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Using population genomics to catch up with climate change

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Local adaptation of populations to historic climatic conditions is common for widespread conifer species, and maladaptation is an inevitable result of rapid climate change. While long-term field experiments such as provenance trials provide excellent phenotypic data on the extent of maladaptation that results from moving genotypes away from their historic climates, they require considerable time, land, and other resources to complete. Population genomic analysis including genotype-environment associations, genome-wide associations studies, and genetic offset projections all provide insights into climate adaptation in these species and can inform assisted migration for reforestation.

I will use examples from Douglas-fir (*Pseudotsuga menziesii*) and lodgepole pine (*Pinus contorta*) to illustrate and compare phenotypic and genomic evidence for local adaptation to climate. While adaptation to temperatures (and especially low temperatures) is a common signal in phenotypic and genomic data, local adaptation to precipitation regimes and phenotypic variation for adaptation to drought is considerably weaker in the species we study. Genetic gains from selection for faster growth in regional breeding programs are stronger in treatments with adequate moisture, but there does not appear to be a trade-off between seedling growth and drought tolerance. However, selection for faster growth does in some cases result in reduced cold tolerance. Genomic offset approaches such as *GradientForests* that project the mismatch of local conifer populations to future climatic conditions have promise for identifying populations and regions likely to experience the greatest maladaptation. The results of these analyses can vary considerably depending on the geographic scope of populations used to build offset models.

Keywords: Climate change, genotype-environment associations, GWAS, offset prediction, assisted migration

Genetic mechanism of hybrid speciation and the methods to identify genes

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It is increasingly realized that homoploid hybrid speciation (HHS), which involves no change in chromosome number, is an important mechanism of speciation. HHS will likely increase in frequency as ecological and geographical barriers between species are continuing to be disrupted by human activities. HHS requires the establishment of reproductive isolation between a hybrid and its parents, but the underlying genes and genetic mechanisms remain largely unknown. In this study, we reveal by integrated approaches that reproductive isolation originates in one homoploid hybrid shrub species through the inheritance of alternate alleles at genes that determine parental premating isolation. The parent species of this hybrid species are reproductively isolated by differences in flowering time and survivorship on soils containing high concentrations of iron. We found that the hybrid species inherits alleles of parental isolating major genes related to flowering time from one parent and alleles of major genes related to iron tolerance from the other parent. In this way, it became reproductively isolated from one parent by the difference in flowering time and from the other by habitat adaptation (iron tolerance). These findings and further modeling results suggest that HHS may occur relatively easily via the inheritance of alternate parental premating isolating genes and barriers

Keywords: HHS, genetic mechanism, alternate alleles, reproductive isolation, speciation genes

Mycorrhizal associations improve biomass production and alter cell wall chemistry in poplar

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Fuels produced from renewable lignocellulosic material are an attractive alternative to fossil fuels. However, large scale production of lignocellulosic biofuels depends on the reduction of financial and environmental costs of biofuel production and the resilience of these plants to environmental stress, particularly under a changing climate. Symbiotic associations with mycorrhizal fungi represent a potential solution to the challenge of expanding feedstock production, especially in marginal lands. Characterizing mycorrhizal impacts on the production and composition of wood is a necessary step in leveraging these associations to promote sustainable production of biofuels and other bioproducts.

Focusing on mycorrhizal fungi as a key driver of plant biomass production, we assessed the growth parameters and secondary cell wall composition of greenhouse grown *Populus tremuloides* inoculated with either arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) fungi and grown under four nutrient regimes. Our results show that associations with both AM and EM fungi increased plant biomass production by an average of 30% depending on the nutrient conditions. Mycorrhizal plants, especially those inoculated with EM fungi, also allocated a greater portion of their biomass to roots, which could be beneficial in the field where plants are likely to experience water and nutrient stress. Only AM associations altered cell wall composition. Thus, the benefit of increased biomass for biofuel production may be partially offset by increased lignin content in AM plants. By comparing mycorrhizal effects on productivity and chemical composition of lignocellulosic tissue, this work provides insight into the role of mycorrhizal fungi in generating feedstocks for biofuel production.

Key Words: biofuels, lignin, mycorrhizal fungi, *Populus*, secondary cell wall

Effects of extreme drought on plant carbon and water processes in subtropical forests

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The occurrence of extreme drought events will be more and more frequent in the future, which may cause tree mortality in global forests and largely influence the carbon and water fluxes. The water shortage induced by drought could affect many plant physiological processes, such as photosynthesis, biomass allocation, litter decomposition, water potential, hydraulic conductivity, transpiration and leaf shedding. Two mechanisms have been highlighted to be the major driver of tree death and changes in plant physiological processes: hydraulic failure and carbon starvation. Hydraulic failure is regarded as the primary reason causing tree death due to water deficit. However, how the related hydraulic traits may regulate the tree mortality and physiological rates of trees in subtropical evergreen broadleaf forest (SEBF) remains unknown, despite of the fact that subtropical area in China is one of the hot spots of extreme drought events. Therefore, we conducted a drought-rewatering experiment on tree seedlings of five dominant species to investigate how the hydraulic traits were related to the tree mortality and the resistance and recovery of photosynthesis and transpiration under different drought severities. In general, species with greater embolism resistance (P_{50}) survived longer than those with weaker embolism resistance. The photosynthesis and transpiration of tree species with greater P_{50} were more resistant to and recovered faster from drought than those with lower P_{50}. Other plant traits could not explain the interspecific variation in tree mortality and drought resistance and recovery. These results suggested that the SEBF might be highly vulnerable to future drought.

Key words: hydraulic failure, tree mortality, evergreen broadleaf forest, drought resistance, embolism

Inferring Future Climate Vulnerability Using Genomics: Lessons from three clades of conifers

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Understanding how species will respond to climate change is paramount for maintaining conservation and management priorities throughout the next century. However, while increasing in recent popularity, the methods often used to predict population vulnerability to future climate change remain largely unvalidated and there is need for further exploration into (hyper)parameterization, training, and inference. Here, we use sets of range-wide genetic data from jack pine (*Pinus banksiana*) and two varieties of Douglas-fir (*Pseudotsuga menziesii*) to train distinct models of multiple genetic offset methods, and then fit these models to the climate of common gardens where an independent sample of individuals from these same populations was grown and phenotyped. We quantify the performance of these offset methods using the relationship of predicted offset with 1) two-year-old seedling phenotypes in a single common garden of Douglas-fir (increment growth, shoot biomass), and 2) 52-year percent mortality and growth in jack pine from two long-term provenance trials. Additionally, we explore the use of climatic and geographic distances as the sole measure of genetic offset and compare these results with those from genomic modelling. We propose best practices for using offset predictions, and discuss some of the nuances that could increase or decrease the predictive accuracy of these offset methods.

Keywords: climate vulnerability, climate change, machine learning, genetic offset

Accessory chromosome loss contributes to increased symbiotic effectiveness of a tree root fungus

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Genomic plasticity contributes to evolution of diverse lifestyles. Studies of genome plasticity in fungi with non-pathogenic life styles are scarce. Here, we delineate the unique genetic divergence in co-existing isolates (16B and 16W) of a novel fungus *Stagonosporopsis rhizophilae*, which vary considerably in phenotypes, metabolic repertoires, and overall effects on tree growth and salinity tolerance. Intriguingly, 16W harbors a ~0.6 Mb accessory chromosome (AC), and exhibited subtle differences on 18 core chromosomes (CCs) compared to 16B. Intriguingly, almost AC-associated genes share homologues in distant lineages (falling outside the defined family), pointing to a possible horizontal acquisition. 16W tends to adopt a rhizosphere lifestyle on tree roots. Notably, AC deletion mutant (Δ16W) instead resembles 16B in several aspects, particularly in enhanced melanization and to be a dark septate endophyte (DSE), and significantly promoting root development. AC loss results in upregulation of genes related to indole acetic acid and melanin biosynthesis, and root colonization (mainly cellulose and pectin-degrading enzymes and candidate effectors). Globally, a moderate to strong decrease of chromatin accessibility in 16B and Δ16W is identified, indicating an impressive of *Cis*-regulatory elements remodeling. There are weak but significant correlations between differences in chromatin and transcriptional landscape, though only few above differentially expressed genes worked in such a manner. Together, AC loss leads to an increase of symbiotic effectiveness, while AC may function to maintain a more open chromatin status for improving fitness. This discovery highlights the role of ACs in origin and evolution of DSEs, which are major non-mycorrhizal root guilds.

Keywords: chromatin accessibility; Cis-regulation; dark septate endophytes; intraspecific variations; melanin

Gene *sdaB* is essential for the high nematocidal activity of *Enterobacter ludwigii* AA4 against the pine wood nematode *Bursaphelenchus xylophilus*

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Bursaphelenchus xylophilus, a plant parasitic nematode, is the causal agent of pine wilt, a devastating forest tree disease. Essentially no efficient methods for controlling *B. xylophilus* and pine wilt disease have yet been developed. *Enterobacter ludwigii* AA4 isolated from the root of maize has powerful nematocidal activity against *B. xylophilus* in a new *in vitro* dye exclusion test. The corrected mortality of the *B. xylophilus* treated by *E. ludwigii* AA4 or its cell extract reached 98.3% and 98.6%, respectively. Morphological changes in *B. xylophilus*, treated with a cell extract from strain AA4 suggested that the death of *B. xylophilus* might be caused by an increased number of vacuoles in nonapoptotic cell death and the damage to tissues of the nematodes. In a greenhouse test, the disease index of the seedlings of Scots pine (*Pinus sylvestris*) treated with the cells of strain AA4 plus *B. xylophilus* or those treated by AA4 cell extract plus *B. xylophilus* was 38.2 and 30.3, that was significantly lower than 92.5 in the control plants treated with distilled water and *B. xylophilus*. We created a *sdaB* gene knockout in strain AA4 by deleting the gene putatively encoding the beta-subunit of L-serine dehydratase through Red homologous recombination. The nematocidal and disease-suppressing activities of the knockout strain were remarkably impaired. Finally, we revealed robust colonization of *P. sylvestris* seedling needles by *E. ludwigii* AA4, which is supposed to contribute to the disease-controlling efficacy of strain AA4. Therefore, *E. ludwigii* AA4 has significant potential to serve as an agent for the biological control of pine wilt disease caused by *B. xylophilus*.

Keywords: *sdaB,* L-serine dehydratase, *Enterobacter ludwigii,* nematocidal activity, *Bursaphelenchus xylophilus,* pine wilt disease, methuosis.

The combined effect of abiotic and biotic stress in *Eucalyptus*

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In recent years, the importance of biotic and abiotic stresses in forestry has increased as a result of climate change enhanced frequency and intensity of weather extremes. International travel and trade has inadvertently mobilized pathogens and pests across the globe, increasing incidence. Forest trees are exposed to both types of stresses in combination or sequentially over their lifetime. In this study, we aimed to characterise the response of *Eucalyptus grandis* two-year-old trees to combined drought stress and pathogen challenge with a fungus that causes stem canker. In our greenhouse experiment, well-watered and drought-stressed ramets, were either mock-inoculated or inoculated with *Chrysoporthe austroafricana*. We measured stem lesion length and harvested stem samples for RNA-seq analysis at different time points. We found significantly longer stem lesions in drought stressed plants at three days post inoculation (dpi) and 10 days after withholding water. There was no significant difference in lesion length after seven dpi and 14 days of drought. Mild drought stress predisposed the plants to pathogen attack while more severe drought at later time points curbed pathogen spread. At the transcriptome level, we found host responses, unique to the simultaneous occurrence of the two stressors. This includes changes in the enzymatic detoxification of reactive oxygen species (ROS), which indicate varied sources of ROS and scavenging enzymes affecting the redox homeostasis under multiple stress conditions. Exposure to combined stress altered crosstalk among abscisic acid, salicylic acid, and jasmonic acid signaling pathways, suggesting that concentration-dependent antagonism among these phytohormones may play a key role to the outcome of tree response to multiple stressors. Our transcriptomic data also showed that fungal infection may compromise accumulation of soluble sugars in drought stressed trees thereby reducing osmotic adjustment and aggravating the effect of drought. Furthermore, the combination of the two stressors resulted in a rewiring of terpenoid and phenylpropanoid biosynthesis. Overall, this work provides insight into the molecular reprogramming incited by combinatorial stress in forest trees, enabling predictions on *Eucalyptus* forest resilience under changing climates.

Keywords: Combined stress; Forest Tree; Signaling pathways; Transcriptome

Increasing plantation tree health challenges require a need for speed and smart solutions

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Plantation forestry, using non-native tree genera such as *Eucalyptus* and *Pinus*, has experienced increasing threats over the past few decades. Initially this was mostly due to increasing numbers of insects and pathogens affecting these trees in their new countries. Annually, more insects and pathogens of plantation forestry are appearing and at much shorter intervals. In South Africa, previously unknown insect pests and pathogens have been detected regularly over the past decade. Climate change also poses an increasing risk to plantation forestry, both in terms of impact on growth conditions for trees and impact on insects and pathogens. The past season has seen the discovery of novel insect and disease problems in South Africa, as well as unpredicted behaviour of pathogens driven by increased temperatures, unseasonally high rainfall and cloud days. To manage pests, early detection is critical and breeding for disease tolerance is key to sustainable management. Due to the size of plantations and resource constraints, ground surveillance and monitoring of pests and diseases mostly fail to detect problems before they have reached epidemic proportions. Similarly, relying on traditional breeding and screening against pests and diseases based on natural infection, has a significant chance of false negatives, resulting in significant losses after commercialization of a variety. The need for implementation of new technologies, such as remote sensing, molecular breeding and the use of e. g. NIR and related technologies for early screening of disease and pest resistance has become increasingly important. Similarly, the need for finer scale, climate and weather prediction and modelling, considering seasonal variation and microclimate in relation to site and other factors, are critical for short to medium term management of pest and disease outbreaks. Multi-disciplinary research, and implementation of new technologies are required sooner, rather than later to ensure sustainable plantations.

Keywords: climate change, early detection, new pests/diseases, molecular breeding, prediction

Pinus pinaster resistance to pine wilt disease: uncovering early molecular responses and mechanisms involved

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Pine wilt disease (PWD) is a serious threat to conifer forests in Europe and Asia, caused by the parasitic nematode *Bursaphelenchus xylophilus* (pinewood nematode-PWN). Despite its high susceptibility to the disease, *Pinus pinaster* has been reported as showing heritable resistance. Early transcriptional responses in PWN inoculated plants have been previously analysed, uncovering the importance of lignin synthesis and jasmonic acid defence pathway in resistance phenotypes.

To complement previous results, we aimed at (i) the identification of miRNAs involved in the *P. pinaster*-PWN interaction and (ii) the detection of SNPs in the pine transcriptome and their association with PWN resistance. Seventy-two hours post-inoculation, the 105 miRNAs found responsive to PWN were associated with jasmonate-response pathway, ROS detoxification, and terpenoid biosynthesis. The generated data further suggest that post-transcriptional regulation of RLK/RLP receptors and L-fucose synthase by miRNAs might be relevant in the resistance to PWD. The analysis of putative bidirectional trans-kingdom RNA silencing led to the identification of several *P. pinaster* genes potentially targeted by PWN miRNAs, which was supported by degradome analysis. Targets for *P. pinaster* miRNAs were also predicted in PWN, suggesting a role for trans-kingdom miRNA transfer and gene silencing in PWN parasitism and plant resistance to PWD. Previously generated RNA-seq data were used for SNP discovery resulting in the identification of 31 SNPs highly differentiated between resistant and susceptible plants, including SNPs in genes involved in cell wall lignification. Part of the validated SNPs were used to test for SNP-phenotype associations in a set of inoculated half-sib plants and associations with phenotype after PWN inoculation were found for SNPs in two genes.

Overall, our results provide new insights into previously unexplored roles of miRNA regulation in *P. pinaster* response to PWN, as well as valuable SNP data for the development of robust molecular markers associated with PWD resistance.

Keywords: Maritime pine, microRNA, pine wood nematode, Single nucleotide polymorphism

Comparative transcriptional dynamics in scion and rootstock stems of grafted maritime pines

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Pinus pinaster Ait. is a Mediterranean native conifer that shows a high genetic variability at inter- and intra-population level, as well as intraspecific variation in response to abiotic and biotic stress factors, such as drought or pathogen/pest outbreaks. Grafting is an excellent research system to study molecular regulation of traits of interest, as well as drought tolerance. In angiosperms, the use of drought tolerant rootstocks improves the drought response of the whole tree. Despite the anatomical differences between angiosperms and gymnosperms, studies on the effects of grafting in gymnosperms, in general, and in conifers, in particular, are scarce.

In this study, rootstock/scion interactions were studied using transcriptomic approaches to analyze grafted stems of *P. pinaster*. Four constructs combining genotypes with contrasting response to drought were studied: Gal1056 (drought-sensitive) and Oria6 (drought-tolerant) used as scions that were grafted onto vegetative propagated F1 siblings of the controlled cross Gal1056 x Oria6, which were used as rootstocks, R1S (drought-sensitive) and R18T (drought-tolerant) (López-Hinojosa *et al.* 2021, Scientific Reports 11:11582). Transcriptomic profiles of scion and rootstock stems were characterized and used to identify differentially expressed genes (DEGs). This study revealed that drought-sensitive scion stems showed significantly higher number of DEGs, regardless of rootstock, affecting several biological functions, such as external stimulus and cell wall modification in Gal1056/R1S scion stems. Activation of terpenoid, ROS, and amino acid metabolism pathways was quantified in Gal1056/R18T scion stems. Furthermore, the effect of drought-tolerant rootstocks was associated with increased expression of genes related to flavonol biosynthesis, transport and epigenetic regulation in drought-sensitive scions (Gal1056), reaching expression levels similar to those quantified in drought-tolerant scions (Oria6). In contrast to the modulation of gene expression quantified in scion stems, no functional effect was observed in rootstock stems associated with the different scion genotypes grafted onto them.

Keywords: *P. pinaster*, grafting, stem transcriptome, drought-tolerance, conifer xylem,

Heat and Osmotic stress have contrasting effects on periderm and endodermis suberization of cork oak roots

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Plant adaptation to water stress involves remodeling of root apoplastic barriers, including cell-wall suberization. Suberin is a natural biopolymer deposited in root endodermis cells, but also in periderm (or cork). Cell wall suberization is induced in endodermis by ABA and other abiotic stresses, and several molecular regulators have been identified in this process. Yet no studies have been conducted in mature root regions undergoing secondary growth, where endodermis is replaced by periderm. This work aimed to assess the potential of cork oak (*Quercus suber*) roots as a tool for functional studies in this species, and assess the role of abiotic stress in root periderm suberization. We investigated the spatio-temporal characteristics of both endodermis and periderm, and found that the onset of periderm formation occurs very early in mature regions of cork oak taproots (approx. 8 days after sowing) (Leal *et al*., 2021). We further performed a comparative transcriptomic analysis using roots grown under control, osmotic and heat stress conditions. We selected root segments undergoing secondary (SD) and primary development, to assess the differential impact of the tested conditions. Independently of the stress, the transcriptomic landscape of SD-region showed enrichment in the expression of genes related to cell-wall modifications, mainly lignification and suberization. These included multiple transcription factors and enzymes related to suberization, phenylpropanoid and lipid metabolism. Osmotic and heat stress had contrasting effects on phellem and endodermis suberization, and these differences may be partially related to differential regulation of ABA signaling genes. Our work provided an unprecedented resolution of the regulatory networks acting during periderm development, which might further contribute to design new strategies to improve plant resilience. The morphological characterization also creates new opportunities to allow a faster assessment of cork development (as compared to studies using stem tissues) in this species, and to tackle fundamental questions regarding its regulation.

Keywords: *Quercus suber*; taproots; abiotic stress; transcriptome; suberization regulatory-networks

References: Ana Rita Leal, Helena Sapeta, Tom Beeckman, Pedro M Barros, M Margarida Oliveira, Spatiotemporal development of suberized barriers in cork oak taproots, *Tree Physiology*, 2021; tpab176, https://doi.org/10.1093/treephys/tpab176

Over-expression of larch *DAL1* accelerates life-cycle progression in *Arabidopsis*

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Homologs of *Larix kaempferi DEFICIENS-AGAMOUS-LIKE 1* (*LaDAL1*) promote flowering in *Arabidopsis*. However, their functional role in the whole life-cycle is limited. Here, we analyzed the phenotypes and transcriptomes of *Arabidopsis* plants over-expressing *LaDAL1*. With respect to the defined life-cycle stage of *Arabidopsis* based on the meristem state, the results showed that *LaDAL1* promoted seed germination, the timing of bolting, flower initiation, and global proliferative arrest, indicating that *LaDAL1* accelerates the meristem reactivation, the transitions of vegetative meristem to inflorescence and flower meristem, and meristem arrest. As a marker gene of meristem, *TERMINAL FLOWER 1* was down-regulated after *LaDAL1* over-expression. These results reveal that *LaDAL1* accelerates the life-cycle progression in *Arabidopsis* by promoting the transition of meristem fate, providing more and novel functional information about the conifer age-related gene *DAL1*.

Keywords: Life-cycle; MADS-box; *AGL6*; reproductive development; gymnosperm

Identification of cork oak genetic modules regulating phellem development in response to drought

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The longevity and high activity of the cork cambium (phellogen) in the bark of cork oak are the cornerstones for the sustainable exploitation of cork (phellem), a unique raw material enriched in suberin. To mitigate the negative impacts on tree vitality and productivity imposed by extreme drought events, novel silviculture practices using optimized fertigation are being developed. In this context, a targeted selection of more productive/resilient genotypes could further minimize water use, yet fundamental knowledge on the genetic regulation of cork development is still scarce. Aiming to identify key genetic pathways acting on the cross-talk between cork development and drought adaptation, 1-year-old plants were grown for 6 months under well-watered (WW) or water-deficit (WD) conditions. Main stems were further targeted for histological characterization, transcriptomic analysis, and chemical profiling. When compared to WD, WW treatment resulted in a 2-fold increase in stem diameter, with an increase in phellogen activity to cope with stem enlargement, as determined by the presence of a continuous layer of phellem. Despite these differences, no changes in phellem chemical composition were found between treatments. Following a tissue-specific approach, we extracted RNA from phellem, phloem, and xylem from WD and WW groups and found an upregulation of genes related to drought adaptation ad photosynthesis in WD, while genes related to growth, cell wall biogenesis, and suberization were downregulated. The overall increase in expression of photosynthesis-related genes in these tissues suggests a determinant role of stem photosynthesis in the adaptation of young plants to long-term drought. Based on gene co-expression analysis we identified important genetic modules and candidate transcriptional regulators related to phellem growth and differentiation, which are regulated by drought. The data gathered will allow harnessing the diverse genetic heritage of this species for the development of optimized management practices that could maximize plant resilience and productivity.

Keywords: *Quercus suber*, Gene regulatory networks; Suberin; Physiology; Abiotic stress

Concerted control of the *LaRAV1-LaCDKB1;3* module by temperature during dormancy release and reactivation of larch

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Dormancy release and reactivation of temperate-zone trees involve the temperature-modulated expression of cell-cycle genes. However, information on the detailed regulatory mechanism is limited. Here, we compared the transcriptomes of the stems of active and dormant larch trees, emphasizing the expression patterns of cell-cycle genes and transcription factors and assessed their relationships and responses to temperatures. Twelve cell-cycle genes and 31 transcription factors were strongly expressed in the active stage. Promoter analysis suggested that these 12 genes might be regulated by transcription factors from 10 families. Altogether, 73 cases of regulation between 16 transcription factors and 12 cell-cycle genes were predicted, while the regulatory interactions between *LaMYB20* and *LaCYCB1;1*, and *LaRAV1* and *LaCDKB1;3* were confirmed by yeast one-hybrid and dual-luciferase assays. Last, we found that *LaRAV1* and *LaCDKB1;3* had almost the same expression patterns during dormancy release and reactivation induced naturally or artificially by temperature, indicating that the *LaRAV1-LaCDKB1;3* module functions in the temperature-modulated dormancy release and reactivation of larch trees. These results provide new insights into the link between temperature and cell-cycle gene expression, helping to understand the temperature control of tree growth and development in the context of climate change.

Keywords: cell-cycle gene, dormancy release, reactivation, temperature, transcription factor

Genomic insights into present local adaptation and future climate change vulnerability of a keystone forest tree species in East Asian

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Rapid global climate change is posing numerous threats to Earth's biodiversity, forcing organisms either to rapid adapt and evolve or face maladaptation and possibly extinction. Assessing the adaptive capacity of ecosystem keystone species, like forest tree species, is fundamental for preserving biodiversity and informing where conservation efforts should be most effective. In this study, we first perform a de novo chromosome-level genome assembly of *Populus koreana*, a pioneer and keystone forest tree species in local ecosystems in East Asian. We then characterize the genomic diversity of 230 individuals collected from 24 native populations of *P. koreana*. A combination of genome-wide environmental association studies and whole-genome selection scans was performed to reveal the genomic basis of local adaptation to diverse climates. We identify a set of single nucleotide polymorphisms (SNPs), indels and structural variations (SVs) strongly associated with various environmental variables and under strong natural selection. Finally, we integrate climate-associated genomic variation with environmental modelling and machine learning approaches to investigate spatiotemporal response to future climate change. The gradient forest (GF) and risk of non-adaptedness (RONA) analyses help us to identify populations being most vulnerable to future climate change. To our knowledge, our work is one of the very few studies that associate whole-genome variations (SNPs, indels and SVs) with local environmental variables and predictions of climate change vulnerability across the landscape of an ecologically important forest tree species. We believe our work will provide a key reference for how to use genomic resources to better understand the climate change consequences of other ecologically and economically important non-model species.

Transcriptome evaluation of the salt-treated roots of *Rhizophora mucronata* and *R. apiculate*

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Mangroves are an ecologically important component of the coastal ecosystem that is well adapted in intertidal zones. *Rhizophora* sp. have developed various morphological and physiological characteristics to adapt to high salinity. They are categorized as salt excluders as they limit sodium uptake from the roots. To understand the genetic determinants that help in limiting salt uptake from the roots, the salt tolerant *Rhizophora mucronata* and the relatively salt susceptible *R. apiculata* were compared. The shoot/ root ratio of Na^+ in propagules subjected to 250 mM NaCl for 24h were found to be higher at 0.97 ppm for *R. apiculata* when compared to 0.83 for *R. mucronata* under NaCl stress indicating restricted Na^+ uptake by *R. mucronata*. To assess the early effects of salt stress (250mM-24 hrs) on the root transcriptome of the two species, RNA-sequencing was carried out using the Illumina platform followed by de novo assembly. Tools like Trinity, CD-hit, Blast+, and DESeq2 were used for assembling raw reads, clustering, annotation, and differential expression of transcripts. Final transcriptome analysis identified 3253 and 23 Differentially Expressed Genes (DEGs) in salt treated roots of *R. apiculata* and *R. mucronata*. We then compared the gene expression profiles between the two species. Out of 34170 transcripts, 9216 transcripts were differentially regulated between *R. apiculata* and *R. mucronata.* The Gene Ontology (GO) analysis revealed key functions modulated during the early stage of salt stress. These includes ATP binding, metal ion binding, protein serine/threonine kinase activity, and water channel activity. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that biosynthesis of secondary metabolites, Metabolic pathways, and Phenylpropanoid biosynthesis pathways are tightly regulated. Further, this study provides a salt induced root transcriptomic resource in two species of Rhizophora for future research on Rhizophora under salt stress.

Keywords: mangroves, abiotic stress, salt excluders, RNA-Seq, gene expression profiling.

