years old produce a leaf litter of about 3.3 tonnes ha<sup>-1</sup> year<sup>-1</sup> (Mailly and Margolis, 1992). Farmers compost *Casuarina* leaf litter into organic fertilizer and apply to their crops for improving vegetable production (Fig. 2). The composted litter and ramial-wood chips increase



Figure 2. Production of *C. equisetifolia* compost with leaf litter to improve production of flowers and vegetables (Photo: M. Ngom)

organic matter content and water holding capacity of the soil and improve vegetable growth and yield (Soumaré *et al.*, 2002). Leaf litter is also used in Dakar region for cultivating flower crops. Soils amended by *C. equisetifolia* leaf litter have a high mineral N content (Diallo *et al.*, 2005). *C. equisetifolia* plantation increased the accumulation of N up to 1567 kg ha<sup>-1</sup>;

an average increment of 45 kg ha<sup>-1</sup> year<sup>-1</sup> in forest floor (Mailly and Morgolis, 1992). In addition to these services, *C. equisetifolia* produces high quality firewood and minor timber (Cisse and Gourbiere, 1993).

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## Transgenic approaches to study valuable genes in Casuarinaceae trees: A review

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### Introduction

Species from the Casuarinaceae family are fast-growing with a number of remarkable characteristics including outstanding ability to grow on harsh and poor soils, and to provide wood of high calorific value and suitability for pulp extraction. In addition, some species thrive in areas exposed to salt spray or polluted by heavy metals (Diagne *et al.*, 2013). To understand the molecular basis of some of these important properties, biotechnological and genetic engineering tools were developed using *C. glauca* as a major actinorhizal model species (Zhong *et al.*, 2013). This review will highlight the major achievements resulting from the use of the biological vector *Agrobacterium* to transfer gene constructs into *Casuarinaceae* trees and the contribution of transgenic plants to improved knowledge in these perennial species.

### Transgenic Casuarinaceae trees: state of the art

In the last decades, *Agrobacterium tumefaciens* has been used as an essential tool for research in plant biology and biotechnology. This alpha-proteobacterium has the ability to transfer and integrate genes into the genome of a broad range of plants, including some forest trees. The genetic transformation process requires the activation of a virulence (*vir*) region located on a large Ti plasmid of the agrobacteria (Lacroix and Citovsky, 2013). The *vir* genes encode proteins that will contribute to the production of a transferable DNA molecule (T-DNA) that, together with bacterial protein effectors, will be transported from the bacteria to the plant nucleus and targeted into the host chromatin. Valuable foreign genes of interest are located on the T-DNA that is flanked by conserved right and left border sequences of 24-25 base pairs each.

The first attempts of genetic transformation in the Casuarinaceae family were reported in 1991 with the biological vector *Agrobacterium rhizogenes* (Table 1) (Phelep *et al.*, 1991). Transgenic plants exhibiting a modified phenotype were obtained from hairy roots induced by *A. rhizogenes* A4 on hypocotyls of *Allocasuarina verticillata*.

However, due to the expression of the oncogenes from the T-DNA, transformed plants were characterized by a root-system that was more developed, plagiotropic and branched than the non-transformed control plants. In addition, the aerial system showed reduced apical dominance and highly branched shoots. Six years later, the  $\beta$ -glucuronidase (GUS) gene under

**Table 1. Genetic transformation of Casuarinaceae species by the biological vector *Agrobacterium***

Plant species	Bacterial strain	Data
<i>Allocasuarina verticillata</i>	Disarmed <i>Agrobacterium tumefaciens</i> C58C1 <i>A. rhizogenes</i> A4RS	Fully transformed plant Fully transformed plant
<i>Casuarina glauca</i>	Disarmed <i>A. tumefaciens</i> C58C1 <i>A. rhizogenes</i> A4RS <i>A. rhizogenes</i> Arqua1	Fully transformed plant Composite plant with transformed roots Composite plant with transformed roots
<i>Casuarina cunninghamiana</i>	Disarmed <i>A. tumefaciens</i> C58C1	Fully transformed plant
<i>Casuarina equisetifolia</i>	Disarmed <i>A. tumefaciens</i> C58C1	Transformed calli

the control of the constitutive 35S promoter was transferred into *A. verticillata* by means of the disarmed *A. tumefaciens* strain C58C1 (Franche *et al.*, 1997). This success was the result of the optimization of numerous factors including tissue culture aspects, appropriate induction of the *vir* genes and the selection of the transformed cells. *A. verticillata* is now routinely transformed and transgenic plants can be selected on nutrient medium with kanamycin following the expression of the neomycin transferase gene (*nptII*). The regeneration procedure for rooted plants takes about four to six months. They exhibit a phenotype and nodulation efficiency by *Frankia* that are similar to that observed in the non-transformed control trees. The transformation was confirmed by PCR analysis and Southern blot. Stable expression of the *GUS* gene was recorded for at least one year of growth in a greenhouse.