Achievements and Future Perspectives of conservation genetic studies on Indian teak (*Tectona grandis L. f.)*

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Teak (*Tectona grandis* L. f.) is a tree species known worldwide for quality and durability of its timber products. Teak originated in India and the country represents the largest proportion of its natural population. Ironically, the country has dubious distinction of importing quality wood material due to low productivity and quality of teak plantation in India. As a matter of fact, the planting stock is mostly raised from unimproved and heterogenous genetic resources of the species. This warrants sincere effort for genetically improved and elite stock material to confer a uniform high growth and assured quality of teak plantation. Consequently, the genetic structure and adaptive fitness of Indian teak was evaluated sampling its Indian populations. Genotyping through DNA-based dominant and co-dominant markers and phenotyping for the traits related to wood quality (wood density), growth (height and girth at breast height), and pest-resistance (against leaf skeletonizer) were performed on a country-wide assemblage of phenotypically superior (plus) trees clones. DNA markers significantly linked to the quantitative trait loci of desirable traits were identified through association mapping. Ecological niche modeling predicted the population fitness to adapt the projected climatic regimes. After validation, the identified DNA markers will help on marker-assisted selection of elite stock material. The identified and evaluated elite clonal material will be used for commercial multiplication and to develop advanced generations of breeding populations.

Key Words: Genetic Diversity, ISSR, Microsatellite, Climate Change, ENM

Integrated approach to combat illicit trade and conservation of medicinal plants

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Overexploitation and unscientific extraction of medicinal plants owing to huge market demand has resulted in the depletion of the existing natural resources which demand immediate conservation/ restoration efforts. The Convention on Biological Diversity and the Convention on International Trade of Endangered Species have been established for the sustainable conservation by regulating trade in threatened species. Since medicinal plants are usually traded as dried, shredded or powdered plant parts, it is hard to trace the exact identity of endangered species involved in trade, which consequently result in the extinction of wild resources. DNA barcoding offers a novel prospective for taxonomists and a reliable alternative to morphological identification which has greatly transformed the species identification process (Hebert et al 2003; CBOL 2009). Recently, amalgamation of DNA barcoding and Machine Learning Algorithm (MLA) has been reported as precise means for species authentication. In this background, a case study has been conducted to track the illegal trade and chain of custody validation of medicinal plants endemic to the Western Ghats of India. Original as well as traded raw drugs were collected, CBOL recommended standard plastid barcode gene regions and nuclear gene region, were employed for developing the reference library of barcode sequence database. In MLA, DNA barcoding analysis is performed with a reference library sequences of known species (BRM) and query sequence of traded samples. Different supervised learning methods were tested on DNA barcodes with 10-fold cross validation. The best classifier with 100 % accuracy was further utilised for the authenticity of traded samples. Our study could identify illegal trade and rampant adulteration of raw drugs in herbal market and herbal industries. Therefore, besides species authentication, restoration, cultivation and conservation measures have to be initiated to enhance the quality of ayurvedic formulations and to reduce the decline of wild resources.

Keywords: DNA barcoding, Raw drug, Machine Learning Algorithm

Reference

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Transcriptome analysis reveals potential transcripts involved in climate adaptation in *Santalum album* L. (Indian Sandalwood)

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An increasing concern among conservationists and tree breeders is the decline in genetic diversity which adversely affects tree health and resilience to changing environmental conditions. Hence, understanding the adaptive diversity of populations is the cornerstone for genetic improvement, conservation and sustainable utilization of tree genetic resources. Indian Sandalwood (*Santalum album* L.), a vulnerable species is a hemi-root parasite which is valued for its essential oil extracted from aromatic heartwood and roots. The threat to this species in India has reached critical level due to over exploitation and hence requires concerted effort to conserve the adaptive gene pool. Variation in gene expression is reported to play a crucial role in determining phenotypes that enable the species to acclimatize to stress conditions. In the present study, leaf transcriptomes of nine *S. album* individuals across three climatic conditions (Tropical Wet, Tropical Dry, Tropical Monsoon) were assembled and functionally annotated. Pair-wise expression patterns were compared and out of 1,54,110 transcripts used for analysis, 727, 1141 and 479 transcripts were significantly differentially expressed in Wet vs. Dry, Monsoon vs. Dry and Wet vs. Monsoon respectively with $\log_2(\text{fold change}) \geq 3$ and a threshold of $\log_{10}(p\text{-value}) < 0.05$. A total of 1517 transcripts were differentially expressed across all the nine individuals and 1459 (96.2%) were functionally annotated with 3978 GO terms. These annotated transcripts were mapped to 105 pathways. The co-expression network analysis revealed several candidate transcripts including ribosomal protein, heat shock proteins, cold stress related transcript and squalene synthase and HMG-CoA reductase from sesquiterpene pathway as probable regulators of climate adaptation in *S. album*. The transcripts and pathways identified in the present study provide a genomic resource to understand adaptation in *S. album* in their natural environment.

Keywords: Adaptation, climatic conditions, genetic diversity, differentially expressed, co-expression network analysis

Walnut ethylene response factor JrERF2-2 interact with JrWRKY7 to regulate the *GSTs* in plant drought tolerance

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Juglans regia is a world-famous woody oil plant, whose yield and quality are affected by drought stress. Ethylene responsive factors (ERFs) play vital role in plant stress response. In current study, to comprehend the walnut molecular mechanism of drought stress response, an ERF transcription factor was clarified from *J. regia* (*JrERF2–2*) and its potential function mechanism to drought was clarified. The results showed that *JrERF2–2* could be induced significantly by drought. The transgenic *Arabidopsis* over-expression of *JrERF2–2* displayed enhanced growth, antioxidant enzyme vitalities, reactive oxygen species scavenging and proline produce under drought stress. Especial the glutathione-S-transferase (GST) activity and most *GST genes*' transcription were elevated obviously. Yeast one-hybrid (Y1H) and co-transient expression (CTE) methods revealed that *JrERF2–2* could recognize *JrGST4*, *JrGST6*, *JrGST7*, *JrGST8*, and *JrGSTF8* by binding to GCC-box, and recognize *JrGST11*, *JrGST12*, and *JrGSTN2* by binding to DRE motif. Meanwhile, the binding activity was strengthened by drought stress. Moreover, JrERF2–2 could interact with JrWRKY7 to promote plant drought tolerance; JrWRKY7 could also distinguish *JrGST4*, *JrGST7*, *JrGST8*, *JrGST11*, *JrGST12*, and *JrGSTF8* via binding to W-Box motif. These results suggested that *JrERF2–2* could effectively improve plant drought tolerance through interacting with JrWRKY7 to control the expression of GSTs. *JrERF2–2* is a useful plant representative gene for drought response in molecular breeding.

Keywords: Drought stress; Transcriptional regulation; Promoter recognition; Ethylene signaling; Protein interaction

Modeling the impact of climate change on clonal recommendation using environmental covariates

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Climate changes should impact cultivar recommendation given the effects of genotype by environment interaction (GxE). GxE can be modeled with the use of environmental covariates (climate, soil, and geographic). Thus, the objective of this work was to predict changes in the recommendation of commercial *Eucalyptus* clones planted in Brazil, under different future scenarios of climate change, using GxE modeling with environmental covariates. For this, a dataset of 13,483 forest inventory data points of five commercial clones planted in eight Brazilian states was provided by the company Suzano S.A. Partial least square regression was adjusted for each clone using 26 environmental covariates, including 19 WorldClim bioclimatic variables, three geographic factors (latitude, longitude, and altitude), soil physics data (clay, sand, soil density), and cation exchange capacity (CEC), from SoilGrids. Future climate data comprise the median of the values predicted by twenty-four climate change models for 2041-2060, obtained from WorldClim for two different scenarios: ssp126 (sustainability) and ssp585 (fossil fueled development). For each scenario, productivity maps were generated and used for pixel-by-pixel ranking (Which-Won-Where maps) via geographic information system (GIS) in the special resolution of 2.5' (~5km^2). These future clonal recommendations were compared with the current one performed using the past WorldClim average data (1970-2000). Under an optimistic scenario (ssp126) 31.72% of the map changed clonal recommendation. This number increased to 35.88% under a pessimistic scenario (ssp585). The Brazilian state that experienced the most changed in recommendation was Mato Grosso do Sul, with 50.08% and 50.18% under the ssp126 and ssp585. Conversely, the most stable state was Maranhão, with only 21.83% and 22.64%. Regardless of the scenario, our results show that recommendation of *Eucalyptus* clones could be widely affected by climate changes in Brazil. This study reinforces the importance of continuous breeding programs for releasing new clones adapted to the changing climate.

Keywords: CO2 emission, eucalyptus breeding, GxE prediction, shared socioeconomic paths

Selection of *Eucalyptus* spp. clones for bioenergetic production in water deficit regions.

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Eucalyptus cultivation plays an important role in the national economy since the wood generated in this activity supplies most of the national forest industries. Recently, forest plantations have expanded, reaching areas present in the Cerrado biome and in transition with the Amazon biome, with very different climate and soils from the regions where Brazilian forestry has developed over the years. This new forest frontier present new challenges to genetic improvement programs, which must develop genotypes adapted to new climatic and silvicultural conditions. Given this need, our objective was to evaluate the performance of 109 eucalyptus clones throughout the production cycle in different locations in the State of Goiás to select clones adapted in the region. The experiment area is characterized as tropical with dry winter climate type (Aw), with precipitation irregularly distributed throughout the year, with 5 months of water deficit. For that, three clonal tests were planted in Catalão, Corumbá-de-Goiás and Luziânia. The experimental design was a randomized complete block with single tree plots and 29 replicates. Estimates of variance components were obtained via REML (Restricted maximum likelihood) and genotypic values via BLUP (Best Linear Unbiased Prediction) for these traits. In this way, it was possible to evaluate the magnitude of the genetic, environmental, and the interaction these effects. The best clones at two and four years are, on average, 65% equivalent to the best clones at six years, when the average productivity was 255.8 m^3 ha^{-1}. All six most productive clones (CCL29, CCL35, CLR454, CCL27, CCL07, AEC144) have *E. urophylla* in their constitution. shows great adaptation in regions with seasonal water stress. Thus, the results indicate Eucalyptus genotypes able to develop well in regions with seasonal water deficit, which should become more frequent, with the intensification of climate change.

Keywords: Biomass; Climate Change; Clonal tests; Forest Breeding; Silviculture.

A rapid and sensitive enzymatic assay to assess the genetic resistance to stem borers using *chitinase* activity in *Eucalyptus*

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Stem borer (Celosterna scabrator), an insect pest is increasing becoming a threat to Eucalyptus plantations in India. The current study reports a novel method developed to screen stem borer resistance by engaging tree's inherent Chitinase enzyme activity in bark tissues of Eucalyptus. Chitinase plays an important role to hydrolyse the chitin, a major structural component of the gut-lining of insects, hence Chitinase activity in host becomes the basis for potential genetic resistance to insect pests. A study to optimize an efficient assay was carried out with fifty three Eucalyptus genotypes (4 families) from two different locations. The crude enzyme extract was assayed for liberation of 4-methylumbelliferone (4MU) released by Chitinolytic activity using a fluorimetric assay. Based on the assay the genotypes under study were categorised for borer resistance under natural field infestation conditions. The specific enzyme activity (U/g) was found to be higher in the bark tissues of resistant genotypes (2.2 U/g and above) compared to susceptible counterparts. Further, a positive correlation (R2= 0.7) of the same enzyme activity was found between the bark and leaf tissue, demonstrating the feasibility of engaging this method early selection in nursery stage. The paper would further discuss the detailed methodology, results from resistance screening methods for their usefulness in developing resilient genetic stocks.

Keywords: Stem borer; Chitinase assay; 4-methylumbelliferone; resistance screening; early selection

Epigenome Editing for Cell Programming

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The advent of genome engineering technologies, including the RNA-guided CRISPR/Cas9 system, has enabled the precise targeting of genomic locations with molecular machinery. While most widely used for editing DNA sequences, these technologies may have even greater and broader impact by programming other functions at specific genomic locations. For example, CRISPR technologies have been adapted and applied to robustly and precisely manipulate gene expression, program the epigenome, annotate the function of the non-coding genome, and control cell fate decisions. Specifically, we and others have engineered CRISPR/Cas9-based tools to regulate the expression of endogenous genes relevant to disease, development, and differentiation. Genome-wide analysis of the DNA-binding, gene regulation, and chromatin remodeling by these targeted epigenome modifiers has demonstrated their exceptional specificity. We continued to expand the CRISPR epigenome editing toolbox via the characterization of novel CRISPR-Cas systems and the discovery of new effectors that coordinate gene expression changes at targeted loci. We have applied these technologies to control the decisions of stem cells to become specific cell fates and reprogram cell types into other lineages for drug screening, disease modeling, and *in vivo* tissue regeneration. High-throughput pooled CRISPR screening with epigenome editors has enabled the discovery of master regulators of complex cell fate decisions. We have also used epigenome editing to reprogram complex epigenetic states, such as imprinting, at disease-relevant loci. Collectively, these studies demonstrate the potential of modern genome engineering technologies to capitalize on the products of the Genomic Revolution and transform medicine, science, and biotechnology.

Keywords: CRISPR, epigenetics, epigenome editing, gene expression

Boosting plant genome editing with a versatile CRISPR-Combo system

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CRISPR-Cas9, its derived base editors and CRISPR activation systems have greatly aided genome engineering in plants. However, these systems are mostly used separately, leaving their combinational potential largely untapped. Here, we develop a versatile CRISPR-Combo platform, based on a single Cas9 protein, for simultaneous genome editing (targeted mutagenesis or base editing) and gene activation in plants. We showcase CRISPR-Combo's powerful applications for boosting plant genome editing. First, CRISPR-Combo is used to shorten the plant life cycle and reduce the efforts in screening transgene-free genome-edited plants by activation of a florigen gene in *Arabidopsis*. Next, we demonstrate accelerated regeneration and propagation of genome-edited plants by activation of morphogenic genes in poplar. Furthermore, we apply CRISPR-Combo to achieve rice regeneration without exogenous plant hormones, which is established as a novel method to predominately enrich heritable targeted mutations. In conclusion, CRISPR-Combo is a versatile genome engineering tool with promising applications in crop breeding.

Keywords: CRISPR-Combo, plant genome editing, Arabidopsis, poplar, rice

Sex determination genes in *Populus deltoides* and a a molecular model underlying female sex lability of poplars

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Sex determination is the intriguing aspect in plants reproduction and species evolution. *P. deltoides* shows full dioecism with heterogametic males (XY). We discovered that the proximal end of Y-chromatid harbors two hemizyous gene loci, *FERR-R* and *MSL*, which triggered the separation of sexes in *P. deltoides* through different regulation mechanisms. *FERR-R* blocks the femaleness through RNA-directed DNA methylation and siRNA-guided mRNA cleavage of *FERR*, an A-type response regulator whose expression is highly restricted in the early developmental stages of female flower. Whereas *MSL* acts as miRNA sponge soaking miRNAs and functions as a maleness promotor. *Populus* species are normally dioecious, baring catkins consisting of staminate (male) or pistillate (female) florets on different trees. Liable expression of sex was frequently reported by empirical observation in a variety of *Populus* species, and findings in *Populus* species reported greater liability in the female than in the male sex. We carried out a systematic analysis for the *MSL* sequence. Based on our results, we proposed a molecular model triggering the female sex lability in poplars

Keywords: Dioecy; sex determination gene; cosexual plant; sex liability; long non-coding RNA

The genome of *Alsophila spinulosa* and xylem formation and secondary metabolite biosynthesis in tree fern

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To date, little is known about the evolution of fern genomes, with only two small genomes published from the heterosporous Salviniales. The order Cyatheales in fern is one of the few lineages having arborescent trunks outside seed plants. Here we assembled the genome of *Alsophila spinulosa*, known as the flying spider-monkey tree fern, onto 69 pseudo-chromosomes. Two putative whole –genome duplication (WGD) events were identified in *A. spinulosa*. The remarkable preservation of synteny, despite resulting from an ancient WGD over one million years ago, is unprecedented in plants and likely speaks to the uniqueness of tree ferns. We observed that most cells in xylem inside the vascular bundle of stem were tracheids, which were bundled together and had scalarifom thickening in walls. Chemical composition and NMR analysis showed

that there were mainly G lignins in xylem. Two *VND* genes were significantly regulated in xylem, indicating that they are likely key regulators for the formation of tracheids that serve both support and transport functions in *A. spinulosa* arborescence trunks. We identified in xylem a novel phenolic compound, alsophilin, that is derived from hispidin and piceatannol. *In vitro* enzyme assays on selected 8 genes encoding type III polyketide synthases (PKS III) help to provide the molecular basis for Alsophilin biosynthesis. Abundant enzyme members, including PKS III, cytochrome P450 monooxygenases, and oxidases (laccase and peroxidase) were identified in the *A. spinulosa* genome, which might suggest that *A. spinulsa* could be a valuable resource for natural product discovery. Finally, analysis of demographic history revealed two genetic bottlenecks, resulting in rapid demographic declines of *A. spinulosa*. The *A. spinulosa* genome provides a unique reference for inferring the history of genetic diversity, secondary metabolite biosynthesis, and evolution of tree ferns, for better protection and application of tree ferns in the future.

Keywords: Tree fern, genome evolution, secondary metabolite, lignin, xylem

Theanine metabolism and transport in tea plants (*Camellia sinensis* L.)

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Theanine, a tea plant-specific non-proteinogenic amino acid, is the most abundant free amino acids in tea leaves. It is also one of the most important quality components of tea because it endows the “umami” taste, relaxation-promoting and many other health benefits of tea infusion. Its content in tea leaves is directly correlated with the quality and price of green tea. Theanine biosynthesis primarily occurs in roots and is transported to new shoots via the vascular system in tea plants. Recently, we made progresses in theanine metabolism and transport in tea plants. Along with the deciphering of the genomic sequences of tea plants, new genes in theanine metabolic pathway were discovered and functionally characterized. Transcription factors were identified to be critical regulators of theanine biosynthesis. Theanine transporters were also identified and were characterized on the affinity for theanine, substrate specificity, spatiotemporal expression, and the role in theanine root-to-shoot transport. The mechanisms underlying the regulation of theanine accumulation by cultivars, seasons, nutrients and environmental factors are also being rapidly uncovered. In this presentation, we will summarize our progresses in theanine biosynthesis, transport processes and the underlying regulation mechanisms. We will also discuss the future studies on theanine in tea plants, and application of the knowledge to crops to synthesize theanine to improve the health-promoting quality of non-tea crops.

Keywords: Theanine, biosynthesis, catabolism, transport, dynamic accumulation, regulation, *Camellia sinensis*

Epigenetic timer of age in giga-genome of Chinese pine

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Perennial woody plants usually have a long juvenile period, and it takes years or even multiple decades for some trees to enter the reproductive growth phase. There is considerable interest in how perennial trees reckon their growth times and developmental ages and enter the reproductive growth phase at a specific age. This is also a crucial issue for the breeding of trees with long juvenile period. Epigenetics has been revealed to play a crucial role in the long-term memory in plants. However, little is known about whether the epigenetic modifications occur with age progressively in plants especially long-living conifers. We presented the single-base resolution DNA methylation landscapes of the 25-gigabase Chinese pine (*Pinus tabuliformis*) genome at different ages, and revealed the global cytosine DNA methylation gradually increased as age progressed. Compared to genic regions and flanking regions (downstream and further upstream), CG and CHG methylation levels gradually increased more in proximal promoter regions near the transcription start sites as age increased. We scrutinized the single-base methylation levels of the gene bodies and flanking regions of previously identified age-related genes, which are typical ultra-long genes of conifers (200kb-500kb), in samples with different ages, the regular discrepancies of DNA methylation in multiple sites were observed to vary with age, especially at CHG context. Two regions, a 10.5 kb and a 6 kb segment, at the five-prime end of the first ultra-long intron in the DAL1, a known conservative age timer in conifers, showed a gradual reduction of CHG methylation as the age increased, which was highly correlated with its expression profile. Similar high correlation was also observed in nine of other age marker genes. These data provide insights into the roles of methylome in the gene transcription regulation and the formation of chronic memory of developmental stages and ages in long-living conifers.

Keywords: DNA methylation, age, gymnosperms, Chinese pine, giga-genome

Genetic diversity and domestication of cultivated tea plants

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Tea plant is an important economic crop, which is used to produce the world's oldest and most widely consumed tea beverages. Here, we present a high-quality reference genome assembly of the tea plant consisting of 15 pseudo-chromosomes. LTR retrotransposons (LTR-RTs) account for 70.38% of the genome, and we present evidence that LTR-RTs play critical roles in genome size expansion and the transcriptional diversification of tea plant genes through preferential insertion in promoter regions and introns. Genes, particularly those coding for terpene biosynthesis proteins, associated with tea aroma and stress resistance were significantly amplified through recent tandem duplications and exist as gene clusters in tea plant genome. Phylogenetic analysis of the sequences of 81 tea plant accessions with diverse origins revealed three well-differentiated tea plant populations, supporting the proposition for the southwest origin of the Chinese cultivated tea plant and its later spread to western Asia through introduction. Domestication and modern breeding left significant signatures on hundreds of genes in the tea plant genome, particularly those associated with tea quality and stress resistance. The genomic sequences of the reported reference and re-sequenced tea plant accessions provide valuable resources for future functional genomics study and molecular breeding of improved cultivars of tea plants.

Keywords: tea plant; comparative genomics; genome evolution; genetic diversity; domestication

Drought induces epitranscriptome changes in stem-differentiating xylem of *Populus trichocarpa*

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N^6-Methyladenosine (m^6A) in plants is the most prevalent RNA modification, which is implicated in regulating many aspects of gene regulation and development. However, there are no comprehensive methods to identify m^6A at single-base resolution for every single transcript, which is necessary for the estimation of m^6A abundance in *Populus trichocarpa* (*P. trichocarpa*). We develop a method for the identification and quantification of m^6A modification at single-base resolution using Nanopore direct RNA sequencing. We validate our method using methylated RNA immunoprecipitation sequencing (MeRIP-Seq) and m^6A-sensitive RNA-endoribonuclease–facilitated sequencing (m^6A-REF-seq), confirming high accuracy. Using this method, we provide a transcriptome-wide quantification of m^6A modification in stem-differentiating xylem under drought stress. Epitranscriptome analysis of cellulose- and lignin-related genes revealed an increased m^6A ratio, which was accompanied by decreased RNA abundance and translation, under drought stress. Interestingly, usage of the distal poly(A) site increased during drought stress. These findings provide insights into the potential interplay among m^6A and polyadenylation under drought stress in *P. trichocarpa* SDX.

Computational tools and infrastructure to improve forest health

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The emergence of improved sequencing technologies, coupled with decreasing costs, inspired innovative assembly methods for large and complex genomes, such as the conifer megagenomes. And plant genomes of all sizes, not just the megagenomes, present unique challenges associated with ploidy, heterozygosity, and repeat content. Although plant assemblies are increasing in contiguity, accurate structural annotation remains challenging. The accuracy of these products impacts estimates of genome duplication, gene family expansion/contraction, and functional assessments. Applications related to genomic selection, classification of hybrids, and pangenome approaches also require robust annotations. Gene space annotations are complicated by the presence of repetitive elements, large gene families, numerous pseudogenes, and long introns. Existing annotation packages are challenged to differentiate among these features and provide high quality results. Recent efforts have focused on improving strategies and we will present integrated approaches for functional and structural annotation, as well as the impact of misassemblies on downstream interpretations. We discuss the advantages and disadvantages of these methods as well as the downstream applications associated with improved assemblies and annotations. Our interest in open-source and reproducible software extends beyond a single reference genome to tools that support population-scale investigations. As such, we will introduce CartograPlant, the first web-based application that integrates genotype and phenotype data for model and non-model plant systems with global environmental layers. This field to analysis framework connects data collection, data submission, ontology-based metadata annotation, and analytics directly to high performance computing.

Keywords: genome assembly, genome annotation, pangenome, structural variants, repeats, forest health, cyberinfrastructure, TreeGenes, CartograPlant

Highly efficient C-to-T and A-to-G base editing in a Populus hybrid

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CRISPR-Cas based genome editing technology has been widely used in many plant species, including some tree species. However, in tree species, previous studies only focused on target indel mutations through Cas-based NHEJ system. Cytosine base editors (CBEs) and adenine base editors (ABEs) have been applied for introducing C-to-T and A-to-G base changes, respectively, to construct premature stop codons, make amino acid changes, alter RNA splicing sites and edit regulatory cis-elements in promoters. Despite promising functions, base editing systems have not been fully established in trees. In this study, two CBEs, PmCDA1-BE3 and A3A/Y130F-BE3, were applied to target two genes *4CL1* and *PII* at two gRNA sites. With PmCDA1-BE3, 26.3% and 78.9% editing frequencies were obtained for *4CL1*-sgRNA1 and *4CL1*-sgRNA2, respectively, lower than those by A3A/Y130F-BE3, 50.0% and 95.5%, respectively. For *PII* gene, A3A/Y130F-BE3 and PmCDA1-BE3 generated 19.0% and 0% editing frequencies at *PII*-sgRNA1 target site and 81.0% and 100% at *PII*-sgRNA2 target site, respectively. Additionally, A3A/Y130F-BE3 can edit a broader window from C5 to C18 on the protospacer while PmCDA1-BE3's editing window shifted slightly to the 5' end of the protospacer, from C2 to C13. Besides CBEs, two ABEs systems, ABEmax_V1 and ABEmax_V2, were also compared in poplar. Neither ABEs generated edited events with *4CL1*-sgRNA5 driven by the AtU6 promoter, while 84.2% and 95.5% editing frequencies were generated with *4CL1*-sgRNA5 driven by the AtU3 promoter in ABEmax_V1 and ABEmax_V2, respectively. Both ABEs have similar editing windows, with high frequencies at A7 and A9. Notably, there is low-frequency and zero indel byproduct mutations in CBEs and ABEs editing site, respectively, suggesting high base editing purity of both base editors. Taken together, 78.9%-100% editing efficiency were obtained from both CBEs and ABEs using AtU3 promoter driving sgRNA, which will provide promising application potential for precise genome editing in poplar and other trees.

Keywords: CRISPR-Cas9, C to T base editing, A to G base editing, poplar

Transcriptomic response to WPBR infection in whitebark pine seedlings

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Whitebark pine, *P. albicaulis* (Engelm), is a high-elevation, five-needle pine that dominates subalpine forests in western North America, and is severely affected by the exotic fungal pathogen *Cronartium ribicola* (white pine blister rust, WPBR). Infections in early developmental stages have led to high mortality in the species. We identified the early responses after fungal inoculations underlying the transcriptomic response and the diversity using the mRNA of six 2-years old half-sib seedlings under constant and controlled conditions of humidity.

We tested phenotypic divergence among non-inoculated and inoculated individuals. Transcriptomic differences were supported by 114 gene family interactions between inoculated seedlings with an important presence of membrane regulates and transport intermediates. Differential expression analyses were conducted to identify 83 Differentially-expressed (DE) genes that involve the physiological response at the immunity level and detect the initial phase of active responses to abiotic factors and stress regulators in potentially resistant individuals. The inoculated individuals showed active metabolic pathways to respond to stress and abiotic stimulus. Furthermore, we reconstructed a reference transcriptome that included 25, 875 high-quality protein annotations derived from the needles of six seedlings from a *de novo* transcriptome assembly. This will provide relevant information to annotate the whitebark pine reference genome, and a better understanding of the initial responses to biotic stimulus, and defense response in pine species.

Full length transcriptome sequencing and differential expression gene analysis of Hybrid Larch under PEG stress

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Larch is the main afforestation and timber tree species in Northeast China, and drought is one of the main factors limiting the growth of Larch and other organisms in Northeast China. In order to further explore the mechanism of Larch drought resistance, PEG was used to simulate drought stress. The full-length sequencing of Larch embryogenic callus under PEG simulated drought stress was carried out by combining Illumina-Hiseq and SMRT-seq. A total of 20.3Gb clean reads and 786492 CCS reads were obtained from the second and third generation sequencing. The de-redundant transcript sequences were predicted by lncRNA, 2083 lncRNAs were obtained, and the target genes were predicted, and a total of 2712 target genes were obtained. The de-redundant transcripts were further screened and 1654 differentially expressed genes (DEGs)were obtained. Among them, different DEGs respond to drought stress through different ways, such as Oxidation-reduction process, Starch and sucrose metabolism, Plant hormone pathway, Carbon metabolism, lignin catabolic/biosynthetic process and so on. This study provides basic full-length sequencing data for the study of larch drought resistance, and excavates a large number of DEGs in response to drought stress, which helps us to further understand the function of Larch drought resistance genes and provides a reference for in-depth analysis of the molecular mechanism of Larch drought resistance.