Since molecular studies and genomic resources were developed with *C. glauca*, the next efforts were focused on this species. The major limitation was to find the appropriate tissue that could produce transformed calli with the ability to generate buds and shoots. Although transgenic calli were reported in 1996 (Le *et al.*, 1996), the first paper describing the analysis of transgenic nodulated *C. glauca* plants was published in 2002 (Smouni *et al.*). Transformation was achieved following the cocultivation of young epicotyls fragments with the *A. tumefaciens* strain C58C1 containing the binary vector pBIN19 and using the selection marker *nptII*. Regeneration of rooted plants was obtained via adventitious organogenesis on about 25% of the transformed calli within 6 to 9 months. Less than 3% of escapes were noted, providing evidence that kanamycin was an appropriate selective agent.

*Agrobacterium rhizogenes* still proved to be a useful vector for *C. glauca* as an alternative to the labor intensive genetic transformation by *A. tumefaciens* (for review, Benabdoun *et al.*, 2012). This pathogenic bacterium can be used to generate

composite plants consisting of transformed hairy roots induced on a non-transgenic shoot. This approach allows a more rapid analysis of transformed roots and nodules than the methods used to generate plants that are stably transformed by *A. tumefaciens*. Functional studies of symbiotic genes can be achieved within about four to five months in composite *C. glauca* plants.

The genetic transformation procedures in Casuarinaceae make it possible to perform some functional analyses of plant genes involved in key physiological processes such as the symbiosis with *Frankia* and/or mycorrhizal fungi, salt and cold tolerance, lignification and biotic stresses. In this context, numerous promoter studies have already been published

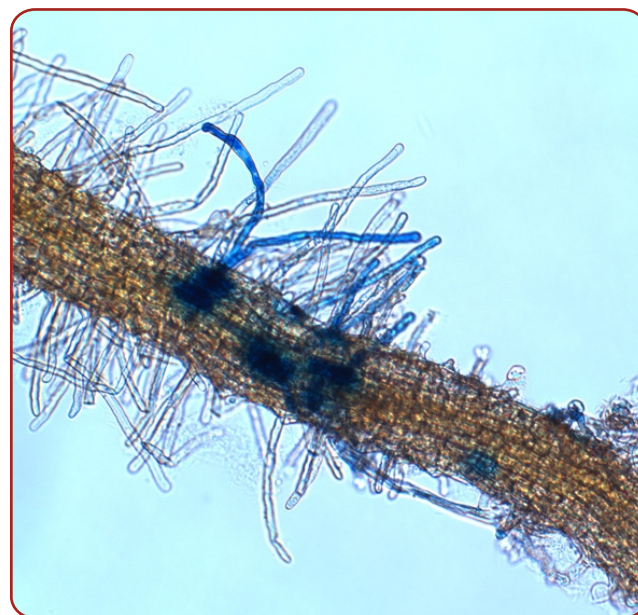


Figure 1.  $\beta$ -glucuronidase (GUS) activity observed in root hairs after contact between transgenic *CCaMK-GUS Casuarina glauca* roots and *Frankia* Cci3.

(Table 2). Promoter-reporter gene fusions have proven to be particularly appropriate when gene expression was limited to discrete subpopulations of cells within a given organ such as a root or a nodule, or temporarily induced by *Frankia* signals

(Figure 1). Whereas down-regulation of candidate genes by antisense gene constructs gave poor results in both *C. glauca* and *A. verticillata* due to the difficulties in identifying appropriate down-regulated transgenic lines.