Keywords: Larch, drought stress, full-length transcriptome sequencing, differentially expressed genes

A method for determining genome editing patterns in T_0 generation tree using Oxford Nanopore Technologies sequencing

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Genome editing technology has been increasingly applied to trees as a method for modifying targeted genes. We have achieved targeted mutagenesis using the CRISPR/Cas9 system in softwood and hardwood trees, such as Japanese cedar (*Cryptomeria japonica*) and hybrid aspen (*Populus tremula* × *Populus tremuloides*). Generally, gene-edited T_0 generation trees are subsequently used for further analyses because of a long crossing time to generate homozygous/biallelic mutants. When using T_0 generation plants, there are two points to be noted before subsequent biological analyses. The first is to determine whether T_0 generation events retain the expected mutation pattern in both alleles. The second is to ensure that the T_0 generation events are not chimeric. T_0 select biallelic- and nonchimeric-events, Sanger sequencing and/or next-generation sequencing (NGS) are commonly used for the determination of the mutation pattern of both alleles in the candidate events. In NGS, Oxford Nanopore Technologies (ONT) provides a cost-effective long read sequencer with a small device, while ONT sequencing shows higher-error-rate data compared to short-read sequencing (e.g. Illumina NovaSeq, MGI DNBseq, etc). In this study, we establish a method to rapidly and accurately determine DNA mutation patterns using ONT sequencing, considering its drawbacks.

As a case study, we generated gene-edited Japanese cedar events, in which magnesium chelatase subunit I (*CjChlI*) was disrupted by the CRISPR/Cas9 system. CjChlI is required for chlorophyll biosynthesis. The DNA mutation patterns were analyzed in two steps; the first step was to identify nucleotide insertion/deletion using Indel Detection by Amplicon Analysis (IDAA), and the second step was to determine mutation patterns of both alleles using ONT sequencing. We will discuss the benefits and issues to be considered in the analysis method using ONT sequencing.

Keywords: genome editing, Oxford Nanopore Technologies sequencing, indel detection by amplicon analysis, *Cryptomeria japonica*

A comparative genomics approach to assess interspecific variability associated with cork development

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Cork oak (*Quercus suber*) is a unique and emblematic resource in the Mediterranean region, with high economical, ecological and social significance in Portugal. While in most plant species the life span of the phellogen is limited, in cork oak it remains active throughout trees life cycle, being fully regenerated after harvest. Phellogen activity in *Q. suber* remains unknown if compared to phellem development in other woody plants. The main goal of this project is to elucidate the evolution of cork oak genome and the genetic elements regulating phellem development and suberin biosynthesis through an integrative analysis conducted using comparative genomics and synteny-guided resolution of gene trees. To explore of the evolution of genetic pathways related to cork formation we initiated a comparative genomic analysis using *Quercus* spp. (*Q. suber*, *Q. robur*, *Q. lobata*, *Q. mongolica* and *Q. rubra*) and other *Fagaceae* (*Fagus sylvatica* and *Castanea mollissima*). We performed a phylogenetic orthology inference of all the protein sequences predicted in these genomes with Orthofinder using DIAMOND algorithm, resulting in 94.3 % of genes being assigned to orthogroups (candidate gene families), of which 14.9% assigned to species-specific orthogroups. A rooted species tree based on this comparative analysis was obtained using the STAG and STRIDE algorithms, which represented the evolution and speciation processes predicted for the *Quercus* genus. We found 937 well-supported gene duplication events for the common ancestor of the *Quercus* genus, some occurring in orthogroups related to suberin synthesis. The biological relevance of orthogroups with specific variability found in *Q. suber* will be further explored by on functional annotations and transcriptomic datasets available for *Q. suber*. This comparative genomic analysis will be the basis for future synteny and collinearity analysis, to further look for inter and intraspecific features (gene content and order) in cork oak and related species genomes.

Keywords: Cork oak, comparative genomics, bioinformatics, suberin

DNA methylation differences between embryogenic and non-embryogenic callus of *Abies nephrolepis*

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Abies nephrolepis (Trautv. ex Maxim.) Maxim. is an evergreen coniferous tree that inhabits subalpine zone. It is susceptible to threats of habitat loss and population decline in South Korea. Somatic embryogenesis is an asexual reproduction process through somatic cell's dedifferentiation to embryogenic callus, and redifferentiation to somatic embryo. It has advantages as morphological and molecular studies of embryogenesis, as well as mass propagation. Somatic embryogenesis studies usually conducted to find optimal conditions for induction, and underlying mechanisms at molecular level, such as embryogenic-competence acquisition, were rarely understood. This study aimed to compare DNA methylation states between embryogenic callus (EC) and non-embryogenic callus (NEC) of *A. nephrolepis*. Zygotic embryos were used as explants, and inoculated to EC induction medium. Induced EC were continuously subcultured, and non-induced ones were subcultured to 5-azacytidine treated medium. 5 sets (each set consists of EC and NEC that derived from same explant) were selected to analyze. MSAP-seq, a technique that uses methylation sensitive restriction enzyme combined with NGS technology, was used to analyze differentially methylated site (DMS), and genes were annotated by BLASTn in DMS. As a result, each five sets of EC and NEC shared average 23% hypomethylated CG methylation region, and remaining 77% showed differentially methylated status. In DMS region, 4 types of genes were annotated as embryogenesis-related genes; stress, cell division and structure, transcription, and kinase related genes. In this study, transition from NEC to EC was observed by the treatment of DNA methylation inhibitor 5-azacytidine. Also, EC and NEC that share the same DNA, but differ in embryogenic competence showed DNA methylation differences, indicating that the acquisition of embryogenic competence and DNA methylation are interrelated. Based on DMS sequences screened in this study, further studies on noncoding sequence methylation and multi-omics approach will enhance understanding of plant regeneration mechanisms.

Keywords: *Abies nephrolepis*, embryogenic callus, plant regeneration, epigenetics, DNA methylation

Genome-wide characterization of plant resistance genes in cork oak (*Quercus suber*)

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Cork oak (*Quercus suber* L.) forests are unique and emblematic resources for Portugal, but the viability of the cork production sector is being challenged by frequent tree dieback events. While cork oak suffers from few major disease problems, its decline is controlled by a combination of several factors, including drought as main driver, and biotic stress which often affects weakened trees. Therefore, it is important to identify the molecular mechanisms behind the tree's native resistance against pathogens as a path towards the resolution of this problem. Nuclear-binding Leucine Rich Repeats (NLRs) are a large gene family that participates in the detection of pathogen effectors and activation of molecular pathways resulting in a hypersensitive response. NLRs have a conserved domain named NB-ARC, often used in phylogenetic studies. Interestingly, specific NLRs (e.g. the ADR1 family) have also been implicated in plant adaptation to drought. This project aims to characterize the NLR family in cork oak and further identify the most relevant players in organ-specific contexts and in response to relevant pathogens and drought.

DRAGO2 – a tool for disease resistance gene search based on sequence homology - and Interproscan - for detecting conserved protein domains - were used for genome-wide identifications of NLRs in 3 Quercus sp. Phylogenetic analysis of NB-ARC revealed no significant difference on evolution between Quercus suber and other Quercus.

Using the aminoacid sequence of reference Arabidopsis NLRs with known function, both the phylogenetic analysis and orthologue identification allowed the a preliminary functional assessment of cork oak NLRs. We also surveyed available transcriptomic data of cork oak subjected to drought conditions, and identified three NLRs that could work against both pathogen invasion and drought conditions. In the future, in silico analysis of promoters of those NLRs and expression validation in controlled assays will validate those NLRs with this double function.

Keywords: R-gene, NLR, pathogen, drought, orthologue

Differential gene expression-based propagule selection for commercial multiplication in *Casuarina junghuhniana* Miq clone ITC 1761

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Casuarina junghuhniana (CJ) clones are widely cultivated for timber, pulp and fuel in south & south east Asia agrarian ecosystems. In India, popular clones such as ITC 1761, CJ9, CH3, CH5 are mainly grown for pulp. CJ clones are primary multiplied through stem cuttings, while the impact of type of cuttings has been unknown. In the present study, we appraise various type of cuttings including apical, nodal and lateral through transcriptome studies and their respective field performance. We also demonstrate the field performance of apical cuttings with 10-15% enhanced growth in the 4 years grown trees, compared to lateral and nodal cuttings. In the transcriptome analysis, we identified 8,900 differentially expressed genes (DEGs) during stem formation, of which there were 4500 upregulated and 4400 downregulated genes between apical, lateral and nodal stem cuttings. Of these, approximately 100 candidate DEGs involved in the auxin-induced pathway. There were 20 auxin responsive genes (ARFs and SAURs), 50 transcription factors (LOB domain-containing protein (LBDs), and 10 auxin transporters (AUX, LAX and PIN-formed (PIN), ATP binding cassette subfamily (ABCB/MDR/PGP)). All these identified DEGs were highly upregulated in apical stem cuttings, indicating their potential roles in better performance of apical cuttings in the field. The biological reasoning & field performance will be presented including the commercial deployment model.

Key Words: *Casuarina junghuhniana,* Differentially expressed genes (DEGs), Auxin-induced pathway, Apical stem cuttings.

Evidence of conserved regulation of the miR160:*ARF18* node during embryo development in conifers

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MiRNAs regulate gene expression mostly post-transcriptionally by leading the cleavage of their complementary target transcripts and/or their translational repression, thereby acting as major regulators during plant development. Somatic embryogenesis (SE) is often the system of choice to study the molecular regulation of plant embryogenesis. In conifers, a differentially expressed miR160 isoform (ppi-miR160) and its predicted target, annotated as *AUXIN RESPONSE FACTOR 18* (*ARF18*), have emerged as potential regulators of embryo development based on a previously published small non-coding RNA transcriptome of *Pinus pinaster* embryos [(1)]. However, the role of specific miRNAs in the regulation of genes essential for conifers SE, such as those encoding the ARF transcription factors, remains mostly unknown. The aim of this study was to characterize the function of ppi-miR160, targeting *ARF18*, based on their respective expression patterns using a conifer *in vitro* embryogenesis model system. As a first step of the characterization of the ppi-miR160-*ARF18* pair, we used a transient expression system in *Arabidopsi*s mesophyll protoplasts for overexpressing the selected miRNA isoform and target mimics to manipulate miRNA levels. Using luciferase reporters for quantifying miRNA activity allowed us to confirm an *in vivo* interaction between ppi-miR160 isoform and the predicted target transcript homologous to Arabidopsis *ARF10*/*ARF16*. Our results in SE of *Picea abies,* used as a conifer model*,* revealed an opposite expression pattern between miR160 and *ARF18* during the same embryo development period, further supporting the interaction between the selected miRNA-target pair. Moreover, the disturbance of auxin homeostasis during embryo development led to differences in the expression profiles of the miR160:*ARF18* pair. Our study reports the first *in vivo* validation of a gymnosperm miRNA with its predicted target, supporting the conservation of the interaction between miR160 and *ARF* transcripts in conifers, and reinforcing their regulator functions during embryo development.

Keywords: auxin, embryogenesis, gymnosperm, miRNA, *Pinus pinaster*

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Unravelling the role of DNA methylation in cork formation

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The periderm protects plants from adverse conditions and is made up of cork cells produced by phellogen. The cork oak phellogen (*Quercus suber*) produces an extraordinarily thick periderm that can be sustainably harvested at 9-years interval. The cork mass is interrupted by lenticular channels, the cork porosity, which, at high levels, strongly depreciates the quality of cork.

The factors that determine the quality of cork are unknown, however, recent studies have shown an association between epigenetics and phenotypes directly linked to phellogen activity: variations in DNA methylation are associated with cork contrasting phenotypes directly linked to phellogen activity[1]; opposite global DNA methylation levels are observed in corks with contrasting phenotypes (higher/lower porosity)[2]; DNA methyltransferases genes expression is correlated with cork traits[3]; and *de novo* DNA methyltransferase gene is highly expressed during cork formation[2]. Due to constraints in using cork oak for functional studies via genetically modified plants, we are testing the hypothesis of a causal relationship between DNA methylation and cork phenotypic variability using potato tuber periderm as a model. To generate loss-of-function mutants of DNA methyltransferase genes specifically in the phellogen, we are using an inducible genome editing system (IGE) that combines CRISPR/Cas9 with a cell-type-specific estrogen inducible system[4]. The allelic composition of *MET1*, *DRM1*, *DRM2*, *CMT2*, and *CMT3* potato genes was determined to design two specific guide RNA spacers targeting the four alleles for each gene. Constructs targeting *DRM1*, *CMT2*, and *CMT3* genes were generated using Golden Gate® and Multisite Gateway® cloning. The IGE construct targeting *CMT3* gene has already been transferred into potato leaves and internodes via *Agrobacterium tumefaciens* to be induced in microtubers from transformed plant lines. The remaining constructs are being prepared for further transformation experiments. Phenotyping of the generated mutants will elucidate on the relevance of DNA methylation in the cork formation process.

Keywords: epigenetics, phellogen, cork quality, cork oak, CRISPR-Cas9

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Mining and development of EST-microsatellite markers in *Leucaena* species

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Leucaena, commonly known as Subabul, is an important multi-purpose tree species used as industrial pulpwood in India, due to its desirable wood properties and other uses. Among 32 classified species, *L. leucocephala* and *L. diversifolia* are the prominent ones used for commercial cultivation and tree improvement programs. Evolutionary mechanisms of the genus *Leucaena* has remained complex with many polyploidy and interspecific hybridization events. With narrowly differentiated morphological variations, identification of different varieties within species and sub-species is challenging. Molecular characterization using microsatellite markers is an efficient and reliable method for identification and assessment of genetic diversity. Simple sequence repeats mined from expressed sequence tags (ESTSSRs) are potentially valuable source of markers as they are rapid and inexpensive in development, and have high cross-species and cross-taxa transferability rate. In this study, we collected 1768 sequences of both nucleotide and EST-sequences reported across twenty-five *Leucaena* species in NCBI database, and mined for the development of SSRs. Using computational tools, the sequences were checked for redundancy and 139 sequences were trimmed. Of 1629 non-redundant sequences used for repeat mining, 726 sequences had repeats and with a total of 715 repeats, with 58 di-, 417 tri-, 166 tetra-, 66 penta- and 8 hexa-repeats. About 574 primer pairs were designed with a maximum of 172 primers in *L. leucocephala* species. Two hundred primers synthesized and were validated by testing in two selected commercial clones of *L. leucocephala*, with 60 to 70% success rate. These new EST-SSRs will help further in marker assisted selection of species including molecular characterization, genetic diversity, DNA fingerprinting of clones and hybrids, and studies on evolutionary relationships in *Leucaena*.

Keywords: *Leucaena*, Molecular characterization, Expressed sequence tags, Simple sequence repeats, Genetic diversity.

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Development of novel genome-wide microsatellites by next generation sequencing in two species of Corymbia

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The advent of research in developing Corymbia has gained importance recently, as the species offers superior pulpwood traits, making it as one of the most suitable hardwood species for paper industries. In molecular tree breeding, microsatellites or simple sequence repeats (SSRs) have become indispensable due to their valuable attributes as they are highly polymorphic, reproducible, multiallelic, co-dominant, ubiquitous and are easily transferable across species. Despite of recent progress in Corymbia genome sequencing efforts, the availability of usable SSRs is limited in Corymbia. The development of new SSRs by conventional methods expensive and time-consuming, but the rapid advancements in sequencing technology has made it cheaper and efficient. In this study, we carried out large-scale development of genomic SSRs in two species of Corymbia, *citriodora* and *torelliana* using next generation sequencing (NGS) by 454 genome sequencing system. Microsatellite repeat-rich sequences were captured separately by enrichment technique using high quality genomic DNA of both species. The NGS with 10x coverage yielded nearly 2 lakh reads, with 30-31 % of reads were assembled in both species using MIRA and CAP3 assembly tools. Citrodora had higher number of contigs (96643) than *torelliana* (69626) with an average contig length of 619 and 464 respectively. Except di-nucleotide repeats, other repeat types were higher in *C. citriodora*, and in contrast, the number of SSR-containing sequences and compound-SSRs types were higher in *C. torelliana*. Among di-and tri- repeats, AG/CT and ACC/GGT were predominant by 7 to 15 times in both species. Nearly 3000 unique and common SSR primers were designed by sorting SSR-containing sequences from both species using i-assembler and primer designing tools. A set of 200 primers were label-synthesized for multiplexing purpose, and validated in both the species with 85% success rate. These novel microsatellite markers offer as handy tools in any marker based selection and deployment in Corymbia breeding programs.

Keywords: *Corymbia citriodora*; *Corymbia torelliana*; Microsatellite markers; NGS technology; Genome sequence

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Populus cathayana genome and population resequencing reveal local adaptation to high elevation

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Populus cathayana is widely distributed across a high elevation range in China and an indigenous poplar species with ecological services and economic value. Exploration of its genomic variation and molecular underpinning of adaptive differentiation is important for elucidating population evolution and development of breeding resource. Here, a high-quality genome assembly for *P. cathayana* species as well as genome resequencing provide insights into its genetic diversity, population structure and local adaptation. Population genomic analysis based on resequencing of 438 *P. cathayana* accessions classified natural population into four geographical groups. Selective sweeps and genome-wide environmental association analysis identified the putative candidate genes associated with local adaptation in *P. cathayana*, especially DNA repair and UV response genes were enriched to help species adaptation to high altitude. We detected 657 environmental association loci (EALs) associated with local adaptation which are correlated with geographic variables and solar radiation, temperature and water variables across heterogeneous environmental scenarios. This study expands our understanding of evolutionary history, population differentiation and environmental adaptation of *P. cathayana*, which would facilitate poplar breeding and efficient utilization of genetic resources.

Keywords: *Populus cathayana*, population genomics, genetic structure, local adaptation

Identification and expression analysis of AP2/ERF superfamily in Pecan (*Carya illinoensis*)

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AP2/ERF is a large transcription factors superfamily whose members are participated in the control of plant development, metabolism and response to various biotic and abiotic stresses. Nevertheless, few studies have been reported in pecan. In this study, a total of 170 *CiAP2/ERF* members were identified and divided into 4 main subfamilies, AP2 (16 genes), RAV (6 genes), Soloist (1 gene), as well as ERF (147 genes) families, and ERF subfamily was further classified into group I to VI-L. Gene structures and conserved motifs indicated that individual groups were highly conservative. A large number of cis-elements such as Box 4, ABRE, and MYB were detected in the promoter region of *CiAP2/ERF* genes. Transcriptional levels in four tissues were analyzed using available transcriptome data. qRT-PCR analysis of the ERF VII group revealed that the subfamily had a positive response to waterlogging stress. The subcellular localization experiments on CiAP2/ERF21, CiAP2/ERF65, and CiAP2/ERF106 manifested that they were localized in nuclear. Additionally, CiAP2/ERF65 and CiAP2/ERF106 had the ability of self-activation in yeast, while the CiAP2/ERF21 gene did not. These results would conduce to understanding the AP2/ERF superfamily and functional roles of CiAP2/ERF TFs under different stresses.

Keywords: *Carya illinoensis*; AP2/ERF; Phylogenetic analysis; Waterlogging stress

Alternative polyadenylation in four species of the Tabebuia Alliance under drought stress

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Alternative polyadenylation (APA) can generate alternative transcripts, regulating gene expression in response to biotic/abiotic stresses in plants. However, in forest species, this mechanism remains poorly understood. We used RNA-Seq and the APAtrap tool to identify genes with significant differential use of polyadenylation sites (DE-APA) in four ipe species (*Tabebuia aurea, Handroanthus ochraceus, Handroanthus impetiginosus and Handroanthus serratifolius*) under drought stress. *H. ochraceus* was the specie that presented the highest number of DE-APA genes (3,530). In contrast, *H. impetiginosus* showed only 120 DE-APA. *H. serratifolius* and *T. aurea* showed similar numbers of DE-APA genes: 368 and 369 respectively. *H. ochraceus* shared 102 and 103 DE-APA with *T. aurea* and *H. serratifolius*, respectively. No DE-APA gene was shared among all four species. DE-APA genes were classified as those affecting coding region (CR-APA) and those in 3'UTR (UTR-APA). *H. impetiginosus* presented 61 UTR-APA and 59 CR-APA; *H. serratifolius* had 197 CR-APA and 171 UTR-APA; *T. aurea* showed 228 CR-APA and 141 UTR-APA; finally, *H. ochraceus* presented 1,089 CR-APA and 2,441 UTR-APA. Regarding the UTR-APA, only *H. ochraceus* had a significant association between 3'UTR length and gene expression levels. In this species, among genes with shorter 3'UTR (proximal APA), 59% were up-regulated, while 62,5% of UTR-APA genes with longer 3'UTR (distal APA) were down-regulated. Furthermore, in the analysis of differentially expressed genes (DEG), *H. ochraceus* presented only eight DEG, while the other three species presented more than 200. As for the Gene Ontology (GO) analysis of DE-APA, only *H. ochraceus* presented enriched categories. These GO enriched categories were involved in transcriptional regulatory pathways, transport/localization of proteins and catabolic processes of macromolecules. These results indicate that response to drought stress may be regulate by APA in ipe species.

Keywords: Alternative polyadenylation; alternative transcripts; Tabebuia Alliance; Drought Stress.

Genome-wide identification and expression analysis of ethylene responsive factor family transcription factors in *Juglans regia*

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[Background] Walnut is an important economic tree species with prominent economic value and ecological functions. However, in recent years, walnuts have become susceptible to drought stress, resulting in a decline in comprehensive benefits. Ethylene responsive factor (ERF) gene family plays important roles in response to stresses, especial drought. Therefore, the identification and characterisation of walnut ERF genes will benefit walnut with regard to the clarification of drought response mechanism as well as the management, production, and quality of plantations.

[Methods] 'ERF' was compared against the walnut transcriptome, The distribution of *JrERFs* in chromosome locations was determined based on walnut genome data from NCBI. The intron-exon structures and conserved domains were analysed using Gene Structure Display Server 2.0 and CD-Search, accordingly. Multi-sequence alignment and a phylogenetic tree were constructed by ClustalX2.1 and MEGA7, respectively. The conserved motifs were acquired using MEME. Total RNA was isolated using the cetyltrimethylammonium ammonium bromide (CTAB) method. Gene expression was determined by using real-time quantitative polymerase chain reaction (qRT-PCR) analysis and calculated according to the $2^{-\Delta\Delta CT}$ method.

[Results] A total of 44 *JrERFs* were identified from the walnut transcriptome, The JrERFs can be divided into six groups (B1–B6), each JrERF contained 1–6 motifs and each motif comprised 9–50 amino acids. Among the motifs, motif1, motif2, and motif3 were the most abundant. More than 40% of *JrERFs* were up-regulated continuously when subjected to ethephon (ETH), PEG_{6000}, and PEG_{6000}+ETH treatments. Of all the *JrERFs*, *JrERF11* showed the highest expression. Therefore, we conclude that walnut ERF genes are highly conserved and involved in the regulation of drought response in the presence of ETH. *JrERFs* are possibly important candidate genes for molecular breeding; hence, the findings of this study provides the theoretical basis for further investigation of ERF genes in walnut and other species.

Keywords: *Juglans regia*, Ethylene response factor, Bioinformatics, Expression analysis

Genome-Wide Identification and Characterization of Long Noncoding RNAs in *Populus*×*canescens* Roots Treated with Different Nitrogen Fertilization

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Nitrate (NO_3^-) and ammonium (NH_4^+) are the primary forms of inorganic nitrogen acquired by plant roots. LncRNAs, as key regulators of gene expression, are a class of noncoding RNAs larger than 200 bp. However, knowledge about the regulatory role of lncRNAs response to different nitrogen forms remains limited, particularly in woody plants. Here, we performed strand-specific RNA sequencing of *P.* × *canescens* roots treated with three different nitrogen fertilization treatments. In total, 324 lncRNAs and 6112 mRNAs were identified as showing significantly differential expression between the NO_3^- and NH_4NO_3 treatments. Moreover, 333 lncRNAs and 6007 mRNAs showed significant differential expression patterns between the NH_4^+ and NH_4NO_3 treatments. Further analysis suggested that these lncRNAs and mRNAs have different response mechanisms for different nitrogen forms. In addition, functional annotation of *cis* and *trans* target mRNAs of differentially expressed lncRNAs indicated that 60 lncRNAs corresponding to 49 differentially expressed *cis* and *trans* target mRNAs were involved in plant nitrogen metabolism and amino acid biosynthesis and metabolism. Furthermore, 42 lncRNAs were identified as putative precursors of 63 miRNAs, and 28 differentially expressed lncRNAs were potential endogenous target mimics targeted by 96 miRNAs. Moreover, ceRNA regulation networks were constructed. MSTRG.6097.1, MSTRG.13550.1, MSTRG.2693.1 and MSTRG.12899.1, as hub lncRNAs in the ceRNA networks, are potential candidate lncRNAs for studying the regulatory mechanism in poplar roots under treatment with different nitrogen fertilization treatments. The results provide a basis for obtaining insight into the molecular mechanisms of lncRNA responses to different nitrogen forms in woody plants.

Keywords: nitrate, ammonium, *Populus* × *canescens*, roots, lncRNAs, ceRNAs

Engineering lignin to improve biomass processing: what have we learned?

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Lignin is an aromatic heteropolymer that is deposited in cell walls of secondary-thickened cells. It provides rigidity to the cell walls allowing plants to resist gravitational forces, and it provides water conducting cells their imperviousness. For certain industrial applications, such as pulp and paper production, or biorefining of lignocellulose to fermentable sugars, lignin is a limiting factor. This has motivated research towards understanding the biosynthesis and polymerization of lignin, and how lignin amount and composition can be modified to improve the efficiency of biomass processing. Lignin is known for decades to be composed of *p*-coumaryl, coniferyl and sinapyl alcohol, but more recent research has demonstrated that other monomers can be incorporated in the polymer to various extents, and genes and enzymes playing a role in the biosynthesis of these new monomers have been identified. This has allowed engineering lignin amount and composition, resulting in large increases in biomass processing efficiency. It is as well possible to engineer completely new lignin structures by expressing exotic genes that vouch for the biosynthesis of lignin-monomer-like compounds. In several cases, the alterations in lignin are associated with a biomass yield penalty, motiving the development of new strategies that avoid the yield penalty while maintaining the improved processing efficiencies. A limited number of transgenic trees with altered lignin and improved biomass processing have been planted in experimental field trials, some for multiple rotations and at multiple locations. The conclusions that can be drawn from these studies will be discussed.

Keywords: lignin, field trials, wood, biorefining

Role of 4-*O*-Me-GlcA decorations on xylan in wood cell walls and in lignocellulose processing

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Wood cell walls are the most important source of renewable biomass on Earth [1] but their architecture at the fine scale is still not fully understood to allow development of technologies aiming at full exploitation of this resource. Xylose yield from *Arabidopsis* stem is increased when xylan glucuronosylation is reduced by mutations in *GUX1* and *GUX2* genes [2]. It is thought that xylan 4-*O*-methyl-glucuronic acid (Me-GlcA) can form γ-ester linkages to lignin, contributing to the formation of lignin-carbohydrate complexes (LCCs) and recalcitrance to bioprocessing. Thus, the removal of Me-GlcA side chains from xylan or removal of their ester links to lignin might be a good strategy to increase saccharification sugar yields in woody plants. To these goals, we used *Aspergillus niger* α-glucuronidase from family GH67 (*An*Agu67A) and *Phanerochaete carnosa* Burt glucuronosyl esterase from family CE15 (*Pc*GCE). The enzymes were expressed in in hybrid aspen (*Populus tremula* L. x *tremuloides* Michx.) and targeted to the apoplast. The growth of transgenic plants expressing these enzymes depended on the promoter used for expression. The 35S promoter induced mild (*AnGH67A*) or severe (*PcGCE*) growth reduction, whereas using the wood promoter [3] avoided all negative effects on development. *AnGH67A* reduced Me-GlcA content of biomass without affecting other cell wall components. Despite a simplified xylan structure, the lignocellulose sugar yields of saccharification with and without acid pretreatment were not improved. In contrast, *WP:PcGCE* improved sugar yields in saccharification without pretreatment, reduced Klason lignin content and increased Updegraff cellulose content. The two enzymes apparently differentially affected γ-ester linkages to lignin and unknown linkages to aliphatic suberin-like compounds. The novel role of Me-GlcA decorations is proposed to be the fastening of these aliphatic suberin-like compounds to cell wall.