**Table 2. Promoters studied in transgenic Casuarinaceae trees using *Agrobacterium*-mediated transformation.**

Promoter	Origin
35S	Sequence from the cauliflower mosaic virus
E35S (70S)	Duplicated 35S from the cauliflower mosaic virus
E35S-OCS	Duplicated 35S containing the transcriptional enhancer <i>ocs</i>
AtUBQ1	Ubiquitin gene 1 from <i>Arabidopsis thaliana</i>
Lbc3	Leghemoglobin gene from <i>Glycine max</i>
Pa	Hemoglobin gene from <i>Parasponia andersonii</i>
Tr	Hemoglobin gene from <i>Trema tomentosa</i>
Cdc2At	Cyclin-dependent kinase gene from <i>A. thaliana</i>
DR5	Auxin responsive synthetic promoter
GH3	Auxin-responsive gene from <i>Glycine max</i>
CgAUX1	Auxin influx carrier gene from <i>C. glauca</i>
CgLAX3	Auxin influx carrier gene from <i>C. glauca</i>
CgIAA7	Indol-3-acetic acid protein gene 7 from <i>C. glauca</i>
AtIAA5	Indol-3-acetic acid protein gene 5 from <i>A. thaliana</i>
PsENOD12	Hydroxyproline-rich protein gene from <i>Pisum sativum</i>
MtENOD11	Early nodulin gene from <i>M. truncatula</i>
GmEnod40	Early nodulin gene from <i>G. max</i>
CgEnod40	Putative <i>Enod40</i> homolog sequence from <i>Casuarina glauca</i>
CgMT1	Metallothionein gene from <i>C. glauca</i>
Cg12	Symbiotic subtilase gene from <i>C. glauca</i>
CgSymRK	Leucine-rich-repeat receptor-like kinase gene from <i>C. glauca</i>
MtDMI2	Leucine-rich-repeat receptor-like kinase gene from <i>Medicago truncatula</i>
CgCCaMK	Calcium and calmodulin-dependent kinase gene from <i>C. glauca</i>
CgNIN	Nodule inception gene from <i>C. glauca</i>

RNA interference (RNAi) gave valuable information on two symbiotic genes isolated from *C. glauca*, *SymRK* and *CCaMK*. They encode respectively a leucine-rich-repeat receptor kinase and a calcium and calmodulin-dependent protein that are necessary for rhizobial and endomycorrhizal infection in legumes. The analysis of the transgenic *SymRK-RNAi* and *CCaMK-RNAi* lines of *C. glauca* provided the evidence that these two genes were necessary for both *Frankia* and *Rhizophagus irregularis* symbiotic processes (Gherbi *et al.*, 2008; Svistoonoff *et al.*, 2013). This data was a major breakthrough in the scientific community working on biological nitrogen fixation since the conservation of the

*SymRK* and *CCaMK* functions between legumes and actinorhizal plants could reflect a common genetic program for endosymbiosis development, thus suggesting that during evolution, root nodule endosymbioses recruited some genes necessary for arbuscular mycorrhizal symbiosis.

### Conclusion

Major progress has been achieved in the understanding of the complex interaction between *Agrobacterium* and its host, thus resulting in improved genetic transformation methods and opening this technology to recalcitrant plants such as the forest trees casuarinas. The genetic transformation of several

Casuarinaceae species has resulted in the possibility of studying key actinorhizal plant genes and has already led to important breakthroughs concerning the knowledge of the symbiotic process with *Frankia* (Zhong *et al.*, 2013). With the development of genomic resources in both *C. glauca* and *C. equisetifolia*, it is expected that new insights will be gained in the future concerning the understanding of the lignification process in Casuarinaceae, and the characterization of genes contributing to improved tolerance to biotic and abiotic stresses. The integration of this knowledge into Casuarina breeding programs has the potential to improve in the future tree performance in plantations.

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## Selection, propagation and testing of *Pongamia pinnata* clones for fruit yield and oil content for biodiesel production

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*Pongamia pinnata* Pierre commonly called as Pongam or Karanj is a commercially important multipurpose tree with potential as a bioenergy crop. It is an evergreen tree widely distributed throughout India growing in all types of soils including dry, stony, clayey and saline soils. It is a nitrogen fixing tree and thereby helps in enhancing soil fertility. Pongam is widely integrated in agroforestry systems and also preferred in other plantation programmes for its high carbon sequestration potential. The pongam oil extracted from seeds has excellent biodiesel properties and is commonly used in irrigation pumps, power generators and automobiles. There is a heavy demand for pongam oil for increasing the share of biodiesel for automobiles and railways. Pongam oil is also

used in tanning industries, soap making, lubricant, medicinal purposes and as a fuel for cooking and lamps. The oil-pressed seed kernels are used as organic fertilizer. The root, bark, leaves and flowers of pongam possess medicinal properties and commonly used in traditional healthcare. The wood is used for making cabinets, cart wheels, agricultural implements and tool handles.

Traditionally pongam tree is grown along roadsides, farm boundaries and in homesteads for various purposes. New plantations of pongam are established now mainly to meet the demand for its oil to be used for biodiesel production. In order to select accessions high yielding in terms of seed production and oil content, an extensive survey was

conducted in different agroclimatic zones covering the States of Tamil Nadu, Kerala and Puducherry in Southern India and 91 trees superior for seed production were selected. All these trees were clonally multiplied by rooting stem cuttings and assembled in a Vegetative Multiplication Garden (VMG). These clones were further multiplied from the accessions in VMG for testing in multilocation clonal trials.

Fruits collected from each of the selected trees were studied for variation in fruit and seed weight, size and oil content. The 100-fruit weight of the selected trees ranged from 107 to 402 g, while 100- seed weight ranged from 75 to 192 g and the oil

content varied from 15 to 33%. Generally trees from dry areas in the Dharmapuri, Krishnagiri and Madurai Districts of Tamil Nadu State showed higher fruit yield (103 to 250 kg per tree) than other locations surveyed. The best 19 clones among the total 91 clones possessed a high fruit yield of 103 to 250 kg fruits per tree and high oil content of 27 to 33%. These clones are made available to different tree growers like Forest Departments, farmers and biodiesel industries for developing high-yielding clonal plantations. Significant variation observed for these characters provides scope for selection and systematic genetic improvement.

## Screening of *Leucaena* germplasm for high pulp wood and low seed production in India

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*Leucaenas* are versatile group of nitrogen-fixing trees widely planted in the tropics for food, fodder, fuelwood and reclaiming degraded land area. *L. leucocephala*, the most

commonly used species was introduced in India during 1970s mainly for fodder production. It gained popularity among farmers very quickly especially in coastal areas. The easy

**Table 1. Growth and seed production of *Leucaena* accessions at 1.5 years' age in south India.**

S.No	Variety	Source	Taxon	Height(m)	DBH(cm)	Seeds per tree
1	K8	BAIF, Maharashtra	LG	5.2	3.42	162
2	K28	TNAU, Tamil Nadu	LG	6.3	2.94	539
3	K636	CRIDA, Andhra Pradesh	LG	6.8	3.34	405
4	K636	BAIF, Maharashtra	LG	5.2	3.46	519
5	K636	Koppal, Karnataka	LG	7.8	4.4	926
6	Indian source	TNAU, Tamil Nadu	LG	4.6	2.88	207
7	Indian source	NAU, Gujarat	LG	5.6	3.46	310
8	Indian source	TNAU, Tamil Nadu	LD	5.6	3.06	567
9	K29	University of Hawaii	LG	7.4	4.2	637
10	K217	University of Hawaii	LG	6.8	4	685
11	K584	University of Hawaii	LG	5.9	3.8	2532
12	K636	University of Hawaii	LG	7.8	4.68	39
13	KX36	University of Hawaii	LGxLG	6.9	3.74	130
14	KX2	University of Hawaii	LPxLG	5.3	3.3	809
	LSD			1.397	0.7036	<b>879.9</b>
	F-prob			< 0.001	< 0.001	<b>&lt; 0.001</b>

Note: LG *L. leucocephala* ssp. *glabrata*; LD *L. diversifolia*; LGxLG intraspecific hybrid of LG; LPxLG interspecific hybrid between *L. pallida* and LG



nursery and plantation methods, fast growth, adaptability to low nutrient and rainfed sites and usefulness of fodder and wood have attracted farmers to grow them in large areas. But the incidence of psyllid attack during the 1980s and their aggressive colonization through seeds restricted the spread of *Leucaena* cultivation. Still around 150,000 ha are cultivated with this crop mostly in the East coast.

Although *Leucaena* was originally introduced to meet the fodder demand, currently the crop is mostly used as pulpwood for paper making. It is regarded as a highly suitable wood for papermaking with a pulp yield of 49.5% coupled with a kappa number of 20.7 (Umesh Kanna et al, 2011) With the acute raw material shortage faced by the paper industry there is a renewed interest in the cultivation and genetic improvement of *Leucaena* in India. In the past, efforts on domesticating *Leucaenas* outside its natural range were towards increasing fodder production and quality and psyllid resistance (Hughes, 1998). The University of Hawaii, USA which has the largest collection of *Leucaena* germplasm has released a few accessions suitable for wood production. Some of them are also low seed producing and tolerant to psyllid attack (Brewbaker, 2013). In order to evaluate the performance of these new accessions a trial was established in Coimbatore, India (11.0183° N, 76.9725° E). Five trees each of six accessions received from the University of Hawaii and eight locally available seed sources were planted in a randomized complete block design at a spacing of 2 x 1 metres during December 2013. They were assessed during June 2015 for height, diameter at breast height (DBH) and seed production (product of number of fruits per tree and number of seeds per fruit). The data was subjected ANOVA using the software Dataplust and Genstat (version 3.22).