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Keywords: biofuels, glucuronoxylan, LCCs, secondary cell wall

Molecular ecophysiology of wood formation under drought

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Woody biomass can substitute many oil-based products because of its multiple usages, for example, as construction material, as raw material for added-value wood products, as feedstock for the chemical industry and as a resource for energy generation. The demand for sustainable production of renewable resources is increasing. Since trees are generally grown on nutrient-limited sites, fertilization can stimulate biomass production but goes along with higher water demand and increased vessel lumina. On the contrary, climate change with increasing periods of drought limits productivity and enhances the risk of mortality for trees species with high water demand. Drought acclimation of trees involves structural changes in wood anatomy with thicker cell walls and smaller vessel lumina to avoid cavitation. Consequently, wood of drought-stressed trees exhibits higher densities than that of well-watered trees. While the ecophysiological responses to drought have been well characterized, the time course of the molecular responses to gradually increasing stress across different tissues that eventually lead to changes in wood anatomy have barely been studied. Here, we show results of poplar ecophysiological, anatomical and molecular acclimation to moderate, gradually increasing drought stress. We uncovered divergent, tissue-specific networks that were correlated with hormonal changes in roots, stem and leaves. To unravel a potential role of abscisic acid, we analyzed a range of transgenic poplar plants with modification in ABA signal perception and response. Our results provide insights into the genomics and ecophysiology of wood formation, which are an essential basis securing sustainable wood production in a future climate.

Keywords: Poplar, ABA, stress, Xylem

Physiological and Molecular Responses of *Populus alba* × *P. glandulosa* to Changes in Light Intensity and Nitrogen Availability

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To investigate physiological and molecular mechanisms of poplars in acclimation to changes in light intensity in combination with N availability, we used saplings of *Populus alba* × *P. glandulosa* treated with either high or control light intensities combined with one of low, normal and high N levels. High light stimulated CO_2 assimilation rate (*A*), xylose concentrations, and led to reduced thickness of fiber double wall and fiber luminar diameter of poplars under normal N condition in comparison with control light. High N enhanced *A*, stem diameter, concentrations of total N and abscissic acid, and resulted in decreased lengths of vessel elements and fibers, whereas low N brought about decreased *A*, higher concentrations of mannose in the wood, and increased lengths of vessel elements and fibers under both light conditions in comparison with normal N. High light and/or changes in N availability led to up- and down-regulation of a number of genes involved in metabolic pathways of carbohydrates, amino acids, and precursors of hemicellulose and other compounds. Particularly, mannose is a key precursor of hemicellulose biosynthesis, which was markedly increased in poplar wood by low N. We found that *cellulose-like synthase 2* (*CSLA2*) was highly likely involved in mannose biosynthesis in poplars. *PagCSLA2*-overexpressing poplars enhanced heights, but decreased stem diameters in comparison with WT supplied with three N levels. High N led to enhanced heights, internodes and stem diameters, and low N resulted in the opposite effects in comparison with normal N. The mannose concentrations were also altered in *PagCSLA2*-overexpressing and RNAi poplars compared to that in WT plants. These results suggest that high light and N availability brought about significantly differentially expressed genes in the cambium, probably contributing to the changes in physiology and wood properties, and that *PagCSLA2* regulates mannose biosynthesis, affecting poplar acclimation to N availability.

Keywords: Poplar, mannose, cellulose-like synthase, physiology, wood quality

Receptor-like kinase SOBIR1/EVR regulates wood development by preventing premature xylem fibers differentiation in *Arabidopsis thaliana*

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Secondary growth brings about wood, the largest portion of terrestrial biomass. Mediated by the vascular cambium, this thickening of stems and roots is an essential feature of tree growth, conferring mechanical support to the plant body and its growing structures, while transporting nutrients and water for plant growth. However, the genetic and molecular mechanisms underlying wood formation and development are still scarcely known. This is mainly because the use of classical genetics is hindered by the challenge of trees long-life cycles and the difficulty in phenotyping traits in the innermost tissues of trees. Taking advantage of the natural variation of secondary growth we performed genome-wide association studies (GWAS)-guided reverse genetics in *Arabidopsis thaliana* to discover new regulators of secondary growth. We identified LRR-RLK EVERSHED (EVR), also named SUPPRESSOR OF BIR-1 (SOBIR1) as a regulator of xylem differentiation. We further investigated the mechanism by which SOBIR1/EVR operates in xylem fiber development and have involved the previously described master regulators BREVIPEDICELLUS (BP) and ERECTA (ER) in this process. We demonstrated that BP binds SOBIR1/EVR promoter and that *SOBIR1/EVR* expression is enhanced in *bp* mutants, suggesting a direct, negative regulation of BP over *SOBIR1/EVR* expression. We also show that SOBIR1/EVR physically interacts with ER and that defects caused by the *sobir1/evr* mutation are aggravated by mutating ER, indicating that SOBIR1/EVR and ERECTA act together in the control of the precocious formation of xylem fiber development. We show the anatomical, genetic, and molecular evidence indicating that SOBIR1/EVR prevents the differentiation of xylem fibers, a key cell type for wood development.

Keywords: Wood, xylem fibers, GWAS, *Arabidopsis thaliana*, *SOBIR1*

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Biomechanics of xylem/woody vessel elements induced ectopically by the VND7 transcriptional switch in Arabidopsis

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Secondary cell wall (SCW) of xylem/woody cells is a main component in maintaining the mechanical strength and rigidity of vascular plants, including trees. We have been studying the structure and biomechanics of xylem/woody vessel elements induced artificially and ectopically by activation of the NAC transcription factor VND7. Using a model culture system in which Arabidopsis T87 cells are differentiated into vessel elements by dexamethasone (DEX) treatment that activates VND7, the mechanical properties of differentiated/differentiating cells were investigated using a multi-scale approach. Large-scale indentation with a micro-compression system under three different osmotic conditions and nanoscale indentation with atomic force microscopy (AFM) in water allowed us to isolate the cell wall response. We propose a spring-based model to deconvolve the competing stiffness contributions from turgor pressure, primary cell wall (PCW), SCW, and cytoplasm in the stiffness of differentiating cells. In addition, we analyzed mechanical properties of ectopically induced vessel elements in Arabidopsis seedlings using AFM. These data provided us the first experimental characterization of the biomechanics of SCW-formation single cells.

Keywords: plant biomechanics, micro-compression, AFM, Arabidopsis thaliana, xylem/woody vessel cells

Advances in The Valorization of Tree Cell Wall Lignin

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Woody feedstocks, such as poplar, willow, and eucalyptus, represent abundant and fast-growing sources of lignocellulosic biomass for use in the production of fibre, biofuels, and other bio-based materials. Lignin, the second most abundant biopolymer, forms a major constituent of the secondary cell walls of terrestrial plants. It has been described as being largely derived from three cinnamyl alcohol derivatives, *p*-coumaryl, coniferyl and sinapyl alcohols, that differ only in the degree of methoxylation on the aromatic ring. However, in recent year, it has been shown that the classical dogma describing lignin chemistry, structure, and composition may be an oversimplification, as several recently described lignins have been shown to naturally incorporate a wide array of non-traditional monolignols. These observations clearly illustrate the inherent plasticity of lignification and provide the impetus for the formal 'design' of lignins. To that end, the introduction of monolignol conjugates into the lignin polymer offers an exciting opportunity to introduce unique bonds and novel pendant groups that valorize lignin, or chemical constituents that alter the size and charge of this biologically and industrially important macromolecule. We have attempted and successfully introduced a variety of such conjugates into the lignin of poplar trees, ultimately producing lignins that are easier to extract and/or generating a stream of highly valuable phenolics that form the basis for chemical or biological upgrading.

Keywords: Lignin, pendant groups, acylation, phenolics, flavonoids, monolignol conjugates

Field-grown transgenic poplar produces unexpected nonconventional lignin and significantly improves biomass saccharification

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Lignin represents a complex biopolymer that makes plant woody biomass processing difficult. Lignin bioengineering holds promise to optimize plant feedstock with reduced biomass recalcitrance. In addition, production of co-products in woody biomass is regarded as a complementary approach to increase biomass value and reduce the costs of biofuel production. Previously, we engineered a monolignol 4-*O*-methyltransferase (MOMT4) that confers novel activity in methylating the *para*-hydroxyl of monolignol. The modified monolignols are denied the propensity for radical generation and further incorporation into lignin polymer. Expression of *MOMT4* in poplar growing in greenhouse condition resulted in significant alteration of lignin composition and the improvement of woody biomass processability. To evaluate the sustainability of lignin compositional change and the resilience of engineered poplar crops in nature environment, we conducted a 3-year field trial experiment with 150 transgenic poplars. The biomass yield, wood density, lignin content and composition, and biomass saccharification were determined with the harvested field-grown trees. Thioacidolysis and/or NMR study revealed an approximately 80% reduction in S-lignin subunits, and an increase in G-lignin subunits in the remaining lignin of transgenic plants. Gel permeation chromatography analysis on the raw biomass unveiled an increased Mw and decreased Mn in the biomass of transgenic plants, leading to a polydispersity index from 3.9 of the control to 5.6 of the transgenic plants. Meanwhile, NMR study revealed a 2~3-fold increase in cinnamyl alcohol end group units of lignin from transgenic lines. These data implicate a hyper branching and more heterogenous lignin/cell wall biopolymer of MOMT4 transgenic poplars. Concomitant with lignin alteration, a number of methanolic-extractable flavonoid biosynthetic intermediates were unexpectedly accumulated in the woody biomass of rotation transgenic poplars. Moreover, those flavonoids were able to act as non-conventional lignin monomers, incorporated into polymer to form novel lignin. As the consequence of the alterations in lignin composition/structure and polymer property, the saccharification efficiency of transgenic woody biomass was increased up to 178%, compared to that of the control lines; and the ethanol yield from Simultaneous Saccharification and Fermentation of transgenic wood without alkaline pretreatment exceeded that of the control wood with intensive pretreatment. Our study suggests an application potential of the MOMT-transgenic poplars in producing the low-cost biofuels.

Genetic Regulation of Vessel Traits Affecting Water Transport and Drought Response in *Populus*

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Angiosperm trees transport water through specialized vessel element cells. Vessel element differentiation is initiated by a dramatic increase in cell diameter, followed by synthesis of a lignified secondary cell wall and programmed cell death. The remaining cell corpse is joined end to end with other vessel elements to make longer structures, vessels, whose properties have major effects on water transport capacity and cavitation under water stress. We used a population of *Populus* hybrids carrying genomically defined chromosomal insertions and deletions to dissect vessel element morphological traits including diameter, grouping index, frequency and lumen fraction. We show that all traits measured are moderately heritable, and that the effect of tree height is genetically separable from vessel diameter. Individual QTL were identified for vessel traits, and together with heritability estimates are consistent with classical quantitative traits controlled by many genes with modest effects. Gene expression data was used in a systems genetics approach to summarize mechanisms and identify individual candidate genes underlying traits.

Keywords: vessel element, drought, forest mortality,

Towards understanding of the signaling involved in regulation of wood formation

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Wood formation follows a consecutive process of cell development, from vascular cambium proliferation, cell expansion and differentiation, secondary cell wall deposition to programmed cell death, which is controlled by multifaceted signals. However, understanding of the signals and their transduction during wood cell development is incomplete. A collection of receptor-like kinases (RLKs) was isolated from developing xylem tissue and their expression specifically localized in secondary xylem indicated that wood formation involves a complex of RLK-mediated signaling. By screening genome-editing knockouts (KOs) of the xylem-specific RLK genes in *Populus*, we identified *ATTENUATION OF SECONDARY XYLEM* (*ASX*) knockouts which displayed defective development of secondary xylem. The *asx* KOs showed abnormal stem secondary growth but little impact on shoot apical primary growth. In stem of the *asx* KOs, fewer layers of the secondary xylem cells were differentiated, while the vascular cambium proliferation was not altered. ASX is phosphorated by SERK through their interaction. By forming a complex, ASK-SERK acts as a module for signal transduction needed in regulation of secondary xylem differentiation in trees. The study provides new insights into understanding of the regulatory signaling in wood formation.

XND1 - the emerging master regulator of xylem cell differentiation

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Wood is the annual accumulation of secondary xylem, which is differentiated from cambial cells in woody plants. Quite a few regulators involved in this process have been identified and the regulatory network underlying wood formation has been preliminarily proposed. *NAM/ATAF/CUC* (*NAC*) domain gene *VND7* (*VASCULAR-RELATED NAC-DOMAIN 7*) is specifically expressed in vascular cells and functions as a transcriptional switch for xylem vessel differentiation. However, the regulation of *VND7* itself is sill elusive. *VND7* was found up-regulated in *XYLEM NAC DOMAIN 1* (*XND1*) overepxressed Arabidopsis plants, though the plants exhibited impaired vascular tissues resulting in dwarf phenotype. In addition, poplar *XND1* gave the similar phenotype when overexpressed in poplar. This indicates that *XND1* could be a regulator upstream VND7. In supporting to this, we have showed that poplar KNOTTED-like homeobox (KNOX) KANT2/6b inhibit the development of xylem by directly up-regulating *XND1* expression, while poplar growth-regulating factors GRF12a and GRF12b impede or enhance xylem differentiation through directly regulating XND1 expression by interacting with GIF1b (GRF-interacting factor 1b) and GIF1a respectively. Furthermore, we recently found that XND1 could directly activate *VND7* expression, and the interaction between XND1 and RETINOBLASTOMA-RELATED (RBR) could affect this regulation. This interaction was attenuated under DNA fragmentation caused by DNase-induced programmed cell death, which resulted in up-regulation of *VND7*. Collectively, these evidences support that XND1 is a master regulator upstream VND7 for xylem cell differentiation.

Keywords: xylem cell, differentiation, regulation, XND1, VND7 expression

Ubiquitinated DA1 negatively regulates vascular cambium activity through modulating the stability of WOX4 in *Populus*

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Activity of the vascular cambium gives rise to secondary xylem for wood formation in trees. The transcription factor WUSCHEL-related HOMEOBOX4 (WOX4) is a central regulator downstream of the hormone and peptide signaling pathways that maintain cambial activity. However, the genetic regulatory network underlying WOX4-mediated wood formation at the post-transcriptional level remains to be elucidated. In this study, we identified the ubiquitin receptor PagDA1 in hybrid poplar (*Populus alba* × *P. glandulosa* clone 84K) as a negative regulator of wood formation, which restricts cambial activity during secondary growth. Overexpression of *PagDA1* in poplar resulted in a relatively reduced xylem due to decreased cambial cell division. By contrast, mutation of *PagDA1* by CRISPR/Cas9 resulted in an increased cambial cell activity and promoted xylem formation. Genetic analysis demonstrated that PagDA1 functions antagonistically in a common pathway as PagWOX4 to regulate cambial activity. We propose that PagDA1 physically associates with PagWOX4 and modulates the degradation of PagWOX4 by the 26S proteasome. Moreover, genetic analysis revealed that PagDA1 exerts its negative effect on cambial development by modulating the stability of PagWOX4 in a ubiquitin-dependent manner mediated by the E3 ubiquitin ligase PagDA2. In sum, we have identified a cambial regulatory protein complex, PagDA1-PagWOX4, as a potential target for wood biomass improvement.

Keywords: vascular cambium, *Populus*, ubiquitin receptor, WOX4, ubiquitinylation

Grass lignin metabolic engineering for sustainable lignocellulose valorization

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Many countries have declared the intention to aim for carbon neutrality by 2050. They are examining various possibilities based on multiple scenarios. Among these, utilization of lignocellulose biomass such as trees and large grasses is indispensable. Trees are essential for the production of wood-based materials and paper, which account for half of the global consumption, while the other half is for burning. Moreover, demand for lignocellulose fuel use is increasing as a substitute of coal in thermal power plants. The majority of the tree biomass used for fuel depends on natural forest logging. Therefore, a reduction of natural deforestation, exploitation of already deforested area, and cultivation of high-productivity biomass in the deforested area are strongly required. In this regard, use of high biomass-productivity species and improvement of lignocellulose usability are the most important factors in establishing systems for sustainable lignocellulose production and utilization. Large grasses greatly surpass trees in terms of lignocellulose biomass productivity and therefore improvement of grass lignocellulose usability, e.g. improvement of lignin content and simplification of lignin structures, is critically important. In this context, using rice as a model of grass biomass plants we produced transgenic plants with increased lignin content, which should be beneficial for the augmentation of heating values and solid fuel use of biomass. We also generated transgenic rice lines with increased content of each of the three aromatic units of lignins (i.e., *p*-hydroxyphenyl, guaiacyl, and syringyl units). This can simplify the composition of lignin-derived aromatic products. Additionally, in-depth lignin analyses of the transgenic rice plants suggested that grasses contain a new biosynthetic pathway leading to the grass-specific -*p*-coumaroylated monolignols. Aiming at a practical application of these results we selected sorghum lines that have higher lignin content, which are suitable for co-combustion with coal in thermal power plant.

Keywords: Lignin, Biosynthesis, Upregulation, Fuel pellet, Sustainable Society

A regulatory network driving shoot lignification based on the population characteristic information of moso bamboo

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Woody bamboo is environmentally friendly, abundant, and an alternative to conventional timber. Moso bamboo (*Phyllostachys edulis*) is one of the representative woody bamboos with great economical and ecological values. However, its further development has been hindered by a lack of population genome information. Here, the genomic variation atlas of moso bamboo was depicted with 5.45 million SNPs from whole-genome resequencing of 427 individuals. We uncover low genetic diversity, high genotype heterozygosity, and genes under balancing selection underlying moso bamboo population adaptation, which was probably originated by a single-origin event from East China. Genome-wide association analysis showed that candidate genes associated with important property-related traits were involved in cell wall, carbohydrate metabolism, and environmental adaptation. Lignin is an important component of cell walls, and the lignification degree affects bamboo timber properties directly. To elucidate the regulatory mechanism of lignification in moso bamboo, we conducted integrated analyses using transcriptome, small RNA, and degradome sequencing followed by experimental verification. The lignification degree and lignin contents increased with bamboo shoot height increasing, and a total of 11,504 differentially expressed genes (DEGs) were identified in different height shoots. Most DEGs associated with cell wall and lignin biosynthesis were up-regulated, whereas some DEGs related to cell growth were down-regulated. Meanwhile, 687 differentially expressed miRNAs (DEMs) were also identified. Additionally, *in silico* and degradome analyses indicated that 5,756 genes were targeted by miRNAs. A regulatory network of lignification was constructed, including 11 DEMs, 22 transcription factors and 36 enzyme genes. Furthermore, *PeLAC20* overexpression increased lignin contents in transgenic Arabidopsis plants. Finally, we proposed a reliable miRNA-mediated 'MYB-*PeLAC20*' module for lignin monomer polymerization. Our findings provide definite insights into the genetic basis of moso bamboo population and property traits, which is helpful for designing strategies to improve bamboo timber properties based on the lignin regulatory mechanism.

Keywords: Moso bamboo, resequencing, genetic basis of property traits, lignification, regulatory network

Phytohormone interactions in regulation of the secondary vascular tissue regeneration after large-scale bark girdling in *Populus*

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Tissue regeneration upon wounding in plants highlights the developmental plasticity of plants. Our previous studies have described the differential regulation of auxin and cytokinin during secondary vascular tissue (SVT) regeneration after large-scale bark girdling in *Populus*. Here, the roles of gibberellin (GA) in SVT regeneration after bark girdling in *Populus* were investigated by using *in vitro* SVT regeneration system combining with exogenous hormone application. The results showed that no SVT formation was induced with exogenous application of GA alone, however, compared with exogenous application of auxin alone, more layers of cambium were induced with exogenous application of auxin and GA. More experiments with transgenic poplar lines, such as *PtoWOX4ap:GUS*, *PtaDR5:GUS*, *35S:PtoKS1* and *PtoKS-CRISPR*, were performed to show that GA promoted the effects of auxin induction by increasing the auxin biosynthesis and response. Furthermore, the interactions of GA, auxin and cytokinin during SVT regeneration after large-scale bark girdling were discussed.

Keywords: gibberellin, phytohormone interaction, secondary vascular tissue regeneration, *Populus*

How do grasses produce lignin differently from woods: case studies with rice

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Monocotyledonous grasses have evolved unique cell wall structures distinctively different from those of typical wood species, i.e., softwoods (gymnosperms) and hardwoods (eudicots). As a prime example, grasses produce lignins highly decorated by hydroxycinnamates (*p*-coumarates and ferulates) and flavonoid tricin units by incorporating grass-specific lignin monomers, i.e., γ-acylated monolignols and a flavone tricin, alongside canonical monolignols for cell wall lignification, which contrasts typical eudicot and gymnosperm species that utilize monolignols as sole lignin monomers. Currently, it remains largely unknown how such grass-specific lignin monomers are biosynthesized and function in grass cell walls. Moreover, how the existence of the grass-specific lignin decorations affects the utility of grass biomass remains poorly understood. In this context, our group has been investigating the biosynthesis of the grass-specific lignin monomers and exploring bioengineering approaches to produce transgenic grass plants with altered lignin decoration units. In this presentation, I will briefly summarize our recent findings regarding the biosynthesis of grass-specific lignin monomers in rice, a model grass species and an economically important grass crop, and some structure and property data of transgenic and mutant rice plants that produce cell walls lacking the grass-specific lignin decorations. Deepening our understanding of the biosynthesis and properties of grass cell walls may contribute to a better understanding of the nature and evolution of wood cell walls.

Keywords: lignin, grass, acylated monolignols, tricin, rice

Conservation and diversification of transcriptional regulatory system for woody cell formation among land plant species

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The VNS (VND, NST/SND, SMB-related) family proteins, which is a subset of NAC transcription factors, are the master regulators of woody cell formation. The evolutional conservation of VNS-based transcriptional regulatory network has been shown in woody cell formation, however, it is unclear how their characteristics as transcription factor have been changed during land plant evolution. Here, we analyzed the binding affinity of VNS proteins from *Physcomitrella patens*, *Selaginella moellendorffii*, *Pinus taeda*, and *Arabidopsis thaliana* to 21 kinds of DNA cis-sequences identified from the VNS downstream genes of these plant species. Hierarchical clustering analysis of Kd values between each VNS and cis-sequence showed a clear separation of moss *P. patens* VNS protein from the VNS proteins of vascular plants, *S. moellendorffii*, *P. taeda*, and *A. thaliana*, suggesting that the DNA binding activities of VNS proteins have been evolutionarily changed. Together with the results of comparative transcriptome analysis, the molecular evolution of transcriptional regulatory system for woody cell formation will be discussed.

Keywords: Woody cells, VNS family protein, Transcription factor, Plant evolution

The regulation of the rapid growth in the Phyllostachys edulis culm internode

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Fast growth is very important for biomass, especially in wood. Moso bamboo (Phyllostachys edulis) is a fast-growing species with uneven growth and lignification from lower to upper segments within one internode. However, the regulation of fast growth in bamboo internodes is poorly understood. Here, First, the miRNA changes in fast-growth stage were studied. One moso bamboo internode was divided into four segments called F4 (bottom) to F1 (upper), then these were analyzed for transcriptomes, miRNAs and degradomes. The bottom segment (F4) had a higher number of actively dividing cells as well as a higher content of auxin (IAA), cytokinin (CK) and gibberellin (GA) compared with the upper segment (F1). RNA-seq analysis showed DNA replication and cell division-associated genes highly expressed in F4 rather than in F1. In total, 63 miRNAs (DEMs) were identified as differentially expressed between F4 and F1. The degradome and the transcriptome indicated that many downstream transcription factors and hormonal responses genes were modulated by DEMs. Second, the growth of Phyllostachys edulis shows a circadian rhythm. The internodes mainly grow slow at the day while they grow fast at the night. Transcriptome analysis revealed that many center oscillator genes such as TOC1 showed circadian rhythm expression. The sugar from the mother bamboo might act as the input signal of the rhythm. These results provide a new perspective for bamboo fast growth.

The metabolic basis of Maize pollen thermo-tolerance

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Sporopollenin is the most resistant physical barrier of pollen wall protecting male gamete under stress conditions, however, the composition of sporopollenin from the pollen of major crops remains unclear. In this study, we investigated the composition of sporopollenin of maize pollen from 117 inbred lines by thioacidolysis coupled with GC-MS analysis. Three common lignin units, p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) monomers were identified as components of sporopollenin of maize pollen. Quantitative analysis suggested that the H unit was the most abundant among the three, which is clearly in contrast to the compositions of the regular secondary cell wall. In addition, we also demonstrated that tropical/subtropical inbred lines exhibited higher lignin content compared to the temperate. mGWAS analysis identified that VAMP726 is associated with H unit accumulation of sporopollenin. Loss-of-function mutations of VAMP726 led to reduced H unit deposition on sporopollenin in maize, and decreased pollen viability upon heat and ultraviolet treatment, whereas, overexpression of AtVAMP726 in Arabidopsis confers increased resistance to tested stress conditions. Taken together, this work shows lignin units are essential for maize pollen viability under stress conditions, and provides insights into the evolutionary and environmental adaptations of maize plants.

PtoWRKY40 interact with CuZn-Superoxide dismutase to modulate drought resistance by regulating lignin synthesis in *Populus tomentosa*

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Wood is a dominant terrestrial biomass and mainly made up of secondary cell wall in trees. Although the biosynthesis of lignin, cellulose and semi-cellulose of secondary walls are well documented in plants, its regulatory mechanism in tree species under drought stress remains largely unknown. In this study, we demonstrate that the *Populus tomentosa PtoWRKY40* functions as a negative regulator of secondary cell wall deposition through directly targeting to the lignin synthesis genes *PtoCCoAOMT1* and *Pto4CL3*. The *PtoWRKY40* gene is expressed predominantly in fibers and vessels in stems and its coded protein localizes to the nucleus. Downregulation of *PtoWRKY40* in poplar resulted in an increase in secondary xylem width and secondary cell wall thickness, associated with ectopic deposition of lignin in developing secondary xylem, leading to an increase in dry weight biomass. In contrast, overexpression of *PtoWRKY40* had the opposite phenotype. PtoWRKY40 could bind directly to the *PtoCCoAOMT1* and *Pto4CL3* promoters and repress their expression. In addition, we showed that PtoWRKY40 physically interacted with CuZn-Superoxide dismutase 4 (PtoSOD4) both in vitro and in vivo. The interaction could attenuate the translocation of WRKY from cytoplasm to the nucleus, as shown by the fluorescence imaging of WRKY nucleocytoplasmic transportation, leading to the inhibition of *PtoCCoAOMT1* and *Pto4CL3* transcription by PtoWRKY40. Interestingly, drought stresses enhanced the interaction of PtoWRKY40 and PtoSOD4, and promoted the lignin biosynthesis. These results demonstrate that PtoWRKY40 is a key regulator mediating secondary wall biosynthesis and drought resistance in poplar.

Keywords: *Populus tomentosa*, *PtoWRKY40*, lignin biosynthesis, drought resistance, secondary wall biosynthesis

Single-cell transcriptomic analysis of the shoot apex reveals conserved and divergent features between *Populus* and *Arabidopsis*

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Stem cell differentiation in the apex gives rise to aerial tissues and organs in plants. Presently we lack a lineage map of the shoot apex cells in woody perennials – a critical gap considering their role in determining primary and secondary growth. In the present work, we applied the nuclei isolation protocol we developed to perform the single-nuclei RNA sequencing technology, to determine the cell type-specific transcriptome of the *Populus* vegetative shoot apex. The data analyses identified highly heterogeneous cell populations clustered into seven groups represented by 18 transcriptionally distinct cell clusters. Clustering annotation served as the foundation to establish the developmental trajectories of the epidermis, leaf mesophyll, and vascular tissue. Motivated by the high similarities observed between *Populus* and *Arabidopsis* cell populations in the vegetative apex, we applied a pipeline to integrate interspecific single-cell gene expression data. We contrasted the developmental trajectories of primary phloem and xylem formation in both species, to establish the comparison of vascular development between a model annual herbaceous and a woody perennial plant species. Our results offer a valuable resource for investigating the mechanisms underlying cell division and differentiation conserved between herbaceous and perennial species while also allowing us to examine species-specific differences at single-cell resolution.

Keywords: *Populus*, vascular development, scRNA-seq, developmental trajectory, shoot apex

Cell-type specific transcription factors form regulatory nexus with a histone modification system for stem cambium development in *Populus*

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Stem vascular cambium cells in forest trees give rise to wood for materials and energy production. WOX4 affects the proliferation of such cells in *Populus*. *PtrWOX4a* is the most abundant stem "vascular cambium-specific" (VCS) gene in *Populus trichocarpa*. We discovered that its function on cambium cell proliferation is controlled by the second most abundant VCS, the PtrVCS2, a novel zinc finger protein. The two form an association through PtrWOX13a direct binding to *PtrWOX4a* as a PtrVCS2:PtrWOX13a:PtrGCN5-1:PtrADA2b-3 protein tetramer. PtrVCS2 affects the interaction intensity of the tetramer's PtrGCN5-1:PtrADA2b-3 component for hyperacetylation (without PtrVCS2) or hypoacetylation (with PtrVCS2) of histone 3 lysine 9 (H3K9) on *PtrWOX4a* promoter, leading to 4-6 more or 6-8 fewer cambium cell layers, respectively, in transgenic *P. trichocarpa*. We tested the tetramer-*PtrWOX4a* path's function in over 20 genotypes of overexpression, CRISPR-knockout, RNAi transgenesis in *P. trichocarpa* and corroborated its effects on cambium cell proliferation. Thus, the tetramer-*PtrWOX4a* system may coordinate genetic and epigenetic regulations in maintaining normal vascular cambium development for wood formation.

Keywords: *Populus trichocarpa*, stem vascular cambium, transcription factor, histone acetylation, transgenesis

An Evolutionarily Conserved Long-distance Migrating Peptide Regulates Monolignol Biosynthesis in Woody Angiosperms

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Peptides act as hormones to deliver intercellular signals to govern intracellular transcriptional networks during complex developmental processes. Lignin in vascular tissues plays a critical role in plant terrestrialization for water-conducting and structural-supporting functions. Comprehensive knowledge has been established on the transcriptional networks for the regulation of vascular development. Little is known on the regulatory functions of long-distance migrating peptides for the development of vasculature. Here, we used peptidomic analyses in two woody eudicots, *Populus trichocarpa* and *Eucalyptus grandis*, to reveal a group of conserved long-distance migrating peptides in vascular sap. Transcriptomic analyses from different tissues demonstrated that these conserved peptides were majorly generated from precursor genes specifically expressed in xylem. Such peptides were further found to be evolutionarily conserved in the vascular sap of an ancient woody species in magnoliids of angiosperms, *Cinnamomum kanehirae*. We found that one of the conserved peptides, named as angiosperm sap peptide (ASAP), can regulate the metabolic fluxes of monolignol biosynthesis through the enhancement of S-lignin pathway. Sequence identity analysis showed that ASAP emerged in land plants, suggesting its regulatory role on lignin to facilitate plant terrestrialization.

Keywords: Peptidomic profiling, vascular sap, monolignol biosynthesis, woody angiosperms, mass spectrometry

Crosstalk regulation of auxin and gibberellin on cambial activity in poplar

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In trees, the vascular cambium is the population of stem cells that laterally divide and as a consequence, produce secondary xylem, which is commonly known as wood. The continuous activity of cambial cells determines the quantity of wood production. Multiple hormonal signals are known to rigorously regulate cambial activity. The exogenous application of auxin and gibberellin (GA) can promote cambium activity in poplar stems, and common transcriptome changes occur via treatment with these two phytohormones. However, how auxin and GA combinatorially regulate cambial activity in trees remains unknown. We show that the DELLA protein REPRESSOR of ga1-3 Like 1 (RGL1), AUXIN RESPONSE FACTOR 7 (ARF7), and Aux/INDOLE-3-ACETIC ACID 9 (IAA9) form a ternary complex that mediates crosstalk between the auxin and GA signaling pathways in poplar stems during cambial development. Biochemical analysis revealed that ARF7 physically interacts with RGL1 and IAA9 through distinct domains. The *arf7* loss-of-function mutant showed markedly attenuated responses to auxin and GA, whereas transgenic poplar plants overexpressing ARF7 displayed strongly improved cambial activity. ARF7 directly binds to the promoter region of the cambial stem cell regulator WOX4 to modulate its expression, thus integrating auxin and GA signaling to regulate cambial activity. Furthermore, the direct activation of *PIN-FORMED 1* expression by ARF7 in the RGL1–ARF7–IAA9 module increased GA-dependent cambial activity via polar auxin transport. Collectively, these findings reveal that the crosstalk between auxin and GA signaling mediated by the RGL1–ARF7–IAA9 module is crucial for the precise regulation of cambial development in poplar.

Keywords: Cambium, auxin, gibberellin, auxin response factor 7, poplar.

Members of a *Populus* wood interaction network: roles in growth and wood chemistry revealed by a three-year field study

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We previously identified *Populus* wood protein-protein interaction networks and also incorporated protein-DNA interactions with promoters of genes involved in lignin biosynthesis. We generated transgenic *Populus* containing 17 transgenes designed to alter gene expression or activity of members of this wood interaction network. We selected genes encoding regulatory proteins that were hubs in the wood network and some of their interactors that included genes newly implicated in wood development. These included the hub protein DIVARICATA (DIV) and RADIALIS (RAD) INTERACTING FACTOR1 (DRIF1) for which we identified 21 interacting proteins. We also selected known transcriptional regulators, such as SECONDARY NAC DOMAIN2 (SND2) and SND3, whose endogenous functions in *Populus* had not been fully defined. As tree biomass accumulates over multiple years, field studies are arguably the most efficient approach for revealing unanticipated phenotypes that result from both climatic and episodic environmental conditions, validating predictions from omics analyses and to further hone in on key regulatory genes. Hence, to characterize these trees, we used a direct to field approach. We will report on growth effects and for 8 transgenic types, wood chemistry analysis showed significant differences in lignin and/or carbohydrates compared to wild-type. Additional phenotypes included an altered tension wood (TW) response to gravity and delayed autumn leaf senescence.

Keywords: *Populus*, transgenics, wood, field trial

Multiplex CRISPR-Editing in *Populus trichocarpa* for Sustainable Fiber Production

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The domestication of forest trees for a more sustainable fiber bioeconomy has long been hindered by the complexity and plasticity of lignin, a biopolymer in wood that is recalcitrant to chemical and enzymatic degradation. Here, we show that multiplex CRISPR-editing enables precise woody-feedstock design for combinatorial improvement in lignin composition and wood properties. By assessing every possible combination of 69,123 multigenic-editing strategies for 21 genes in the lignin pathway, we deduced 7 unique genome-editing strategies targeting the concurrent alteration of up to 6 genes, and produced 163 edited poplar variants. CRISPR-editing increased the wood carbohydrate-to-lignin ratio to 239% of wildtype, leading to more efficient pulping for fibers. The edited wood alleviates a major fiber production bottleneck, bringing unprecedented operational efficiencies, bioeconomic opportunities, and environmental benefits.

Characterization of Xyloglucan Fucosyltransferase in Poplar

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Xyloglucan (XG) exists throughout the plant kingdom and is the dominant hemicellulose in primary wall of type I cell wall (mostly dicot species). It cross-links cellulose microfiber and plays a pivotal role in the plant growth and development. The typical structure of XG is a β-1,4-glucan backbone substituted with xylose which is further decorated with galactose and fucose. The biosynthesis of XG is relatively well characterized in Arabidopsis while the mechanism in woody species in under investigated. XG biosynthetic enzymes include glycosyltransferase (GT) 2 family members CSLC, α-1,6-xylosyltransferase (XylT) from GT34, β-1,2-galactosyltransferase (GalT) from GT47 and α-1,2-fucosyltransferase (FUT1) from GT37. Phylogenetic analysis of GT37 family reveals the segregated evolution in higher plants. In this study, we cloned and expressed all seven GT37 members from poplar 84K (*Populus alba* × *Populus tremula var. glandulosa*). The poplar XG FUT1 was identified via enzymatic assay and High-pH Anion Exchange Chromatography analysis. The gene is highly expressed in developing xylem, suggesting it may affect the formation of secondary wall and wood quality. The physiological roles of the polar XG FUT1 is under investigation via genetic analysis using CRISPR-Cas9 technique. The data identified new member of GT37 family and provides insights of the significance of hemicellulose in woody species.

Keywords: cell wall, xyloglucan, fucosyltransferase, poplar, wood

Abscisic acid regulates secondary cell-wall formation through phosphorylation of NST1 in *Arabidopsis* and its ortholog in *Poplar*

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Plant secondary cell-wall (SCW) deposition and lignification are affected by both seasonal factors and abiotic stress, and these responses may involve the hormone abscisic acid (ABA). However, the mechanisms involved are not clear. Here we show that mutations that limit ABA synthesis or signaling reduce the extent of SCW thickness and lignification in Arabidopsis thaliana through the core ABA-signaling pathway involving SnRK2 kinases. SnRK2.2. 3 and 6 physically interact with the SCW regulator NAC SECONDARY WALL THICKENING PROMOTING FACTOR 1 (NST1), a NAC family transcription factor that orchestrates the transcriptional activation of a suite of downstream SCW biosynthesis genes, some of which are involved in the biosynthesis of cellulose and lignin. This interaction leads to phosphorylation of NST1 at Ser316, a residue that is highly conserved among NST1 proteins from dicots, but not monocots, and is required for transcriptional activation of downstream SCW-related gene promoters. Loss of function of NST1 in the snd1 mutant background results in lack of SCWs in the interfascicular fiber region of the stem, and the Ser316Ala mutant of NST1 fails to complement this phenotype and ABA-induced lignin pathway gene expression.
Based on a gene-editing/complementation strategy, our team has generated the modified poplar lines of the conserved phosphorylation site of PtrWND2, the poplar ortholog of NST1. We observed less lignin accumulation and the improvement of the cellulose-to-glucose conversion efficiency in the editing lines than the wild-type under drought stress. Our findings would provide the theoretical basis for cultivating new poplar germplasm that maintains excellent fiber wood quality in the arid environment.

Keywords: secondary cell wall, ABA, post translational modification, lignification, drought.

nst/snd multiple mutants raise new questions on xylem cell differentiation and secondary cell wall formation

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Cell walls, especially secondary cell walls (SCW), maintain cell shape and reinforce wood, but their structure and shape can be altered in response to gravity. In hardwood trees, tension wood forms along the upper side of a bending stem and contains wood fiber cells that have a gelatinous layer (G-layer) inside the SCW. In our recent papers[1,2], we generated *nst/snd* quadruple-knockout aspens, in which SCW formation was impaired in 99% of the wood fiber cells, and *nst/snd* triple-knockout aspens, in which many wood fibers had thinner SCW than the wild-type (WT) and some had no SCW. Because SCW layers are always formed prior to G-layer deposition, the *nst/snd* mutants raise interesting questions of whether the mutants can form G-layers without SCW and whether they can control their postures in response to changes in gravitational direction. The *nst/snd* mutants and the WT plants showed growth eccentricity and vessel frequency reduction when grown on an incline, but the triple mutants recovered their upright growth only slightly, and the quadruple mutants were unable to maintain their postures. The mutants clearly showed that the G-layers were formed in SCW-containing wood fibers but not in those lacking the SCW. Our results indicate that SCW are essential for G-layer formation and posture control. Furthermore, each wood fiber cell may be able to recognize its cell wall developmental stage to initiate the formation of the G-layer as a response to gravistimulation.

Keywords: NST/SND, secondary cell wall, tension wood, G-layer, wood fiber

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Spacing effect on growth and wood parameters of willow clones.

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The intra-clonal competition was studied in short-rotation willow clones to assess the effects of spacing on growth, wood properties, volume and biomass production. The three clones (J-799, J-194 and Kashmiri) were planted in plantation densities of 1 × 1 m (10000 plants/ha), 1 × 2 m (5000 plants/ha) and 1 × 3 m (3333 plants/ha) in randomized complete block design in the year 2017 in the Khaltoo experimental field of the department of Tree Improvement and Genetic Resources. Three-year-old growth and coppice growth of one year was recorded. The plant height (5.86 m) recorded in three-year-old plantation significantly higher in closest spacing (1 × 1 m) and lowest (3.93 m) in widest spacing (1 × 3 m). The plants planted in widest spacing recorded maximum (6.82 cm) basal diameter. Volume index per hectare basis was maximum (173.61 m^3 ha^{-1}) in spacing of 1 × 1 m. Wood physical properties, fibre length (0.72 mm) and moisture content (84.14 %) were observed maximum in Kashmiri clone. Lignin content (21.69 %) was recorded maximum in 1 × 3 m. The maximum height (3.88 m) in one year old coppice recorded in 1 × 1 m and maximum coppice diameter (2.86 cm) was recorded in 1 × 3 m. Volume index of coppice per hectare (13.37 m^3/ha) was recorded maximum in 1 × 1 m and minimum (7.71 m^3/ha) in 1 × 3 m. Therefore, the spacing of 1 × 1 m was found more suitable for maximising growth for raising the willow crops in short rotation forestry.

Keywords: Volume index, Short rotation, Coppice growth, Clones

Unveiling the impact of endophytic microbiome on cork development and suberin biosynthesis

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Cork oak (*Quercus suber* L.) is a characteristic species of the South of Portugal's landscape but it also has a great impact on the country's economy. This is closely related to the species' unique capacity to produce cork, a versatile and highly valuable material mainly composed of suberin. However, cork production is associated with long waiting periods: around 25 years are needed before the first cork extraction, followed by a 9-year period between extractions. Consequently, there has been much interest in finding ways to shorten these intervals, mainly through the use of irrigation and fertilizers. Nevertheless, improper application of mineral fertilizers has negative impacts on the environment, contributing to soil degradation, leaching, and other serious problems. One alternative to the use of mineral fertilizers is to take advantage of the potential of microorganisms present in the soil, known to play significant roles in soil fertility. This work aims to uncover the impact of endophytic microbiome on cork oak root periderm development and deposition of suberin. We are identifying endophytic microorganisms present in the roots of germinating cork oaks for a first look into the microbial diversity surrounding cork oaks and its putative beneficial role in plant development. Additionally, the development of efficient systems for the functional validation of genes in cork oak is important for the elucidation of the mechanisms regulating the species' growth and development. Thus, a protocol for the infection with *Agrobacterium rhizogenes* and consequent transformation of cork oak is being optimized to develop a "hairy root" system. Also, expression vectors have been constructed to be used in this system for the study of genes related to cork development and suberization.

Keywords: *Agrobacterium rhizogenes*; Cork oak; endophytic microorganisms; Root periderm; Suberin

PtaERF194 inhibits plant growth and enhances drought tolerance in poplar

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The water deficits limit the growth and development of agricultural and forest organisms. The AP2/ERF family has been identified as one of the largest plant-specific transcription factors (TFs) essential for plant development and stress response. The function of PtaERF194 in growth and drought tolerance was detected in the overexpression (OX) and RNAi transgenic poplar 717 hybrids. Plant growth, stem vessels, water-use efficiency (WUE), chlorophyll content and PtaERF194 co-expressed genes were analyzed using morphological, physiological and molecular methods. Overexpression seedlings showed a shorter and smaller phenotype along with smaller and more vessels compared with the wild-type (WT). Physiological indices indicated that OX with low transpiration and stomatal conductance improved the tolerance to drought by enhancing WUE, limiting water loss and maintaining high water potential. A total of 12 differentially expressed genes co-expressed with PtaERF194 were identified, and they worked together to regulate drought tolerance through the abscisic acid signaling and reactive oxygen species scavenging processes. However, RNAi plants showed similar morphology and physiology to WT, suggesting that the function of PtaERF194 was redundant with other ERF TFs. The findings of the current study may shed new light on the positive function of ERF TFs in plant drought stress tolerance.

Keywords: drought stress, ERF194, plant growth, transgenic poplar, water-use efficiency

Wood transcriptome analysis of *Pinus densiflora* identifies genes critical for secondary wall formation and NAC transcription factors involved in tracheid formation

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Although conifers have significant ecological and economic value, information on transcriptional regulation of wood formation in conifers is still limited. Here, to gain insight into secondary cell wall (SCW) biosynthesis and tracheid formation in conifers, we performed wood tissue-specific transcriptome analyses of *Pinus densiflora* (Korean red pine) using RNA sequencing. In addition, to obtain full-length transcriptome information, PacBio single molecule real-time iso-sequencing was carried out using RNAs from 28 tissues of *P. densiflora*. Subsequent comparative tissue-specific transcriptome analysis successfully pinpointed critical genes encoding key proteins involved in biosynthesis of the major secondary wall components (cellulose, galactoglucomannan, xylan and lignin). Furthermore, we predicted a total of 62 NAC (NAM, ATAF1/2 and CUC2) family transcription factor members and identified seven PdeNAC genes preferentially expressed in developing xylem tissues in *P. densiflora*. Protoplast-based transcriptional activation analysis found that four *PdeNAC* genes, homologous to VND, NST and SND/ANAC075, upregulated GUS activity driven by an SCW-specific cellulose synthase promoter. Consistently, transient overexpression of the four *PdeNACs* induced xylem vessel cell-like SCW deposition in both tobacco (*Nicotiana benthamiana*) and Arabidopsis leaves. Taken together, our data provide a foundation for further research to unravel transcriptional regulation of wood formation in conifers, especially SCW formation and tracheid differentiation.

Keywords: conifer, NAC transcription factor, *Pinus densiflora*, tracheid, wood formation

Molecular cloning and analysis of gene encoding a monosaccharide uptake transporter in *Cryptomeria japonica*

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Sugars are essential organic substances for plants, acting as carbon skeletons for the synthesis of cellular compounds, energy sources, osmotic regulators, and signaling molecules. Uptake of sugars, such as monosaccharides and disaccharides, in plant cells is closely linked to the movement of sugars across biological membranes, such as the plasma membrane and the tonoplast. This transmembrane transport of sugars is mediated by membrane proteins classified as transporters. Elucidating the mechanism and roles of sugar membrane transport is important for a better understanding of growth, including wood formation, and physiology in trees. In this study, we isolated a gene (cDNA), *CjSTP3*, encoding a monosaccharide transporter from sugi (*Cryptomeria japonica*), which is one of the most important forestry conifer species in Japan. The translated sequence of the *CjSTP3* cDNA was found to be homologous to proteins belonging to the sugar transport protein (STP) family. Sequence analysis indicated that the CjSTP3 protein has 12 transmembrane domains with the N- and C-terminus located inside the membrane, which is consistent with other known STP transporters. To investigate the monosaccharide uptake function of CjSTP3, we conducted complementation tests using a yeast (*Saccharomyces cerevisiae*) strain, which is deficient in hexose uptake activity. The yeast strain transformed with CjSTP3 was able to grow in a medium containing hexose (glucose, fructose, galactose, or mannose) as the sole sugar source. This suggests that the heterologous expression of CjSTP3 complements the deficiency of this strain, and therefore, CjSTP3 has a function in hexose uptake. Expression analysis showed that *CjSTP3* was expressed in all parts of sugi trees examined in this study, including differentiating xylem, inner bark, roots, and male strobili. It is likely that CjSTP3 is involved in monosaccharide membrane transport in the cells of various parts in sugi trees, including differentiating xylem.

Keywords: Biological membrane, Conifer, Hexose uptake, Monosaccharide transporter, Sugar transport protein

Field evaluation of transgenic hybrid poplars with desirable wood properties and enhanced growth for biofuel production by bicistronic expression of *PdGA20ox1* and *PtrMYB3* in wood-forming tissue

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To create an ideotype woody bioenergy crop with desirable growth and biomass properties, we utilized the viral 2A-meidated bicistronic expression strategy to express both *PtrMYB3* (*MYB46* ortholog of *Populus trichocarpa*, a master regulator of secondary wall biosynthesis) and *PdGA20ox1* (a GA20-oxidase from *Pinus densiflora* that produces gibberellins) in wood-forming tissue (i.e., developing xylem). Transgenic Arabidopsis plants expressing the gene construct DX15::PdGA20ox1-2A-PtrMYB3 showed a significant increase in both stem fresh weight (threefold) and secondary wall thickening (1.27-fold) relative to wild-type (WT) plants. Transgenic poplars harboring the same gene construct grown in a greenhouse for 60 days had a stem fresh weight up to 2.6-fold greater than that of WT plants. In a living modified organism (LMO) field test conducted for 3 months of active growing season, the stem height and diameter growth of the transgenic poplars were 1.7- and 1.6-fold higher than those of WT plants, respectively, with minimal adverse growth defects. Although no significant changes in secondary wall thickening of the stem tissue of the transgenic poplars were observed, cellulose content was increased up to 14.4 wt% compared to WT, resulting in improved saccharification efficiency of the transgenic poplars. Moreover, enhanced woody biomass production by the transgenic poplars was further validated by re-planting in the same LMO field for additional two growing seasons. Taken together, these results show considerably enhanced wood formation of our transgenic poplars, with improved wood quality for biofuel production.

Keywords: Bicistronic gene expression, Developing xylem promoter, Hybrid poplar, LMO field experiment, Saccharification

Overexpression of *EgrIAA20* from *Eucalyptus grandis*, a Non-Canonical *Aux/IAA* Gene, Specifically Decouples Lignification of the Different Cell-Types in *Arabidopsis* Secondary Xylem

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Wood (secondary xylem) formation is regulated by auxin, which plays a pivotal role as an integrator of developmental and environmental cues. However, our current knowledge of auxin-signaling during wood formation is incomplete. Our previous genome-wide analysis of *Aux*/*IAAs* in *Eucalyptus grandis* showed the presence of the non-canonical paralog member *EgrIAA20* that is preferentially expressed in cambium. We analyzed its cellular localization using a GFP fusion protein and its transcriptional activity using transactivation assays, and demonstrated its nuclear localization and strong auxin response repressor activity. In addition, we functionally tested the role of *EgrIAA20* by constitutive overexpression in *Arabidopsis* to investigate for phenotypic changes in secondary xylem formation. Transgenic *Arabidopsis* plants overexpressing *EgrIAA20* were smaller and displayed impaired development of secondary fibers, but not of other wood cell types. The inhibition in fiber development specifically affected their cell wall lignification. We performed yeast-two-hybrid assays to identify EgrIAA20 protein partners during wood formation in *Eucalyptus*, and identified EgrIAA9A, whose ortholog PtoIAA9 in poplar is also known to be involved in wood formation. Altogether, we showed that EgrIAA20 is an important auxin signaling component specifically involved in controlling the lignification of wood fibers.

Keywords: lignification; non-canonical Aux/IAA; secondary fiber; Eucalyptus; Cambium differentiation.

MiR395c Regulates Secondary Xylem Development through Sulfate Metabolism in Poplar

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Secondary xylem development requires the coordination of multiple regulatory factors, including plant hormones, transcription factors and microRNAs (miRNAs). MiR395 is an important regulator involved in sulfate metabolism, but its function in plant development is unclear. This study investigated the functions of miR395c in the secondary xylem development in *Populus alba* × *P. glandulosa*. MiR395c was highly expressed in shoot apex and secondary xylem. Overexpression of miR395c resulted in an increase in both secondary xylem width and vessel dimension, as well as a decrease in the thickness of secondary cell wall of xylem fiber. Further analysis showed that miR395c inhibited biosynthesis of sulfate metabolic products by targeting *ATPS* genes, which led to the reduction of ABA synthesis and down-regulation of *MYB46* expression. Our results indicate that miR395c regulates the secondary xylem development process via sulfate metabolism in *Populus*.

Keywords: miR395, *ATPS*, sulfate metabolism, secondary xylem development, *Populus alba* × *P. glandulosa*

SAR-test: finding new bud break genetic pathways through a novel functional screening in Poplar

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To sustain proper vegetative growth, development, flowering, and fruit production throughout the years, perennial trees of temperate and boreal climates have adapted to unfavorable climatic conditions through annual growth-dormancy cycles. In autumn, short days signal drives to the cessation of apical meristem growth, culminating with the development of winter buds and reaching a dormancy state. To be released from dormancy, perennial trees possess growth-promoting factors that trigger bud break and the restoration of vegetative growth during late winter and early spring. Nowadays, functional studies of these bud break and dormancy release factors are limited. We propose SAR-test (Shoot Apex Reactivation Test) as a screening tool to identify *bud breaker* genes[1]. SAR-test is based on FT2 (FLOWERING LOCUS T2) CRISPR/Cas9 mutant lines in Poplar, which we have previously described as plants that cannot maintain vegetative growth and form premature winter buds even under long days conditions[2]. Thereby, *ft2* mutant presents an ideal phenotypic background on which we can overexpress potential dormancy release factors to study the bud break potential of the candidate's genes. Currently, we are implementing SAR-test to study SOC1 (SUPPRESSOR OF CONSTANS 1)-like family members of poplar, apple and peach, as we have previously shown that overexpressing a member of SOC1-like genes promotes bud break in ecodormant poplars[3]. Furthermore, we are screening the shoot growth reactivation genes since we previously identified them as DNA methylation repressed during dormancy[4]. We present SAR-test as a tool to investigate our hypothesis: these genes represent novel pathways that control dormancy release. Preliminary results and future goals are discussed.

Keywords: Dormancy, bud break, SAR-test, *ft2* and poplar.

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Key roles of Populus Flowering Locus T2 (FT2) and TEMPRANILLO like (TEM like) in annual tree growth transitions

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Perennial trees adapt their growth-dormancy cycles to environmental changes. Flowering locus T (FT) has been described as a regulator of these transitions in multiple species[1]. In Populus, FT1 and FT2 have been associated with the reproductive onset and vegetative growth, respectively[2], but functional studies are needed to dissect their specific roles finely. We generated FT2 loss of function plants to study its role in transitions. We demonstrated that Populus FT2 plays a dual role. It is essential to maintain shoot growth and limits internode elongation under long days. In both cases, it acts upstream of gibberellins (GA) pathway and controls optimal levels of bioactive GAs. On one side, in the apical shoot, the absence of FT2s triggers growth cessation and bud set through deregulation of GA metabolism genes. In leaves, FT2 is required to regulate the levels of GA1 by inducing the deactivated gene *GA2ox1* and repressing the biosynthetic gene *GA3ox2*. We associate this function to the limitation of the internode growth in poplar. Furthermore, we found that Populus orthologs to Arabidopsis FT repressor, TEMPRANILLO like 1 and 2 (TEM-like 1 and TEM-like 2), do not conserve FT repressive function among species. We show that poplars overexpressing TEM-L1 or TEM-L2 does not affect to the growth-dormancy transition. Here, we present a new role of Populus TEM like genes in the transition from dormancy to growth.