Highly significant ( $p < 0.001$ ) differences were found in terms of growth and seed production among the accessions tested at 1.5 year's age. The Hawaiian accessions recorded on an average 13% better height growth and 17% DBH than the local accessions (Table 1). The best growth was recorded by the Hawaiian giant variety (K636) with a height growth of 7.8 m and DBH of 4.68 cm which was 32 and 38% respectively more than the mean value for local accessions. This variety also possessed a straight stem and produced the lowest number of seeds per tree (39; Fig. 1). If the most prolific seed producing accession (K584) and the least producing one are omitted the mean number of seeds per tree for the local and Hawaiian accessions is similar around 450. High level of correlation for wood yield in age one and four has been reported for *Leucaena* (Brewbaker, 1995). These early trends indicate that considerable gain in wood production can be achieved through deploying accessions like K636 from Hawaii which will also reduce the weediness in the planting areas. The outstanding individuals may be exploited as clones after adequate field testing. In the long term, breeding populations involving more accessions from Hawaii and natural distribution range selected for wood production may be considered to realize increased genetic gain for pulpwood production.

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Fig. 1. Variation in growth, tree form and seed production among *Leucaena* accessions in south India.

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## Reports

### A Report on the Fifth International Casuarina Workshop

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Over two million hectares of Casuarina plantations protect human habitats and agricultural fields, help in reclaiming degraded sites and meet industrial raw material requirements for paper and plywood making and biomass-based energy generation. Cultivation and harvesting of Casuarina generate livelihood opportunities for farmers and agriculture-dependent labour force in rural areas. Following four earlier successful international meetings in Canberra (1981), Cairo (1990), Da Nang (1996) and Haikou (2010) the Fifth International Casuarina Workshop was held in Mamallapuram, Chennai, India, between 3 and 7 February 2014 under the aegis of IUFRO Working Party 2.08.02, *Improvement and Culture of Nitrogen-Fixing Trees*.

The objective of the Workshop was to bring together researchers and managers to update the knowledge on this important group of species so that the results are effectively used for improving livelihood opportunities in rural areas as envisaged by the working title of the Workshop: *Casuarina improvement for securing rural livelihoods*. More than 80 participants from Australia, Bangladesh, China, France, India, Mali, Philippines, Senegal, Thailand and USA attended the workshop and presented papers on various aspects of improvement and utilization of Casuarina. The following are the major outputs from the Workshop:

a) The current status of casuarina planting, utilization and research for improved products and services in the 10 countries represented in the workshop were presented. Strategies to meet the diverse end-use and environmental requirements were discussed.



Participants of the Casuarina Workshop

## Acknowledgement

We are grateful to Dr. James L. Brewbaker, Dr. Charles T. Sorenson, Department of Tropical plant and Soil Sciences, University of Hawaii, Honolulu, Hawaii USA for providing the seedlots and guidance. Mr R.S. Prashanth, Director, Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu, India is thanked for providing facilities to conduct the experiment.



- b) The importance of assemblage, testing and conservation of Casuarina genetic resources both in its natural and planted habitats was emphasized to maintain a reservoir of variability which can cater to all products and services expected from Casuarina plantations.
- c) The extent of genetic gain realized from the ongoing systematic breeding programmes and its impact on securing rural livelihoods and industrial raw material

were discussed. It was decided to place more efforts on improving the accessibility and affordability of genetically improved planting material to smallholding farmers.

- d) A major part of the meeting was devoted to discuss the need to utilize the nitrogen-fixing ability of *Casuarina* to meet the challenges of cultivating trees in salt-affected, moisture-stressed and nutrient-deficient sites. It calls for further research on host-microbe interactions and the molecular basis of biological nitrogen fixation.

Based on presentations made and group discussions, participants drafted recommendations under four broad areas for follow-up action. Accelerating breeding

programmes, improving cultivation techniques and pest management, molecular biological approaches to *Casuarina-Frankia* symbiosis and biological nitrogen fixation, applied research on wood quality and harvesting methods to meet industrial requirements are the major recommendations. The meeting also called for international cooperation in germplasm exchange to sustain the ongoing genetic improvement programmes. Eighty-one abstracts were submitted prior to the workshop which were compiled, printed and provided to all participants of the workshop. The full papers of those papers presented in the workshop have been peer-reviewed and publication as proceedings of the workshop is in the advance stage.