Keywords: Poplar, flowering, transition, FT2, TEM

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Efficient plant regeneration protocol for superior *Eucalyptus camaldulensis x E. urophylla* hybrid and field performance at an early stage

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Micropropagation is an efficient technique for plant tissue rejuvenation and rejuvenated plants with improved rooting properties can be used as mother plants for commercial scale production. In present study, we developed a method for cloning elite *Eucalyptus camaldulensis* x *E. urophylla* hybrid using a micropropagation method. Micropropagated plants were used to raise a clonal micro-garden and for field planting. Nodal segments from epicormics shoots initiated at the base of a 4-year-old standing tree were used as explants. *In-vitro* cultures were established on Murashige and Skoog (MS) media with different combination of plant growth regulators (PGR's) and optimal combination were determined and followed further experiments. Primary shoots were initiated *in-vitro* using nodal explants and optimal results were achieved on MS media with 4.4 µM BA, 0.46 µM Kinetin and 0.54 µM NAA which were further multiplied on media with 2.2 µM BA and 0.054 µM NAA and shoot elongation carried on media with 1 µM BA + 1 µM Kinetin. *In-vitro* rooting was initially 60% with 7.4 µM IBA and addition of Riboflavin in rooting media improved rooting by another 10%. Base media contained 3% Sucrose, 50 mg/l Ascorbic acid, 50 mg/l Riboflavin, 100 mg/l PVP and pH adjusted to 5.7-5.8. Hardened micro-propagated plants were used to establish hedge garden to produce mini cuttings. In addition, well developed micro-propagated plants were planted directly in the field along with clonally propagated plants. The hedge garden plants stared producing 10-12 apical shoots per harvest once in 10 days at the age of six months. Growth and stem form of tissue cultured plants were on par with clonally propagated plants of the same elite hybrid at the age of 12 months.

Keywords: Eucalyptus Hybrid, In-Vitro propagation, Hedge garden, Rooting, Plant growth and stem form, Plant growth regulators.

Trees need closure, too – Do trees heal or seal bark wounds? Is there a change coming in bark treatments?

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Bark wounds abound all ecosystems and usually remove the bark and damage underlying cambium/meristem and vascular tissues (xylem and phloem). Wounds that are not maintained could potentially be hazardous because injuries expose trees to microbes that infect and cause discoloration and decay of the wood resulting in unhealthy, unsightly, and unsafe trees with shortened lifespans. Wound treatments must enhance vascular tissue regeneration and no external treatments are as effective and as strong as the tree's new wood. Our knowledge about these innate wound-induced wood formation processes remains scarce. Therefore, we developed a novel model based on Induced Somatic Sector Analysis (ISSA) technique. Our model acts as a genetic tool that can be used to study wound signaling at the cellular level. It also allows us to explore anatomical variations during different stages of wound-induced tissue regeneration. Unveiling these developmental and molecular mechanisms during plant wound responses takes us one step closer to devising improved treatment options for wounded trees.

First, we conducted our experiments in a time series and representative samples were sectioned and observed under an optical microscope. The microscopic imaging provided insights into the different developmental stages of wound-induced tissue regeneration. We identified five tissue types that are either functioning or reformed during the process. Different tree species showed different regeneration capacities, and newly formed tissues acquired their functional capacities within 30 days irrespective of the species. Therefore, our model provided valuable clues about tissue and time-specific wound signals. In conclusion, bark wounds do have the ability to heal if the damage is controlled. Having understood these anatomical stages, our genetic experiments to assist healing processes hold much promise for developing rapid, cheap, reliable, localized and tissue-specific treatments of wounds in mature tree stems; the change is coming!

Keywords: Cambium, Wound-induced tissue regeneration, Genetic models, ISSA, Plant vascular tissues

Transcription factor OfMYB21 regulates the synthesis of *OfTPS6*-mediated linalool in *Osmanthus fragrans*

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Terpenoids are the most characteristic aroma components of *Osmanthus fragrans*, and terpene synthase (TPS) is the first key enzyme at the branch point of terpenoid metabolism. In this study, the volatile components of *O. fragrans* were determined by gas chromatograph-mass spectrometer (GC-MS). Linalool, a monoterpene aromatic compound, was found to be the main volatile component of *O. fragrans*. Based on the transcriptome data of different tissues and florescence of *O. fragrans* and phylogenetic tree analysis, a highly expressed terpene synthase gene *OfTPS6* at florescence was isolated. Meanwhile, *OfTPS6* was proved to be a key linalool synthase gene by prokaryotic expression and enzyme activity assay in vitro. Through weighted gene co-expression network analysis, an upstream regulator of *OfTPS6*, OfMYB21, was further screened. Yeast one hybridization, double luciferase activity analysis and *O. fragrans* flower petal transient transformation experiment all proved that OfMYB21 can activate the expression of *OfTPS6*. Overall, our results provide an important scientific significance for elucidating the molecular mechanism of linalool enrichment in *O. fragrans* and for exploring the germplasm resources of high-quality *O. fragrans*.

Keywords: *Osmanthus fragrans*, Linalool, Transcriptional regulation, OfMYB21

Risk Assessment of *Prunus yedoensis* Street Trees in Daegu and Gyeongbuk Province

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We conducted risk assessment of *Prunus yedoensis* street trees in Daegu and Gyeongbuk Province in July 2021. A total of 60 trees were subjected to assessment in terms of tree attributes, wood-decaying fungi, and defects. Internal defect analysis was performed using sonic tomography at the height of 30 cm from the ground surface. Fungal fruiting bodies were collected in the street trees and identified based on DNA sequence data. Visual inspection revealed the ‘very good’ (48%), ‘good’ (46.4%) and 'bad' (1.6%) groups among the trees. The majority of external defects was derived from boring insects (64%), fungal fruiting bodies (18%), uncalloused wounds (13%), and cavity (2%). Using sonic tomography, either cavity or decay estimated as damaged area was found in 12 trees, where the average damaged percent was 3.3%. Wood-decaying fungi were identified as *Ganoderma gibbosum* (20%), *Trametes versicolor* (20%), and *Daedaleopsis confragosa* (13%). These results could provide insights into the effective management of *Prunus yedoensis* street trees in Daegu and Gyeongbuk Province.

Keywords: *Prunus yedoensis*, street trees, sonic tomography, internal defect analysis, wood decay

Arabidopsis thaliana root endodermis suberization in *WOX 9* mutants

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Suberin is a complex biopolymer deposited in the bark of trees. An extreme example of suberin deposition can be found in the bark (cork) of cork oak trees. In the Arabidopsis thaliana root tissues suberization also occurs, protecting the root from biotic/abiotic stresses. There are mainly two suberized tissues in the *Arabidopsis thaliana* roots: (1) the endodermis, which differentiates inwards to the cortex, close to the RAM, and progresses along the root axis giving rise to (2) the periderm, a protective outer tissue more prominent at the root-hypocotyl transition. Recently, a transcriptomic study performed at our lab revealed several promising candidate genes to be exclusively involved in cork differentiation in cork oak[1]. By making use of a simpler model system[2], we analyzed the loss of function mutants for the homolog candidate genes as to their periderm development phenotypes at the root-hypocotyl border in Arabidopsis. We found that mutants for the candidate *WUSCHEL-RELATED HOMEOBOX 9 (WOX9)*, a homeobox gene required for SAM growth showed significant changes in the width of periderm tissues when compared to the wild type. To investigate if the changes observed in the mutant periderm were caused by alterations in suberin deposition patterns during primary growth at initial stages of endodermis development, we are analysing the root patterns of suberization using the fluorescent Fluorol Yellow stain to 7-day old wild-type and mutant seedlings[3]. By examining the suberization patterns of the root endodermis our preliminary results indicate that *wox9* shows delayed suberization of the endodermal cells, when compared to the wild type or the overexpression mutant. We are currently artificially-inducing suberization in the mutants using hormones[4] to further explore the possible regulatory roles for *WOX9* gene and its positioning in potential existing regulatory pathways.

Keywords: cork, endodermis, periderm, suberin, *WUSCHEL-RELATED HOMEOBOX 9*

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Internal defect analysis of American sycamore infected by *Perenniporia fraxinea* using sonic and electrical resistance tomography

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Urban trees under abiotic and biotic stresses are prone to having various defects, raising a concern about tree hazard. Internal defects in trunk are mainly caused by wood-decaying fungi and might lead to unexpected tree failure. We conducted the non-invasive internal defect analysis of American sycamore (*Platanus occidentalis*) street trees with fungal fruiting bodies in Daegu, Korea. Internal defect analysis in 2020 was performed at the height of 30 and 70 cm from the ground surface using sonic tomography (SoT). Electrical resistance tomography (ERT) was additionally performed in 2021. The orange fruiting bodies at the trunk were identified as *Perenniporia fraxinea* based on DNA sequence data. SoT in 2020 detected the abnormal wood area which was estimated as decay only at the height of 30 cm, where damaged area percent was 11%. In 2021, the damaged area percent at the height of 30 cm was 14%. ERT showed a low resistance at the analogous location as the damaged area of 30 cm height, supporting the presence of decayed area. These results provide the insights into temporal expansion and distribution of the internal defects of American sycamore infected by *P. fraxinea.*

Keywords: American sycamore, *Perenniporia fraxinea*, non-invasive internal defect analysis, sonic tomography, electrical resisitance tomography

MicroRNA-mediated post-transcriptional regulation of sesquiterpene pathway genes in *Santalum album*

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Santalum album (Indian sandalwood) is an obligate evergreen hemi-parasitic tree, indigenous to India, eastern Indonesia and northern Australia. The essential oil extracted from the heartwood is an important commodity in the International market. *S. album* yields the highest oil content of 6-7% compared to *S. austrocalendocium, S. spicatum* and *S. yasi.* The essential oil is composed of four major sesquiterpenols: (*Z*)-α-santalol, (*Z*)-β-santalol, (*Z*)-*epi*-β-santalol and (*Z*)-α-*exo*-bergamotol synthesized through mevalonate (MVA) or methylerythritol phosphate (MEP) pathway. The present study was undertaken to document the post-transcriptional regulation of sesquitepene pathway in *S. album*. The wood tissue from a ~15-year-old tree was harvested for transcriptome and small RNA sequencing. *De novo* assembly and annotation of transcriptome predicted a total of 40,604 transcripts with GO terms assigned to 26,329 transcripts. A total of 47 transcripts from the sesquiterpene pathway were mined for further analysis. Small RNA data analysis led to the discovery of 55 novel miRNA in the wood of *S. album,* which has not been reported hitherto. An integrated analysis of small RNA and transcriptome data revealed that major sequiterpene pathway genes including farnesyl diphosphate synthase, santalene synthase, sesquisabinene synthase, bergamotene oxidase and cytochrome P450 reductase were regulated by miR156, miR159 and miR319. Three miRNA families were found to target santalene synthase, the major catalytic gene in the sesquiterpene pathway. The abundance of miRNA and their putative gene targets was further confirmed by quantitative real-time PCR. This study provides the first insight into the post-transcriptional regulation of sesquiterpene pathway in the genus *Santalum* and further analysis across genotypes will predict the probable role of miRNA in determining the oil yield and santalol content in Indian sandalwood.

Keywords: MicroRNA, transcriptome, sesquiterpenols, santalene synthase, real-time PCR

Insights into Scots pine and black pine timber identification in timber trade and presence of hybrids in natural populations

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During last years, the major timber consuming markets have introduced laws to ensure the legality of the timber traded an avoid frauds, such as the EU Timber Regulation, US Lacey Act or Australian Illegal Logging Prohibition Regulation. In many cases, this implies the need to validate whether or not the timber belongs to the species listed in the documentation accompanying the shipment. However, timber species identification is still and unsolved problem, since for many timber species do not yet exist the tools needed. In many cases, anatomical characterization allows only assigning at genus level, and then genetic, isotopic, spectrometric or machine learning approaches are needed to identify the species.

This is the case with the wood of *Pinus sylvestris* L. and *Pinus nigra* Anr., the two most relevant pine species in the Spanish market. Compared to Scots pine, black pine wood have a higher resistance to bending and a higher modulus of elasticity, having the disadvantage of being much more prone to deformation after drying. However, it also has the advantage that the rotting of standing trees is significantly lower than in Scots pine. Despite these technological differences, the timbers of Scots and black pines are anatomically indistinguishable.

The Timber Species Identification Service of INIA (CSIC) is working on molecular marker techniques to differentiate the timber of these species. The analyses developed have resulted in the identification of several genetic patterns compatible with the presence of natural hybrids of both species in certain populations, which will be the subject of this communication. Since the wood of these species are commonly used in structural applications where strength and quality are the primary factors, If confirmed, this result could have impact in the way in which forests are managed and how these woods should be used.

Keywords: *Pinus, P. sylvestris*. *P. nigra*, species identification, hybridization.

Transcriptome analysis of developing xylem with shade response in three poplar hybrids

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Poplars have a high response to light, and shading is one of the main environmental factors that limits the growth and development of poplars. Exploring the response mechanism of developing xylem of poplar under shading is of great reference significance for improving wood yield. In this study, three excellent poplar hybrids (*Populus euramericana* 'Zhonglin46'(Pe), *Populus deltoides* '27-17'(Pd), *Populus* × 'Wq156' (Pw)) under shading conditions were studied. Based on phenotypic data and developing xylem transcriptome analysis, the molecular mechanism of poplar to shade was preliminarily revealed, and the core regulatory genes responding to shade were identified by weighted co-expression network analysis (WGCNA). The results showed that Pw growth was significantly affected by shade, while Pe growth was slightly affected by shade. Enrichment analysis of 13,675 differentially expressed genes (DEGs) indicated that different poplar hybrids had specific biological functions and regulatory processes within and between species. WGCNA analysis identified two modules ("Brown" and "Purple") related to shading response, and discovered seven hub genes. These hub genes were related to xylem development, vascular cambium division, stomatal development and *Phytochrome A* signal transduction, respectively. These results provide important basic information for gaining insight into the molecular response to shading in different poplar hybrids.

Keywords: Poplar, Shade, Developing xylem, Transcriptome, Co-expression network

Gender differences in physiological tolerance, enrichment and transport characteristics of *Hippophae rhamnoides* subsp. *sinensis* seedlings to soil cadmium

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Using male and female biennial seedlings of *Hippophae rhamnoides* subsp. *sinensis* as research materials in this study. A pot experiment was conducted to study the growth, antioxidant system, osmoregulation substances, Cd content and accumulation characteristics of *Hippophae rhamnoides* subsp. *sinensis* male and female seedlings under the treatment of soil Cd concentration (mg · kg^{-1}) of 0 (CK), 25 (C1), 50 (C2), 100 (C3) and 200 (C4). It is expected to lay a theoretical foundation for the application of *Hippophae rhamnoides* subsp. *sinensis* in the remediation of Cd contaminated soil, and also provide scientific guidance for the sex selection of *Hippophae* in the remediation of Cd contaminated soil. The results showed that: (1) compared with CK, the treatment of low concentration (C1-C2) had no significant effect on the growth of plant height and basal diameter of *Hippophae rhamnoides* subsp. *sinensis* seedlings ($P > 0.05$)；The growth of plant height and basal diameter decreased significantly ($P < 0.05$) under the treatment of high concentration (C3-C4), and the decline was slightly lower in females than in males. (2) With the increase of Cd concentration, the activities of SOD, POD, CAT and APX both male and female plants tended to increase first and then decrease, and the activities in female plants were higher than in male plants, among which the activity of SOD in female plants was significantly higher than in male plants ($P < 0.05$)；The MDA content of male and female plants showed an upward trend, and the MDA content of male plants was always slightly higher than that in female plants. (3) With the increase of Cd concentration, the contents of Soluble Protein (SP), Soluble Sugar (SS) and Proline (Pro) were increasing in both males and females, and the contents of three osmoregulatory substances in female plants were higher than in male plants. (4) With the increase of Cd concentration, the Cd content in various organs of male and female plants increased significantly, among which the roots accumulated the most, and the Cd content in various organs of female plants was higher than that of male plants, but the Cd content of female plants aboveground was significantly higher than that of male plants ($P < 0.05$)；Bioconcentration factors (BCF) of female and male roots were between 2.33 ~ 3.57 and 2.04 ~ 2.91, respectively, and BCF of female and male aboveground parts were between 0.59 ~ 1.05 and 0.38 ~ 0.76, respectively；However, Translocation factor (TF) of male and female plants were less than 1, ranging between 0.21 ~ 0.48 and 0.14 ~ 0.33, respectively. With the increase of Cd concentration, BCF of male and female plants tended to increase first and then decrease, while TF decreased, but BCF and TF of the root and aboveground of female plants were higher than those of male plants. In conclusion, *Hippophae rhamnoides* subsp. *sinensis* seedlings a have strong tolerance to Cd stress. Under the treatment of low concentration (C1-C2), it have stronger physiological tolerance and enrichment and transport capacity, while female plants have stronger physiological tolerance and enrichment and transport capacity to Cd stress than male plants. Therefore, this study suggests that female plants of *Hippophae rhamnoides* subsp. *sinensis* are more conducive to be used as a Phytoremediation tree species for soil Cd pollution than male plants.

Keywords: *Hippophae rhamnoides* subsp. *Sinensis*; Cadmium stres; Gender difference; Physiological tolerance; Enrichment and transport capacity

Study on the coarse root distribution of standing trees in Northeast China based on ground penetrating radar (GPR)

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This study is to quantify the root distribution and root accuracy of GPR detection of live standing trees under clay condition, and to determine the specific factors that affect the accuracy of the GPR detection. It was conducted in Xinganling forest region. GPR was used to scan the coarse root distribution of 3 *Piceas* and 3 *Pinus koraiensiss*. The actual roots of a *Picea* sample were excavated for further analysis and the point cloud of the coarse roots were subsequently obtained with a three-dimensional laser scanner. GPR test shows that all samples' root scope is wider horizontally than that vertically, the accuracy of GPR detection of *Picea*'s is 58.52%. The accuracy is positively correlated with the diameter of coarse roots, and negatively correlated with the depth of soil and the density of coarse roots. The health status of coarse roots has a great influence on the accuracy of GPR detection. All samples' coarse roots morphology is horizontal in study site. GPR performs well in detecting the underground distribution of coarse roots in clay condition. The detection accuracy was mainly affected by the diameter, density and the health status of coarse roots, and the depth and clay content of soil.

Keywords: Dark brown forest soil; Clay content; GPR; Coarse roots; Nondestructive root detection

Nano-FTIR and Nanomechanical Mapping of Secondary Cell Walls and Compound Middle Lamella in Poplar Hardwoods of Varying Recalcitrance

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Nano-FTIR and PeakForce quantitative nanomechanical mapping (PF QNM) are new AFM-based nanoscale techniques that can be applied to plant tissues to get high-resolution in spatial distribution of cell wall components that are unavailable from bulk characterization. These imaging and mapping approaches can be useful for finding phenotypic changes to plant cell walls by genotype and environment. Three natural *Populus trichocarpa* variants of varying recalcitrance and composition, i.e., high lignin, low lignin, and *epsps* low recalcitrance mutant associated with 5-enolpyruvylshikimate-3-phosphate synthase biosynthetic gene that lowers the lignin content, were investigated by nano-FTIR and PF QNM. In nano-FTIR spectra of secondary cell walls (SCW) and compound middle lamella (CML), the 1162 cm^{-1} and 1269 cm^{-1} peaks could distinctly identify polysaccharides and lignin, respectively. The differences between SCW and CML in intermolecular hydrogen bonding of cellulose based on the 997 cm^{-1} peak indicated that cellulose in CML might have a different ordering of cellulose nanofibrils than crystalline cellulose in SCW. Spatial variability in content of polysaccharides and lignin was significantly larger in CML than SCW for all three poplar genotypes. PF QNM measurements revealed that *epsps* mutant poplar had significantly reduced cell wall adhesion compared to its high lignin content counterpart. A lowered adhesive force may be a highly beneficial trait associated with cell wall disintegration during the carbohydrate deconstruction aimed at the production of biofuels. The rare mutation, however, did not affect any other nanomechanical properties important to the growth of the poplar trees such as cell wall stiffness quantified by the reduced Young's modulus of elasticity, cell wall toughness quantified by dissipation energy, or cell wall deformation. These findings provide new insights into differences among poplar plants associated with a recalcitrance to the deconstruction of lignified cell walls.

Keywords: Cell Wall Nanomechanics, Cell Wall Polysaccharides, Cellulose, Lignin.

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Cell Wall Compositional Assessment of Grey Poplar Hybrids

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Interspecific hybrid poplars, such as grey poplar (*Populus* × *canescens*), are rated among the most promising tree species for biofuel and bioenergy production due to a minimal requirement for fertilizer and an ability to grow on marginal infertile lands. In this study, we focused on the assessment of the chemical profiles for the artificial interspecific hybrids of grey poplar in terms of the content of main woody cell wall components and extractives, lignin monomer composition and neutral saccharide composition. The clone T-9, (*P.* × *canescens*) × *P. grandidentata*, showed the reduced lignin content (down to 17.6%) with the abundance of syringyl units (syringyl-to-guaiacyl ratio in lignin equals 2.3), and the increased proportion of exctractives (4.9%). From the saccharides, the proportion of D-glucose achieved 51.3%, whereas for D-xylose and D-galactose it was 18.1 and 4.1%, respectively. The proportions of L-arabinose and D-mannose were not higher than 2.2% for each. The micropropagated plants of the T-14 clone, *P. tremula* × (*P.* × *canescens*), also showed the lignin content lower than 20% (i.e., 18.2%), almost identical syringyl-to-guaiacyl ratio in lignin (2.3), but the proportion of exctractives was lowered (down to 3.3%). From the saccharides, the proportion of D-glucose achieved up to 69.8% that was the most distinctive difference between the two examined clones. Also, the proportions of D-xylose and D-mannose were increased (22.4 and 4.2%, respectively), whereas for D-galactose and L-arabinose it was less than 2.0% for each. The cell wall compositional characteristics of the T-14 micropropagated plants were then compared to those of the T-14 plants propagated from root cuttings. These plants were used as the counterparts to the micropropagated plants because root cuttings are the preferred option of source material over the poorly rooting stem cuttings for conventional vegetative propagation of grey poplar and its artificial interspecific hybrids. The performance of the T-14 micropropagated plants was higher for the content of cellulose, D-glucose and D-mannose. On the other hand, the performance of the T-14 plants propagated from root cuttings was superior for the content of hemicelluloses, D-xylose and L-arabinose. The T-14 micropropagated plants were found to be a promising renewable resource of fermentable sugars for the biofuel industry.

Keywords: Biofuels, Cellulose, D-Glucose, Extractives, Lignin.

Acknowledgements: This work was supported by the Slovak scientific grant agency VEGA (1/0450/19).

CRISPR-Knockout of *CSE* Gene Improves Saccharification Efficiency by Reducing Lignin Content in Hybrid Poplar

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Caffeoyl shikimate esterase (CSE) has been shown to play an important role in lignin biosynthesis in plants and is, therefore, a promising target for generating improved lignocellulosic biomass crops for sustainable biofuel production. Populus spp. has two *CSE* genes (*CSE1* and *CSE2*) and, thus, the hybrid poplar (*Populus alba* x *P. glandulosa*) investigated in this study has four *CSE* genes. Here, we present transgenic hybrid poplars with knockouts of each *CSE* gene achieved by CRISPR/Cas9. To knockout the *CSE* genes of the hybrid poplar, we designed three single guide RNAs (sg1–sg3), and produced three different transgenic poplars with either CSE1 (CSE1-sg2), CSE2 (CSE2-sg3), or both genes (CSE1/2-sg1) mutated. CSE1-sg2 and CSE2-sg3 poplars showed up to 29.1% reduction in lignin deposition with irregularly shaped xylem vessels. However, CSE1-sg2 and CSE2-sg3 poplars were morphologically indistinguishable from WT and showed no significant differences in growth in a long-term living modified organism (LMO) field-test covering four seasons. Gene expression analysis revealed that many lignin biosynthetic genes were downregulated in CSE1-sg2 and CSE2-sg3 poplars. Indeed, the CSE1-sg2 and CSE2-sg3 poplars had up to 25% higher saccharification efficiency than the WT control. Our results demonstrate that precise editing of *CSE* by CRISPR/Cas9 technology can improve lignocellulosic biomass without a growth penalty.

Keywords: biofuels, caffeoyl shikimate esterase (CSE), CRISPR/Cas9, lignin, saccharification

Genome-wide identification of the AAAP gene family in *Populus* and functional analysis of *PsAAAP21* in adventitious root growth

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Adventitious root (AR) is the basis for the successful propagation of plant cuttings and tissue culture and is essential for maintaining the traits of excellent varieties and expanding plant roots. Members of the amino acid/auxin permease (AAAP) gene family play indispensable roles in various plant metabolism and biosynthesis processes. Analysis and functional verification of the AAAP genes in poplar will increase understanding of this important gene family and is of great significance for improving the root development of woody plants. In this study, a systematic bioinformatics analysis of the poplar AAAP family was carried out, and the potential gene *AAAP21* regulating root development was screened by combining the results of RNA-Seq and QTL. 83 *PtrAAAPs*, divided into 8 subfamilies, were identified in the poplar genome. *PsAAAP21* interacted with the root development gene *LST6* gene, and the overexpression lines of *PsAAAP21* were proved to promote adventitious root formation and improve the survival rate of cuttings. The results of this study are important for understanding the structure and expression patterns of *PtrAAAPs* and further predicting their functions. Meanwhile, the discovery of the promotion effect of *PsAAAP21* on rooting of *Populus* cuttings provides a theoretical basis for understanding the functions of AAAP genes on AR formation and breeding new woody plant species with strong rooting ability.

Keywords: Poplar, *PtrAAAPs*, Adventitious root, Genetic transformation, QTL

PeCLH2 gene positively regulate salt tolerance in transgenic *Populus alba* × *Populus glandulosa*

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Salt is an important environmental stress factor, which seriously affects the growth, development and distribution of plants. Chlorophyllase (Chlase, CLH) plays an important role in stress response. Nevertheless, little is known about the physiological and molecular mechanism of CLH genes to plants. We cloned *PeCLH2* from *Populus euphratica*, and found that *PeCLH2* was differentially expressed in different tissues, especially in the root of *P. euphratica*. PeCLH2 protein localized to the nucleus. To further study the role of *PeCLH2* in salt tolerance, *PeCLH2* overexpression and RNA interference transgenic lines were established in *Populus alba* × *Populus glandulosa*, and used for salt stress treatment and physiologic indexes studies. Overexpressing lines significantly improved tolerance to salt treatment and reduced reactive oxygen species production. RNA interference lines showed the opposite. Transcriptome analysis was performed on leaves of control and transgenic lines under normal growth conditions and salt stress to predict genes regulated during salt stress. This provides a basis for elucidating the molecular regulation mechanism of *PeCLH2* in response to salt stress and improving the tolerance of poplar under salt stress.

Keywords: *Populus euphratica*, *PeCLH2*, Salt stress, Physiologic indexes, Transcriptome

Genomic selection in forest tree breeding: climbing the slope of enlightenment

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Almost 12 years have passed since the early prospects of applying genomic selection (GS) to forest tree breeding were published, initially based on deterministic simulations, and quickly followed by empirical reports in species of *Eucalyptus*, *Pinus* and *Picea*. Given its solid prospects for causing a paradigm shift in the way that tree breeding will be done in the years to come, GS passed from a hot topic to a fast-moving area of applied research and operational implementation in several organizations worldwide, both public and private. Following the pioneering steps of GS in animal breeding, we have passed the initial phases of the Gartner's hype cycle of emerging technologies and we are now definitely climbing the slope of enlightenment. GS in tree breeding is a real thing and not one more hyped "biotech bandwagon". By converging modern high-throughput DNA typing and time-proven quantitative genetics methods, GS moved the focus away from the unrealistic concept of dissecting a complex trait in its individual components. Instead of trying to find the needle in a haystack, i.e., the "magic" gene in the complex and fluid genome, GS "buys the whole haystack" of genomic effects to predict complex phenotypes, similarly to an exchange-traded fund that more efficiently "buys the whole market". Tens of studies have now been published in forest trees showing that GS matches the accuracy of phenotypic selection for growth and wood traits, enhancing the rate of genetic gain by increasing selection intensity, radically reducing generation interval and improving the accuracy of breeding values. Breeder-friendly and cost-effective SNP genotyping "chips" are now available for all mainstream plantation forest trees, but even more efficient methods based on low-pass whole genome sequencing with imputation are expected to further reduce genotyping costs. Yet, predictive abilities are impacted by GxE interaction and driven mainly by relatedness, such that population-specific predictive models are necessary. While the fundamental aspects of GS are now solidly established in tree breeding, strategic and logistics aspects for the optimized adoption of GS are now the challenges to fully integrate this new breeding technology into routine tree improvement.

Key words: Genomic selection, tree breeding, SNPs

Large-scale sequencing of Eucalypt genomes to unravel genome variation for woody biomass production

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Eucalypt species including >900 members of the sister genera *Eucalyptus, Corymbia* and *Angophora*, harbor vast amounts of genetic, chemical and adaptive variation, most of which has not been unraveled at the genomic level for breeding and genetic studies. In particular, the extent of pan-genome variation within and between eucalypt species is largely unknown. To overcome challenges with the assembly of highly outbred eucalypt genomes and obtain the first detailed views of pan-genome variation in eucalypt species, we (Lötter, Candotti et al.) used a trio-binning approach to sequence (60-100X Oxford nanopore, 100X Illumina) and assemble the *E. grandis* and *E. urophylla* haplogenomes in three F1 hybrids of these species revealing tens of thousands of structural variants that have not been described before. In parallel, we (O'Neill-Mostert et al.) used genome-wide SNP genotyping of a range-wide collection of *E. grandis*, as well as three breeding populations, to obtain the first views of natural genomic variation and of the genomic consequences of 100 years of early domestication and selective breeding of a eucalypt species. To expand these views, we (PIs Myburg, Wegrzyn and Borevitz) initiated a US-Department of Energy - Joint Genome Institute (JGI) funded project aimed at reference sequencing (60X PacBio HiFi, HiC) of 10 eucalypt species representing diverse phylogenetic lineages, diversity sequencing (40X Illumina) of over 900 eucalypt species, pan-genome sequencing (30X HiFi) of 24 individuals each of *E. grandis* and *E. melliodora*, and skim-seq (10X Illumina) of 2800 trees of these two species hosted in common gardens established in South Africa and Australia. The project will produce genome sequences for 3700 trees, which we propose to make available for a community effort to produce genomic data for a 10,000-eucalypt genome initiative (10KEGI). We invite the tree biotechnology community to participate in the 10KEGI effort to unlock the vast genome diversity of the eucalypts for next-generation breeding and genetic studies.

Keywords: *Eucalyptus*, *Corymbia, Angophora,* pan-genome, structural variants, common gardens, woody biomass

Genomic selection: an effective tool for *Eucalyptus globulus* clonal selection

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Arauco is one of the largest forest companies in Chile and it has an intensive clonal breeding selection program for *Eucalyptus globulus* with the main objective to increase forest productivity for pulp production. As a new challenge to improve and accelerate gain capture, the company is incorporating genomic selection as part of the clonal selection strategy. To implement this new strategy, an *E. globulus* clonal population was used to train a model to predict genetic merit at rotation age, based on single nucleotide polymorphism (SNP) markers from DNA extracted at juvenile age from plants in the nursery. A total of 600 clones were used to train the model where pulp productivity, measured as Air Dry Tones of cellulose per tree (ADt), was measured on all clones and it was the trait to be correlated with the SNP marker genotypes. The average predictive ability estimates for ADt was 0.71 and the standard deviation of the cross-validation iterations was 0.04. A genomic best linear unbiased prediction model (GBLUP) was used to predict genomic estimated breeding values (GEBVs) for a different prediction population, consisting of 3,368 new rooted clones recently developed at the Arauco nursery. The clones from the training and prediction populations were genotyped at 20K SNPs. The prediction of new clones with unknown phenotypes allowed the pre-selection of 90 top clones that will be propagated at the nursery to establish validation field trials. Based on the results of these trials, new operational clones will be selected in order to increase genetic gain.

Keywords: *Eucalyptus globulus*, genomic selection, SNP, clonal selection, pulp productivity (ADt).

Haplotype and structural variant-based dissection of quantitative traits in *Eucalyptus*

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Eucalyptus, an important genus of woody perennials for the forestry industry, harbours vast genetic, chemical and morphological diversity. Interspecific hybrids are commonly planted as they combine favourable traits from two species into a single genetic background. Currently, genome-wide SNP markers are being used for complex trait dissection. Haplotype and structural variation, key components of pan-genome variation, are relatively unexplored as sources of complex trait variation in these outbred organisms. To address this, we are characterising haplotype and structural variant (SV) diversity in *Eucalyptus* species and hybrids. Using Flex-seq technology (RAPiD Genomics), we developed a haplotype mining and genotyping panel with 8,915 high quality probe sets targeting 4,637 genes with multiple evidence lines supporting their involvement in growth, wood properties and biotic and abiotic interactions. We used the haplotype marker panel to identify 88,968 discrete haplotypes in *E. grandis, E. nitens, E. urophylla* and *E. dunnii* samples (average of three to four haplotypes per target region). To perform a genome-wide assessment of haplotypes inherited by F_1 progeny of *E. grandis* and *E. urophylla*, we sequenced and assembled the parental haplogenomes of three F_1 hybrids through a trio-binning approach using ~100X short-read (Illumina) of the parents and ~200X long read (Oxford Nanopore) and ~100X short-read (Illumina) data of the hybrid individuals. We scaffolded the phased haplocontigs against high-density genetic linkage maps of the parents to obtain chromosome-scale haplogenomes. SVs were identified within and between the parental species. 384 F_1 hybrid progeny from each of a series of interconnected full-sib families from a multi-parent F_1 mapping population are being used for QTL detection within families and nested association mapping across families. We aim to identify haplotypes and SVs underlying significant associations as a first step towards assessing and understanding how these two types of pan-genome variation may affect quantitative trait variation in *Eucalyptus*.

Keywords: Genetic dissection; Haplogenome; Hybrid genetics; Structural variant

Genomic tools for species restoration: the case of American chestnut

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Introduction of *Cryphonectria parasitica* to North America in 1904, and its subsequent spread, functionally extirpated American chestnut (*Castanea dentata*) from its natural range by the middle of the 20th century. The loss of American chestnut was catastrophic from ecological, economic, and societal perspectives. Two approaches to developing blight resistant chestnuts currently show promise. First, The American Chestnut Foundation has developed segregating populations by hybridizing Chinese chestnut (*Castanea mollissima*), which is resistant to the blight, with American chestnut, followed by repeated backcrossing to American chestnut using pollen from rare, wild, flowering trees. Second, genetically modified trees expressing a wheat oxalate oxidase (OxO) transgene have been developed, which have resistance approaching that of Chinese chestnut. However, each of these methods pose challenges in a species restoration conext. For backcross breeding, polygenic inheritance combined with low heritability makes selection of the best families difficult. For transgenics, a uniform genetic background in the founding line means multiple generation of outcrossing are required to recover a reasonable effective population size and to incorporate adaptive genomic variation. To address the selection bottleneck, we developed a genomic prediction model that circumvents time-consuming and costly progeny tests. To aid in diversifying transgenic lines, we sequenced whole-genomes of many wild *C. dentata* stump sprouts, and applied a variety of modern population genomic tools to understand patterns of neutral and adaptive variation. These results will guide *ex situ* conservation of wild germplasm for breeding with transgenic lines. Finally, we have sequenced a panel of 96 additional *Castanea* species that vary in blight resistance, which will inform selection of additional targets for transgenic manipulation or genome editing. I will summarize results and progress from each of these projects, which support our long-term goal of developing disease resistant, locally adapted American chestnut populations for restoration of the species.

Keywords: Forest health, genomics, restoration, *Castanea*, adaptation

The *Litsea* genome and the evolution of the laurel family

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The laurel family within the Magnoliids has attracted attention owing to its scent, variable inflorescences, and controversial phylogenetic position. *Litsea cubeba*, the core Lauraceae species, is an important species for producing essential oils (roughly 80% citral). Here, we present a chromosome-level assembly of the *L. cubeba* genome, together with low-coverage genomic and transcriptomic data for many other Lauraceae. Phylogenomic analyses show phylogenetic discordance at the position of Magnoliids, suggesting incomplete lineage sorting during the divergence of monocots, eudicots, and Magnoliids. An ancient whole-genome duplication (WGD) event occurred just before the divergence of the Laurales and Magnoliales; subsequently, independent WGDs occurred almost simultaneously in the three Lauralean lineages. The phylogenetic relationships within Lauraceae correspond to the divergence of inflorescences, as evidenced by the phylogeny of FUWA, a conserved gene involved in determining panicle architecture in Lauraceae. Monoterpene synthases responsible for the production of specific volatile compounds in Lauraceae are functionally verified. The Lauraceae-specific terpene biosynthesis gene cluster was identified and investigated using a multifaceted approach combining phylogenetic, collinearity, biochemical, and transgenic *L. cubeba*. Aggregation of specific terpene biosynthetic pathways suggests they may form the basis of important characteristics that enhance adaptability. Furthermore, the metabolic regulatory network underlying terpene biosynthesis and accumulation was investigated. Overall, our work sheds light on the evolution of the Lauraceae, the genetic basis for floral evolution and specific scents.

Keywords: *Litsea cubeba*, genome, evolution, terpene, metabolic regulation

Transcriptional Regulation of Pectin Demethylesterification

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Pectins are synthesized in Golgi and then transported to the primary cell wall in a highly methy-esterified state, where they are demethylesterified by pectin methylesterases (PMEs). The degree and pattern of methylesterification determine the functional properties of pectin, and thus affect plant growth and development, stress response etc. However, the regulation mechanisms of pectin demethylesterification are largely unknown. By employing seed coat mucilage as a model system, we have identified a few transcription factors that regulate pectin demethylesterification process. MYB52 and ZAT5 act as transcriptional activators and repressors to negatively regulate pectin demethylesterification by targeting PMEI and PME genes respectively. While ERF4 and BLH2/4 act as transcriptional activators and repressors to positively regulate pectin demethylesterification by targeting PMEI and PME genes respectively. ERF4 and MYB52 can physically interact to antagonize each other's regulation fuction to fine tune the pectin demethylesterification process. The similar physical interaction was also observed between ZAT5 and BLH2/4. These findings will lay foundation for the exploration of pectin demethylesterification regulatory network.

Keyword： transcription factor; pectin; demethylesterification; PME (pectin methylesterase)

Dynamic genetic architecture reveals natural variation in *PtoP4H9* is a robust factor responsible for perennial stem growth in *Populus*

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Perennial trees need to maintain growth throughout their entire lifespan to promote dynamic stem growth. The genetic basis of perennial stem growth has remained elusive. Here we deciphered the dynamic genetic architecture that underlie the perennial growth trajectories, by using GWAS for annual measurements of growth traits in a natural population of *Populus tomentosa*. By integrating the results with dynamic expression patterns, the candidate genes were divide into three regulatory patterns: (1) 211 time-specific genes, i.e. *PtoAPF2*, *PtoHDG11*; (2) 30 general genes, i.e. *PtoBIGE1B*, *PtoP4H9*; (3) eight pleiotropic genes, i.e. *PtoNST1*, *PtoHB-14*. We then focused on a major general gene *PtoP4H9*, encoding a prolyl 4-hydroxylase 9, contributes to diameter at breast height (DBH) growth at seven successive time points. Overexpression and knock-down of *PtoP4H9* revealed that this gene promotes stem growth by regulating cell wall assembly to alter cell expansion in *Populus*. We show that natural variation in *PtoP4H9* are located in a core-promoter element of *PtoBPC1* that control the expression of *PtoP4H9*. The geographic distribution of natural variation in *PtoP4H9* is consistent to the modes of selection among populations. Taken together, our study provides important genetic insights into dynamic stem growth in *Populus*, and the causal loci or genes we identified can be important for accelerating the genetic improvement in perennial trees.

Keywords: Dynamic genetic architecture, Stem dynamic growth, *PtoP4H9*, Cell expansion, *Populus*

The cytosolic alkaline/neutral invertase, HbNIN2, and 14-3-3 module participates in rubber production of *Hevea* tree

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Natural rubber (NR), *cis*-1, 4-polyisoprene, is an indispensible industrial raw material with wide applications, e.g. for manufacturing aircraft and heavy-duty tires. *Hevea brasiliensis*, a tropical tree species, is currently the sole commercial NR source. In *Hevea* rubber-producing laticifers, a cytosolic alkaline/neutral invertase (CINV), HbNIN2, is critical for fueling the precursor sucrose into rubber production. Here, we show that HbNIN2 directly interacts with 14-3-3 proteins, and a hexamer phosphor-peptide "KRSSpSW" at the C-terminus is the core motif of HbNIN2 for 14-3-3 binding. The 14-3-3 binding significantly activates the invertase activity of recombinant HbNIN2, and such activation may associate with the 14-3-3-induced structural change observed in HbNIN2 catalytic domain. The enzymatic stimulation of 14-3-3 on invertase also occurs in *Hevea* latex, the cytoplasm of laticifers and the rubber-harvesting product, and correlates closely with the tapping-induced latex production. Thus, our findings shed light on the active participation of a CINV-14-3-3 module in rubber production.

An excellent haploid poplar cell line could be the HeLa in woody plant

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HeLa cells have offered an enormous help in advancing human medical and biological research. The HeLa cell in higher plant also has long been desired. Plant cell suspension culture is an important tool for basic studies in cellular and molecular biology research. However, to obtain an ideal suspension cell line is usually hard to achieve because plant cells usually are not susceptible to suspension culture. Also, even now no excellent woody cell line was widely used in molecular biology research of tree. In present study, we obtained a doubled haploid cell line, named Qu-1, from a population of haploid origin from *Populus simonii* × *P. nigra* by anther culture method. This cell line showed some unique genetic characteristics, exceptionally high dispersibility for suspension culture, and quick growth rate, efficient transient and *Agrobacterium*-mediated transformation, ability of regeneration of whole plant, and all of these unique genetic characteristics will be useful for molecular biological experiments. Also, the high-quality whole genome sequencing data of Qu-1 are available. We suppose Qu-1 will be a HeLa cell line in woody plant.

Keywords: *Populus*, suspension cell, haploid

When to bud break: molecular control of dormancy-growth transition in poplar

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Annual dormancy-growth cycle is a developmental and physiological process essential for the survival of deciduous trees in temperate and boreal forests. Seasonal control of shoot growth in woody perennials requires specific genetic programs responding to environmental signals. The mechanisms that regulate the shift between winter dormancy and growth-promoting genetic programs remains under elucidation. Here we present our latest contributions to understand different key regulators involved in this process: **1**) We have found that Populus ortholog to Arabidopsis FT repressors, TEMPRANILLO like 1 and 2 (TEM like 1 and TEM like 2), do not regulate FT2 expression and short day induced growth cessation. Our results show TEM like 1 and TEM like 2 play a role in the transition from dormancy to growth. **2**) Dynamics in genomic DNA methylation levels are involved in the regulation of dormancy-growth cycle in poplar. The reactivation of growth in the apical shoot during bud break process in spring is preceded by a progressive reduction of genomic DNA methylation in apex tissue. The induction in apex tissue of a chilling-dependent poplar DEMETER-LIKE 10 (PtaDML10) DNA demethylase precedes shoot growth reactivation[1]. **3**) We have shown that overexpressing a member of SOC1-like genes promotes bud break in ecodormant poplars. Our results support MADS12 participation in the reactivation of shoot meristem growth during ecodormancy and link MADS12 activation and GA2ox4 downregulation within the temporal events that lead to poplar bud break[2]. Taking into consideration the importance of a precise time for bud break, flowering, fruit development, and vegetative growth in a global warming scenario, the biotechnological use of these key regulators will reduce large economic losses in tree plantations.

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Pitro50K, a genome-wide SNP genotyping resource for data-driven molecular breeding of tropical pines

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Plant breeding has become a data science as a result of advanced phenotyping platforms and the application of next-generation DNA sequencing and genotyping technologies. However, a lack of genome-wide genotyping resources in pine tree species, partly due to the enormous size of their genomes, has hampered progress with the implementation of genomic technologies in pine breeding programmes. We performed genome and gene targeted SNP discovery towards the development of a genome-wide 50,000 marker, multi-species genotyping array for tropical pines (Pitro50K) using the Axiom 384-format SNP array platform (ThermoFisher). This cost-effective resource is serving as a basis for a new data-driven framework supporting genome-assisted breeding of tropical pines. Thousands of pine trees from breeding populations in South Africa, as well as natural populations in Mexico and Central America, are being genotyped towards a genome diversity atlas and the development of species and hybrid identification methods. We recently created the first topical pine genetic linkage maps, of a clonal F1 *P. patula* x *P. tecunumanii* low elevation (LE) family. Combined across the two parental maps, we now have 8,474 SNP markers with positions on a genetic map. When sufficient genetic maps are available, it will be possible to perform genome-wide ancestry mapping to determine the genetic composition of F1 and F2 hybrids at chromosomal level, and perform quantitative trait locus mapping in biparental crosses. Genetic maps will also help to anchor a future tropical pine genome assembly. We are inviting the tree biotechnology community to (i) use the Pitro50K SNP chip and (ii) contribute to producing community resources such as genetic linkage maps. In the longer term, we aim to implement genomic selection in tropical pine breeding programmes and ultimately combine this with artificial intelligence methodology to integrate genotype, environment and phenotype data for the development of superior, climate resilient genotypes.

Keywords: Tropical pines; Molecular breeding; Pitro50K; SNP chip; Genetic linkage maps

Genetic Improvement and analysis of genetic diversity in *Gmelina arborea* Roxb.

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Gmelina arborea Roxb. is a fast-growing tree species of family Lamiaceae, naturally distributed in Eastern sub-Himalayan tract, Aravali Hills, Western Peninsula, Indo-Gangetic Plains, Western Himalaya and Central India. It is commercially important tree species for various wood-based industries and an excellent choice for high quality timber, pulp and paper. The unscientific extraction of wood from forest has been leading to degradation of gene pool. The India State Forest Report indicates that between 2015 to 2021 forest cover has declined, and necessary steps are to be taken to increase green biomass for wood-based industries and also establish quality raw material for wood-based industries. A strategic and systematic tree improvement programme through proper selection of plus trees is essentially needed for assemblage of superior characteristics. A survey of north-western regions was therefore carried out to explore such genotypes. An extensive work was carried out to measure growth characteristics such as height, diameter at breast height (DBH), clear bole height (CBH), collar diameter (CD), stem straightness (SS), branching behavior (BB) and crown diameter (CD). These growth traits were found to be of utmost importance to carry out one of conventional methods of selection through indexing, which ultimately lead to selection of superior trees with higher weightage. During present investigation, attempts were also made to analyze genetic diversity of existing population. Therefore DNA isolation was carried out to analyze genetic diversity through ISSR markers. The ISSR markers were found to be extremely useful and played complementary role in differentiating various genotypes efficiently. The findings were also in agreement with outcomes of other investigators whereby nine genotypes were differentiated using 16 ISSR markers through 174 bands, which exhibited 80% polymorphism.

Keywords: Tree improvement, selection, gene pool, genetic diversity, *Gmelina arborea*

Population genetic study of *Eucalyptus bosistoana* suggests weak genetic structure among populations and isolation by distance

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Eucalyptus bosistoana is a key breeding species of The New Zealand Dryland Forests Initiative (NZDFI) which aims to create plantations of high-value timber species in dry environments on the east coast of New Zealand. Seeds of this species were previously collected in Australia and grown in the NZDFI breeding trials in New Zealand for common garden experiments. To inform the breeding program, we genotyped leaf samples of *E. bosistoana* from 148 breeding families using a *Eucalyptus* 68kSNP Axiom array to understand patterns of genetic diversity and structure within and among natural populations of this species. To our knowledge, the known natural occurrence of *E. bosistoana* is limited to the eastern coast of New South Wales and eastern Victoria, but trees were sampled from western Victoria that are morphologically similar to *E. bosistoana* and plants grown from their seeds were therefore also included in our genotyping analyses. These analyses showed that these plants from western Victoria are also genetically similar to *E. bosistoana*. Although our samples originated from a wide geographic range of localities in Australia, STRUCTURE results only suggest weak genetic structure among *E. bosistoana* collection sites including the western Victorian population. However, a statistically significant signal of isolation by distance was found among the collecting sites. Additionally, evidence of hybridization between *E. bosistoana* and *E. melliodora* was found in some of the populations.

Keywords: Eucalyptus bosistoana, population genetics, SNP array, breeding program

Genetic diversity and Genome-Wide Association Study for growth and wood quality traits in *Eucalyptus camaldulensis*

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Knowing and maintaining genetic diversity in tree breeding programs is crucial in the context of climate change and for molecular breeding approaches such as a Genome-Wide Association Study (GWAS). This work assessed the genetic variation of a *Eucalyptus camaldulensis* Dehnh population and the detection of new marker-trait associations (MTA) for eight growth and wood quality traits.

To achieve these goals, 689 individuals were chosen from a progeny trial (implanted in 1996 by The National University of Santiago del Estero, UNSE), consisting of 2,200 trees (between 1 and 12 individuals per family) from 110 open-pollinated families of 14 different provenances. These selected trees were phenotyped by NIR analysis of wood and conventional measurements of growth, and genotyped by EUChip60K chip, resulting in 19,034 polymorphic SNPs.

The genetic diversity values were consistent with those expected for the species (Polymorphic Information Content, PIC = 0.27; Observed Heterozygosis, Ho = 0.35; Expected Heterozygosis, He = 0.34). An analysis of the population structure showed six subgroups and low differentiation among them (FST = 0.062). GWAS for growth traits identified two MTAs for diameter at breast height (explaining 2 and 2.3% of the variance at five and 12 years, respectively) and one for wood density (2.1% of variance). For wood quality traits GWAS showed one MTA for total and ethanolic extractives (3 and 2.8% of the variance, respectively), one for cellulose (3% of variance), one for syringyl:guaiacyl ratio (3.1% of variance), and three shared between total and Klason lignin (9.1% of the variance for each trait). Sequences of the markers with significant associations were mapped and annotated using the *E. grandis* genome. Potentially interesting genes were identified *in silico* nearest the MTA regions.

The findings of these studies provide valuable information regarding genetic diversity and useful marker-trait associations for genetic improvement in this population.

Keywords: SNP, EUChip60K, breeding population, genetic variability, marker-trait association.

Transcriptome analysis reveals the candidate genes for regulating leaf shape in *Ammopiptanthus mongolicus*

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Ammopiptanthus mongolicus (Maxim. ex Kom) Cheng f., an evergreen broad-leaved shrub growing in Mid-Asian desert, a relict plant of the Tertiary desert flora, plays a vital role in protecting the fragile ecological environment in Northwest China. Previous studies mainly focus on its distribution, cultivation, physiology and biochemistry and adverse resistance molecular biology. However, there are few studies on the molecular regulation mechanism of the leaf shape. Here, we examined a variety of *Ammopiptanthus mongolicus*, which we have named the "narrow-leaf mutant". To find the intrinsic genetic factors for the leaf variation and identify the key genes related to Leaf shape of *Ammopiptanthus mongolicus*, narrow-leaf mutant NM and normal leaf NL were used to transcriptome sequencing. Six cDNA libraries in two groups (NL, and SL)were constructed. After assembly, a total of approximately 39.87 Gb clean data were obtained. There are 29 292(78.80%), 12 422(33.42%), 29 918(80.48%), 35 282(94.91%), 24 309(65.39%) and 28 089(75.56%) genes were annotated by using GO, KEGG, COG, Nr, Swiss-Prot, and Pfam databases, respectively. A total of 1,326 signifificantly up-/down-regulated genes were identifified in NM vs NL. These DEGs were mainly enriched in phenylpropanoid biosynthesis, plant hormone signal transduction and amino sugar and nucleotide sugar metabolism. We characterized 1846 TF genes into 34 TF families, and the most abundant TF family was the MYB family, followed by the bHLH and C2H2 families. We also emphasized the expression levels of differentially 33 transcription factors related to leaf development in NM vs NL. In addition, ten genes were chosen for qRT-PCR verification, and the results showed that the transcriptome sequencing data were consistent with the qRT-PCR results. The results provide insight into the molecular mechanism of leaf shape variation and lay the foundation for altering leaf shape through molecular breeding in *A.mongolicus*.

Keywords: Transcriptome; gene; leaf shape; *Ammopiptanthus mongolicus*

Genome-wide association study of *Pinus pinaster* resistance to pinewood nematode

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The pine wilt disease, caused by the pine wood nematode (PWN), represents a severe threat to *Pinus pinaster* (maritime pine), a species with great relevance in southwestern Europe. Survival after PWN inoculation has been reported as a heritable trait in *P. pinaster* (1), opening the possibility for selecting and breeding for this trait. In this work, we aimed at identifying genomic regions associated to the response to PWN by performing a genome-wide association study (GWAS). We have collected data for progressive wilting over time after PWN inoculation to calculate area under disease progress curve (AUDPC) values in five half-sib families with previously characterized genetic effects on survival to PWN infection (1). A total of 541 3-year-old plants were genotyped using a customized SNP array (4TREE array) including approximately 50k SNPs from 4 pine species. To assess if the observed differences in the AUDPC values were associated with the identified 3521 SNP markers, a mixed linear model accounting for population structure and kinship confounding effects was fitted. Ten SNP markers were found significantly associated to PWN response. These markers were located at coding and non-coding regions of genes previously reported as involved in the response to PWN in one family used in this study (2) but also in other *P. pinaster* populations.

This study suggests that GWAS is a useful approach for identifying genetic factors associated to PWN resistance. The SNP markers identified here could potentially assist in *P. pinaster* breeding programs by identifying resistant plants to PWN at an early stage of development.

(1) Carrasquinho et al. *Ann. For. Sci*. **2018**, 75

(2) Modesto et al. *Front. Plant Sci.* **2021**, *12*, 1–18

Keywords: Single nucleotide polymorphism, genotyping, maritime pine, pine wilt disease, *Bursaphelenchus xylophilus*

Establishment of male sterile lines in Japanese cedar (*Cryptomeria japonica* D. Don) using CRISPR/Cas9

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Japanese cedar (*Cryptomeria japonica* D. Don, or sugi) is distributed across Japan. *C. japonica* is an important tree species in Japan and has been used since ancient times for timber and to produce household items, etc. The national breeding program of forest trees has been in progress since the 1950s, and more than 3,500 plus tree clones of this species have been selected. Now *C. japonica* is accounting for 44% of the planted forest area in Japan (12% of the total land area). Meanwhile, *C. japonica* pollinosis is currently a major problem in Japan due to the widespread dispersal of its pollen, and there is an urgent need to generate superior low pollen or pollen-free lines. To produce male sterile lines in a short time, we attempted to use a genome editing system for the modification of genes involved in the development of male strobilus and pollen. Target genes were selected in terms of expressed in male strobili specifically, and the selected genes were attempted to disrupt using the CRISPR/Cas9 system. As a result, several gene-edited lines were obtained, which inhibited pollen development. Furthermore, by optimizing the CRISPR/Cas9 expression vector into *C. japonica*, we succeeded in efficiently obtaining many gene-edited individuals. This technique will greatly accelerate molecular breeding in conifers.