## New collaboration on *Acacia mangium* provenance trials

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In the past two decades short rotation plantations of *Acacias* have expanded in South and South-East Asia (Harwood & Nambiar, 2014). These have become a major commodity crop providing the raw materials for the manufacture of paper pulp, composite and engineered wood products and sawn timber in many countries. *Acacia mangium* is the principal species being utilised. There are around 2.6 million hectares of *Acacia* plantings in this region.

In the late 1980's and early 1990's, CSIRO's Australian Tree Seed Centre (ATSC) arranged and supported the performance testing of a range of provenances of *Acacia mangium* in tropical countries in Asia. These trials identified that Papua New Guinea (PNG) provenances were better performing for these countries, but only a limited number of provenances were represented in these original trials. Since then the ATSC has collected many more provenances from PNG and the aim of this current series of collaborative trials is to test more of these, the majority of which come from Western Province (WP) sources. These will be compared against previously trialled material and improved material.

Collaborating countries include Cambodia, China, India, Malaysia, Thailand and Vietnam. Most have already received their seed to grow for planting out in the 2015 planting season. The trial includes 11 wild provenances (10 from PNG & 1 from Australia) and 3 improved seed sources. Individual collaborators are also able to include other locally used seed sources in their trials. It is expected that the trials will run for at least 3 years and yearly measurements will be made on growth and form traits. A trial establishment and assessment manual has been prepared by the ATSC to assist in ensuring that the trials are planted and managed in a similar way. The

ATSC will also be providing the trial designs and data analysis so that cross site analyses can be carried out. These trials will provide some additional information on any variation in performance between PNG locations as well as against currently available improved seed.

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### Meeting Information

18<sup>th</sup> International Meeting on *Frankia* and Actinorhizal Plants - ACTINO 2015; Montpellier, France; 25-27 August 2015. Contact: Claudine Franche, Email: [claudine.franche@ird.fr](mailto:claudine.franche@ird.fr)

Forest Genetics 2015: Integrating Tree Breeding, Silviculture, and Growth and Yield Prediction, New Brunswick, Canada. 17-20 August 2015. Contact: Kyu-Suk Kang, Email: [kangks84@snu.ac.kr](mailto:kangks84@snu.ac.kr); Michele Fullarton, Email: [Michele.Fullarton@gnb.ca](mailto:Michele.Fullarton@gnb.ca)

Integrating Genomics Tools in American Chestnut Restoration. Penn State University, Pennsylvania, United States, 23-24 October 2015. Contact: Yousry El-Kassaby, Email: [y.el-kassaby@ubc.ca](mailto:y.el-kassaby@ubc.ca)

Scientific cultivation and green development to enhance the sustainability of eucalypt plantations. Zhanjiang City, Guangdong Province, China, 21-24 October 2015. Contact: Shaoxiong Chen, Email: [sxchen01@163.com](mailto:sxchen01@163.com)

4<sup>th</sup> International Conference on Conservation of Forest Genetic Resources in Siberia Barnaul, Russia, 24-29 August 2015. Contact: Konstantin V. Krutovsky, Email: kkrutovsky@gmail.com

Forest Genetics for Productivity. Rotorua, New Zealand, 14-18 March 2016. Contact: Heidi Dungey, Email: Heidi.Dungey@scionresearch.com

All-Division 2 Conference; Prague, Czech Republic; 17-24 June 2018. Contact: Yousry El-Kassaby, Email: y.el-kassaby@ubc.ca, Milan Lstiburek, Email: lstiburek@gmail.com

### Guidelines for Contributions to NFT News

We are inviting contributions for the forthcoming issue of the NFT News on or before 31st May 2016. Contributions may focus on activities that highlight research, promotion and development of nitrogen fixing trees and shrubs. The newsletter will also carry announcements on new information resources, useful websites, and upcoming relevant events.

Contributions to the newsletter may be written in a simple and reader-friendly language to interest a diverse audience. They should contain new information and not a concise version of an article published already. Please limit your contributions to 1000 to 1500 words in a Word file. Inclusion of good-quality photographs (scanned at 300 dpi) is encouraged. Photographs, graphs and other illustrations may please be sent as separate files. Contributions may be sent to any member of the Editorial Team with a copy to casuarina2014@gmail.com.

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