Keywords: CRISPR/Cas9, *Cryptomeria japonica*, genome editing, male sterility, pollinosis

SNPs markers can distinguish sections, species, some provenances and hybrid composition of *Eucalyptus*

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Eucalyptus is an economically important genus comprising more than 700 species in different subgenera and sections. Forest breeding programs have been exploiting the complementary characteristics of species to develop interspecific hybrids. However, species identification by morphological characteristics is challenging. This study evaluated the ability of SNP markers to distinguish sections, species, and provenances within the main *Eucalyptus* subgenera. We also identify the species compositions of known hybrids derived from controlled crosses. Samples belonging to 15 *Eucalyptus* species of subgenus Symphomyrtus (*E. argophloia, E. brassiana, E. camaldulensis, E. tereticornis, E. grandis, E. longirostrata, E. pellita, E. robusta, E. saligna, E. urophylla, E. benthamii, E. dunnii, E. globulus, E. nitens, E. viminalis),* three additional species of other two subgenera (*E. deglupta, E. cloeziana, E. pilularis*) and 44 interspecific hybrids were used in the study. SNP genotyping was performed using the 72K Eucalyptus Axiom array. After quality control data filtering (call rate> 95%, MAF> 0.05 and LD pruning), the dataset contained 22,230 SNPs genotyped in 528 samples. StructureSelector of Faststructure results indicated K=20 as the best clustering. This model could correctly separate all *Eucalyptus* subgenera and sections, assigned each species to a different cluster (except for *E. pilularis* and *E. cloeziana*) but was able to distinguish only one of the 17 provenances. Faststructure results could predict the ancestral composition of 70% the hybrids. Of all hybrids tested, 28 Faststructure correctly identified all the species known to be involved in their pedigree, 43 at least 50% of crossed parental and one had no parental species identified. Because unknown history and local intermating of some of the *Eucalyptus* species and provenances introductions in Brazil we cannot rule out the possibility that the presumed parental species were misclassified or were not pure species. In any case, SNP marker data can correctly discriminate subgenera, sections and species.

Keywords: Forestry, forest breeding, genetic structure, population structure

A minimal model to explain *FT2* daily expression in poplar.

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The correct cycling between active growth and dormancy is critical for the survival of trees growing in temperate regions. Trees like poplar use photoperiod as a precise indicator of seasonal changes that allows to properly synchronize plant phenology with environment. Daylength signal converges to control the expression of *FLOWERING LOCUS T 2* (*FT2*). *FT2* daily expression level is essential to promote shoot apex development[1], which sets poplar annual growing period. However, only limited evidence exists for molecular factors controlling *FT2* transcription. In this study, we have developed a minimal data-driven model that mimics *FT2* expression changes in response to daylength. This computer model predicts that a regulation driven by the circadian clock core genes is able to explain *FT2* expression. In agreement with experimental findings, the model points to the clock gene *GIGANTEA* (*GI*) as the key promoter of *FT2* expression, creating an activation frame that is delimited earlier in the day by *LATE ELONGATED HYPOCOTYL* (*LHY2*) and *TIMING OF CAB EXPRESSION* (*TOC1*), and later by *CYCLING DOF FACTOR* (*CDF*)[2,3,4]. CRISPR/Cas9 loss of function lines for *LHY2* and *TOC1* show increased *FT2* transcription under long days, similar to previously reported studies in *Arabidopsis*[5,6]. These results support the repressive role of *LHY2* and *TOC1*. Moreover, the model predicts that *FT*2 downregulation when shortening the daylength could be explained by a narrowing of this activation frame due to the phase shift observed for the previous genes. Further research into the interaction between these factors could lead to take advantage of *FT2* expression control to improve tree adaptation and, therefore, plantation forest breeding.

Keywords: Circadian clock, Flowering Locus T (FT), growth-dormancy cycles, photoperiodism, shoot apex development.

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Genetic diversity and population genetic structure in fragmented stands of *Prosopis affinis* (Leguminosae): implications for management and conservation

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Deforestation of native forests is one of the main causes for biodiversity loss. In Argentina, deforestation is mostly linked to forest harvesting and an increasing agricultural expansion. In Entre Ríos province, the resulting fragmentation adversely affects a large part of its forest species. *Prosopis affinis*, also known as "ñandubay", is a native tree with a wide distribution in the "espinal" phytogeographic region. This species has high value for local communities, with logging, medicinal, nutritional and chemical properties. To assess the effect of landscape fragmentation on the genetic diversity and structure of *P. affinis* in Entre Rios (Argentina), we examined 173 individuals distributed in 16 native forest fragments within an agricultural intensification gradient, using 12 SSR markers and 327 AFLP polymorphic loci. Nei's Genetic Diversity was assessed, being low to moderate in both cases (*He*-SSR: 0,734 / *He*-AFLP: 0,214), with higher levels of genetic variability being found in the least fragmented forest fragments within de gradient. The number of exclusive alleles and the number of private bands was low (*Ea*: 0-8 / *NPB*: 0-17, particularly in areas with higher levels of potential isolation. Both markers revealed a moderate to high genetic differentiation among forest fragments (SSR_θp: 0.198 / AFLP_*Fst*: 0.178). The population structure analysis by Bayesian clustering methods, through both molecular markers, allowed us to distinguish two genetic clusters (K: 2). Nei's genetic distance among fragments was moderate, with an average value of 0,394 for SSR markers. These results show the intense effect of landscape fragmentation on the genetic status of *P. affinis* stands. The loss of genetic variability and the levels of genetic structuration observed as a result of habitat anthropization, could limit the ability of *P. affinis* to adapt to changing environmental conditions in the future (e.g. new land use practices, climate change), compromising its management and conservation.

Key Words: *Prosopis affinis*, Argentina, Molecular Markers, Genetic Status, Landscape Fragmentation

Whole genome dissection & genomic selection for growth and wood properties in *Casuarina junghuhniana* Miq.

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Casuarina junghuhniana has high economic and ecological importance, grown for timer, pulp and fuel. While informed tree improvement largely depends on genomic information, however availability is still scanty. Here, we dissect the genome using DArT (diversity Array Technology) sequencing for association mapping and selections. Current study is aimed at developing genomic selection model for economic traits such as wood yield (WY) and pulp yield (PY). A total of 240 individuals, representing 55 half-sib families were evaluated across four different agro-climatic locations. We identified ~58461 SNPs (Single Nucleotide Polymorphism) for WY and 88400 SNPs for PY *in-silco*. Post filtering criteria (MAF >0.05 and missing genotype <10%), Genotyping by sequencing (GBS) approach yielded 10334, 11392 SNP markers for WY and PY respectively. An association mapping using generalised linear model (GLM) at false discovery rate (FDR) adjusted to P<0.05 resulted 8,722 SNPs for WY and 9,862 SNPs for PY. Highly associated markers were used for the development of genomic selection model. Further, RR-BLUP (Ridge regression-best linear unbiased prediction) model was used to estimate the marker effect. The accessions were divided into two sets: training population (80%) and validation population (20%). Genomic selection model was developed based on the marker effects using training data set (Genotype & Phenotype) and the accuracy of the GS model was validated using validation data set (based on genotype only). The GS model was able to predict the phenotype for WY (accuracy of 0.65) and PY (0.55). Application of genomic selection in untested population to predict genetic value of individual genotype based on genomic estimated breeding values (GEBV) for early selection is discussed.

Keywords: Diversity Array Technology, Genomic selection (GS), Genotyping by sequencing (GBS), Pulp yield (PY), Wood Yield (WY), Genomic estimated breeding values (GEBV)

Accuracy of scarcely recorded wood traits in a *Eucalyptus grandis* population is improved by combining genomic selection and predictor traits

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The genomic selection methodology is particularly relevant for traits that are difficult or expensive to measure. In this study, we investigated the impact of using genomic information and/or records on predictor growth traits to increase the breeding value accuracies of scarcely recorded target wood quality traits. The scarcely recorded traits (n=1,214) were pulp yield, cellulose, extractive, and wood density, while the predictor traits (n=3,159) were diameter at breast height and total height. Data was obtained from an open-pollinated progeny trial of *Eucalyptus grandis* (Hill ex Maiden). A total of 548 trees were genotyped with 37,229 single nucleotide polymorphisms (SNPs) using the Axiom Euc72K. The performance of single- (ST) and multiple- (MT) trait single-step genomic best linear unbiased prediction (ssGBLUP) and conventional pedigree-based (ABLUP) models were compared. Theoretical accuracies for estimated breeding values were calculated by ten-fold cross-validation on all the scarcely recorded traits. Consistently, the ssGBLUP approach outperformed the ABLUP model, with accuracies across traits ranging from 5.99% and 8.02% above the latter. When ST and MT models were compared, generally large and significant increments of accuracies (up to 19.60%) in all the target traits were observed when records on predictor traits were available for both, the training and validation populations. On the other hand, when records of predictor traits were only available for the training population, the accuracies generally showed a smaller increase (or not increase at all; from 0.00% to 14.77%). The largest increments in accuracy (up to 27.58%) were achieved when genomic information and records on both predictor traits were included in the analysis. We conclude that the inclusion of predictor traits in the training and validation populations coupled with a multiple-trait ssGBLUP model is a promising breeding tool to improve the accuracy of breeding values in trees that have not been phenotyped for wood quality traits.

Keywords: single- and multiple-trait individual-tree model, single-step GBLUP, theoretical accuracy, scarcely recorded traits.

Investigation of promoter regions in Scots pine using linear DNA amplification and massive parallel sequencing

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The absence of a publicly available high-quality Scots pine reference genome makes studies gene 5' flanking regions challenging. These regions include gene expression regulation motifs, and thus knowledge about the structure of these parts of the genome is important. Initially, we used a multiplex linear DNA amplification approach in combination with massive parallel sequencing to target these regions. The obtained ssDNA template was circularized and used as template for rolling circle amplification, products of which were sequenced using different technologies. For two of the targeted genes we identified contigs with the expected sequences, the rest of the sequences were from Scots pine but not what was expected, for various reasons. Despite the low efficiency, this shows that in principle the method is working and, with optimization, results could be improved.

A second strategy was then adopted – a modified terminal transfer amplification and sequencing (TTAS) -based method. Initially we used a multiplex strategy with TTAS but that was later substituted with single-plex approach followed by pooling of the reactions with the expected linear amplification results. Although we obtained long sequencing reads containing the employed primer binding sites, the flanking sequences were not what was expected based on the transcriptome used as a reference. We are currently investigating the reasons for that.

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Keywords: *Pinus sylvestris*, disease resistance, NGS, transcription regulation, linear DNA amplification

Hybrid *de novo* sequencing and assembly of *Macadamia* accessions Beaumont/ HAES 695 and Santa Anna

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Macadamia is a valuable tree crop with a high-quality nut product, however genomic and molecular breeding resources are still limited. Macadamia nuts are the most expensive in the world and are also nutritious and healthy, with high fat and low sugar content. South Africa is one of the world's largest producers, along with Australia, where macadamia species are native. The macadamia cultivars grown in South Africa are mainly imports from Australia and Hawaii, where it was first commercialised more than 100 years ago. Commercial macadamia cultivars are mainly hybrids derived from two species, *Macadamia integrifolia* and *M. tetraphylla*. Genome assemblies for these species and their hybrids can provide valuable references for developing genomic tools for genetic resource management, breeding and crop improvement. The aim of this study was to generate high-quality genome assemblies of two macadamia accessions, Beaumont/HAES 695 (*M. integrifolia* x *M. tetraphylla* hybrid planted extensively in South Africa) and Santa Anna (*M. tetraphylla* representative). Towards this we produced >80x Illumina HiSeq short-read coverage, >100x Oxford Nanopore PromethION long-read coverage and >100X optical mapping coverage (BioNano Saphyr) of the two genomes. The long and short-read DNA sequence data was combined to successfully assemble the Beaumont and Santa Anna genomes. Heterozygosity was estimated to be above 2.6%. The resulting genome assemblies are contained in fewer than 1,400 contigs with a N50 above 1 Mb, largest contig above 10 Mb and BUSCO completeness greater than 97%. The assembly length for both genomes was approximately 800 Mb, within the range of published macadamia genome sizes. Optical mapping will assist in identifying structural variants present within the genomes. This study will add to the existing genomic resources for macadamia and assist researchers to decipher the genomic composition of elite macadamia accessions and develop tools for accelerated breeding efforts.

Keywords: Genome assembly; Genomic variation; Macadamia; Molecular breeding

Screening of western hemlock genetic resistance to Annosus root and butt rot disease by dual transcriptome profiling

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Annosus root and butt rot caused by *Heterobasidion* spp. complex has affected many important forest tree species, including a wide range of forest conifers and broadleaf species. The BC breeding program has selected parent trees from wild stands of western hemlock (*Tsuga heterophylla*) to develop seed orchards for genetic gain with higher forest performance. However, in western hemlock populations, genetic information on host resistance types and levels against the Annosus root and butt rot disease is unknown. In this study, we evaluated the disease progression and the reprogramming of the host's transcriptome following inoculation with *H. occidentale*, and detected seedlings with quantitative resistance. The resistant seedlings showed a unique molecular defense response as compared with that of susceptible seedlings in a western hemlock composite seed family. The susceptible and resistant seedlings were well separated based on their transcriptomic defense profiles and phylogenetic analysis. Furthermore, we detected genetic variation in defense- and resistance-related genes, providing a direct link between genotypes and phenotypic disease resistance and susceptibility. Therefore, resistant genotypes can be selected in a new breeding cycle to gain higher levels of genetic resistance to Annosus root and butt rot disease. The allelic variants identified within resistance-related genes highlights genomic resources to develop molecular tools for marker-assisted selection for future breeding applications. Our findings provide beneficial insight on western hemlock-*H. occidentale* interactions and provide useful information for decision-making in breeding and silvicultural operations.

Keywords: Annosus root and butt rot disease, non-synonymous single nucleotide polymorphisms (ns-SNPs), quantitative resistance (QR), dual transcriptome profiling, western hemlock.

Transgenic resistance to white pine blister rust conferred by over-expression of a TIR-NBS-LRR gene in eastern white pine

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Eastern white pine (EWP; *Pinus strobus*) and western white pine (WWP; *Pinus monticola*) are two of the most important conifers of North America due to their economic and ecological values. However, the exotic fungal pathogen, *Cronartium ribicola* that causes white pine blister rust (WPBR), has killed up to 95% of native white pine stands in the severely impacted regions since its introduction into North America about 100 years ago. Identification of disease resistance (R) genes, followed by functional verification, allows development of biotechnological strategies to enhance host resistance to plant pathogens and pests. The objective of the present study is to evaluate long-term effects of over-expression of the WWP gene PmTNL2 in transgenic EWP for resistance against *C. ribicola*. PmTNL2 encoded a Toll/interleukin-1 receptor-nucleotide binding site-leucine rich repeat (TIR-NBS-LRR) protein. EWP somatic embryogenic cell line (1054-16) was transformed with PmTNL2 through co-cultivation with Agrobacterium tumefaciens. Following confirmation of PmTNL2 expression, transgenic EWP seedlings were generated and planted in pots with soils in 2010. They have been subjected to recurring infection of *C. ribicola* by placing the pots close to infected Ribes seedlings and cankered white pine plants since 2014. Transgenic plants showed high survival from repeated infections occurred over the last 8 years. It was observed that their needles well infected, fungal mycelia extended into branches and some cases into the main stems. Most cankered branches, however, died before the fungus had reached main stems. In plants where cankers did form on the main stem, most cankers were inactive, or had healed over without spreading further down along the main stem and the plants had survived. Development of these resistance-related traits in transgenic EWP plants demonstrates that PmTNL2 conferred quantitative resistance against *C. ribicola* by causing extensive tissue death of the cankered stems to restrict fungal growth in-planta.

Keywords: Eastern white pine, disease resistance gene, gene transformation, white pine blister rust,

Genomic selection with the pre-selected markers

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Large numbers of random markers distributed across the genome are generally used in genomic selection (GS) studies. Recently genomic imputation was used to increase the number of markers to the whole genome sequence (WGS) level. However, increasing the number of markers to the WGS level did not yield improvement in the genomic prediction accuracies. This is mainly due to several of these markers being either non-causal or not in LD with the causal loci leading to the noise in genomic predictions. Pre-selection of markers associated with the traits using genome-wide association studies (GWAS) was proposed as a strategy to reduce the number of non-causal markers and to improve the accuracy of the genomic predictions. We developed marker panels with pre-selected markers associated with various traits for several tree species such as *Eucalyptus* and *Acacia*. Several GS studies were performed with these marker panels. GS studies in *Eucalyptus pellita* (*E. pellita*) and *Eucalyptus nitens* (*E. nitens*) using pre-selected markers revealed substantial dominant effects and improved genomic predictions. Results from these studies will be discussed.

Keywords: genomic selection, nonadditive effects, single-step GBLUP, GBLUP

Genome-wide association study for resistance to *Leptocybe invasa* and stem canker disease in *Eucalyptus grandis*

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Eucalyptus is the most widespread genus in plantations worldwide. Recently, the productivity of these plantations has been threatened by the emergence of pests and pathogens such as *Leptocybe invasa*, the "*eucalyptus* gall wasp" and *Teratosphaeria gauchensis* and *T. zuluensis*, causal agents of the fungal stem canker disease (formerly *Coniothyrium* canker). In this work, QTL and candidate genes for resistance to these pest and fungal pathogens were identified.

The base population (Paraguay) consists of a trial of 782 *E. grandis* trees (progeny of 382 mothers/families) with five clones per tree replicated in six sites (23460 trees). One to nine half-siblings within each family were selected according to the extreme values of diameter at breast height at 44 months (DBH44) and then these 689 individuals (BiotechII EuropeAid/136-457 project) were genotyped (EUChip60K, 20634 polymorphic SNP markers). For phenotyping *L. invasa* tolerance, a binary scale was used in which 0 was assigned to trees in which no clone showed any damage and 1 to those in which at least one clone showed some level of damage (2 sites). For stem canker, clonal BLUPs were obtained with measures at 27 and 44 months using a categorical scale with values between 0 (no damage) and 6 (high damage).

GWAS analyses detected 3 SNP markers associated with resistance to *L. invasa*, explaining 7.8 % of the variance. For stem canker resistance, it resulted in 4 SNPs associated (2 SNPs at 27 months and 2 SNPs at 44 months) which together explain 10.58% of the phenotypic variation. Additionally, *in silico* analysis of adjacent genomic regions of the associated SNPs showed 8 genes of interest, including 2 disease resistance proteins (TIR-NBS-LRR class) in which one of them localized at 3.9Kb.

This information can be used to further understand the genomic architecture of the resistance to these biotic stresses.

Keywords: single nucleotide polymorphism, linear mixed model, *eucalyptu*s gall wasp, stem canker, candidate genes

Structural variation in the haplogenomes of an F1 hybrid of *Eucalyptus urophylla* and *E. grandis*

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Structural and haplotype variants underlie pan-genome variation and thus phenotypic diversity and plasticity. *De novo* phased long-read genome assemblies are now possible in highly outbred species as long reads span divergent haplotypes. This has allowed analysis of pan-genome variation in crop species. To probe pan-genomic variation within *Eucalyptus*, we analyzed genomic variation between the haplogenomes of an F_1 hybrid of *E. urophylla* and *E. grandis*. We first produced the two parental haplogenomes of the F_1 hybrid using *de novo* assembly of trio-binned long-read data of the F_1 hybrid based on short-read data from the parents. A total of 99.98% of the long reads in the F_1 hybrid could be assigned to a parental haplotype. Independent *de novo* assembly of the haplotype read groups resulted in a 544.5 Mb and 566.7 Mb genome assembly size for the *E. urophylla* and *E. grandis* haplogenomes, respectively (>98% BUSCO completeness). Chromosome-level assemblies were attained by scaffolding with high-density linkage maps. Comparison of the chromosome-level haplogenome assemblies revealed an overall 257 Mb synteny between the *E. urophylla* and *E. grandis* haplogenomes. We detected 48,729 SVs ranging in size from 100 bp to 4.01 Mb. Besides the SVs, a total of 8 million SNPs were identified underlying the high heterozygosity estimate of 3.46%. Genome annotation yielded 37,942 and 39,849 structural gene models (>94.6% BUSCO completeness) of which 33,915 and 35,572 were functionally annotated for the *E. urophylla* and *E. grandis* haplogenome assemblies, respectively. A comparison of 23,390 gene pairs in 238 synteny blocks indicated that 8,114 (34.69%) gene pairs were rearranged between synteny blocks. This study provides a first genome-wide view of structural variation between the haplogenomes of *E. urophylla* and *E. grandis* present in an F_1 hybrid. Future work will include species level variation towards understanding pan-genome variation in species and genus.

Keywords: *Eucalyptus*, Gene Synteny, Haplogenome Assembly, Oxford Nanopore, Structural Variants

Transfer simple sequence repeat (SSR) markers from Eucalyptus spp. to *E. sideroxylon* in a cost-effective multiplex format

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In Argentina, slow-growing forest native species satisfy the demand for natural long-lasting hardwood. This implies a sustainability problem for the native forest, leading their population size to a risk point. *Eucalyptus sideroxylon* (A.Cunn. ex Woolls), is ranked among the most durable species and performs well against cold and drought. According to Standards Australia's AS 5604 2005 (Timber - Natural durability ratings), it is considered Type 1 (very durable without treatment).

An important step in promoting the multiplication and use of *E. sideroxylon* as a source of Type 1 wood in the Argentinean pampas, is to know the genetic variability of the populations present in the region. In this sense, molecular markers are useful tools to address this issue.

Therefore, due to the lack of microsatellite markers (SSR) developed for this species, we carried out the transferability of 28 SSR (neutral and functional markers) developed in *E. grandis* and *E. globulus* using a multiplex format.

As a result, four amplification mixtures containing between seven to five markers were able to successfully transfer 23 SSR (82%) with reproducible and reliable amplicon patterns. Twenty-one of them were polymorphic and two monomorphic (distributed in 10 of the 11 *Eucalyptus* chromosomes) in four samples, the rest of them did not show amplification product.

Thanks to the multiplex PCR format and the successful cross-transferability of the SSR, this development will allow rapid genotyping at a low cost. The knowledge of the genetic variability will boost the establishment of a breeding program for *E. sideroxylon* in Argentina.

Keywords: cross-transferability, genetic variability

QTL and eQTL mapping of wood properties in a poplar F_1 hybrid population

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Poplar is an important timber species in the world, and its wood formation is a complex biological process. Analyzing the genetic regulation of gene expression is very important for understanding phenotypic variation. Wood quality and growth rate have been extensively studied in poplar, but have not been thoroughly investigated. In this study, we aimed to explore novel genomic regions involved in the regulation of wood development by QTL and eQTL mapping in a *Populus deltoides* 'Danhong' × *Populus simonii* 'Tongliao1' F_1 hybrid population. The growth and wood properties of the two parents and hybrid population were measured and statistically analyzed. We detected a total of 1,504,840 highly reliable single nucleotide polymorphisms (SNPs) and 19,619 genes. Through QTL and expression QTL (eQTL) analysis, we identified 237 quantitative trait locus (QTLs) and 48,019 cis-eQTLs and 2,184,889 trans-eQTLs contributing to the whole-genome transcriptome variation in poplar. Co-expression of hotspot12654 with TFs and structural genes participated in RNA binding, catabolic process and spliceosome pathway. It was found that *PdCaM247* was involved in the secondary xylem development and biomass accumulation in poplar. This study generates a large genetic resource for studying xylem development and provides new insights into the genetic basis of secondary cell wall regulatory networks.

Keywords: QTL, eQTL, co-expression network, xylem development, poplar

Draft long read assembly of the *Acacia mearnsii* genome

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Acacia mearnsii (black wattle) is an important plantation forestry species for commercial farmers, small and medium scale timber growers in South Africa occupying ~7% of the forestry estate. To breed for improved woody biomass production, it is important to understand the genetic make-up of the local black wattle breeding populations. A comprehensive understanding of the genome diversity of black wattle is lacking and the availability of DNA markers for molecular breeding is limited to a small number of microsatellite DNA markers. The need for molecular breeding tools in black wattle is further emphasized by an ongoing wattle rust epidemic that has been causing major damage to young plantations. To address this need, we used long-read (Oxford Nanopore) and short-read (Illumina) DNA sequencing technology to sequence the genome of a black wattle genotype. A total of 160 Gbps of long-read nanopore sequencing data and over 70 Gbps of short-read data representing 230X and 100X coverage of the *Acacia mearnsii* genome respectively was obtained. We produced a preliminary genome assembly comprising of 430 contigs with an N50 value of 2.3 Mbps, which accounted for 676 Mbps of the estimated 680 Mbp genome size for the species. Analysis of universal-single copy orthologs (BUSCO) genes suggested that the current assembly is 95% complete. We have also initiated *in-silico* mining for microsatellite DNA sequences suitable for the development of markers for routine DNA fingerprinting and parentage analysis. The genome sequence generated in this study will also serve as a reference for the development of genome-wide genotyping and molecular breeding resources for the species.

Keywords: *Acacia mearnsii*, genome assembly, black wattle, Oxford Nanopore, microsatellite markers

Genetic variations in *Melia dubia* Cav. for a potential approach for wood forensics

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Melia dubia Cav. is a highly valued, multipurpose tree species of Mahogany family in India which has been gaining importance of tree growers for production of quality raw material in timber and panel industry. However, improved planting stock of *Melia* is available in very limited proportion. The species is therefore under pressure of negative impacts by tree cutters and illegal felling. The efforts were directed towards conserving forest genetic resources and forensic research of the species by creating identification systems so that quality material could be kept safe and made available whenever needed. The analysis was therefore conducted in two major geographical areas of *M. dubia* growing, Northern and North-Eastern region of India. Molecular characterization of 70 genotypes using 64 Simple Sequence Repeat (SSR) markers demonstrated that *M. dubia* belongs to two diverse populations, with maximum polymorphic information content (PIC) of 0.94 for SSR5 in northern and maximum PIC of 0.94 for SSR18 in north-eastern region. Mean PIC was greater than 0.5 (0.79 for north-eastern and for 0.83 for northern areas, respectively) suggesting that these markers had high identification ability. The power of discrimination (PD) also corresponded to those of PIC values with mean value of 0.923. The maximum PD was recorded with SSR28 (0.978) for North-Eastern regions and 0.980 for Northern region with SSR5. Moreover, mean number of effective alleles (Ne = 9.456 for Northern and Ne = 9.321 for North-Eastern region) were found to be lower than that of mean number of alleles (Na = 13.785 for Northern Ne = 13.892 for North-Eastern region), which suggested that many of the alleles were relatively rare. Such significant genetic variations across the geographical regions form a positive baseline for future forensic analysis, to develop individual identification system and also to track geographical origin of such valued genetic resources.

Keywords: Felling, identification, PIC, PD, *Melia dubia* Cav.

Identification and Characterization of 5 Walnut *MYB* Genes in response to Drought Stress involved in ABA Signaling

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Walnut is a popular nut tree species and usually suffers from drought stress. However, little information is available on the mechanism of walnut responding to drought stress, resulting in lack of basic understanding for its resistance. In order to excavate more functional genes that can respond to stressors, and enrich the theoretical basis for walnut resistance, in this study, 5 *MYB* genes with complete ORFs were identified from *J. regia* and the basic bio-information as well as expression patterns in different tissues and response to drought and ABA stresses were confirmed using qRT-PCR assay. The results showed that 2 *JrMYB* genes belong to R1-MYB subfamily and 3 *JrMYB*s belong to R2R3-MYB, encoding the proteins from 212 to 362 aa in length. The phylogenetic analysis categorized proteins of 5 JrMYBs and 40 Arabidopsis AtMYBs into 10 subgroups. JrMYBs in the same subgroup exhibited significant similarities in the composition of conserved domains and motifs in amino acid sequences and exon/intron organization in DNA sequences. The results of qRT-PCR analysis revealed that *JrMYB* genes diversely expressed in various tissues. Moreover, the expression values of *JrMYB*s were upregulated or downregulated significantly under drought and ABA stresses. Most attractively, in contrast with suffering from drought stress alone, the treatments with drought and additional ABA greatly enhanced the transcript levels of *JrMYB*s. All these results suggested that *JrMYB* genes play a vital role in plant biological processes and drought as well as ABA stress response, and possibly perform as ABA-dependent drought response transcription factors in plant.

Keywords: Walnut; MYB transcription factors; Drought stress; ABA signaling

Identifying and Expression Analysis of WD40 Transcription Factors in Walnut

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Walnut is an important woody oil plant and will be affected by abiotic and biological stress during its growth and development. The WD40 protein is widely involved in plant growth, development, metabolism, and abiotic stress response. In order to explore the stress response mechanism of walnuts, based on the complete sequencing results of the walnut genome, this study identified and analyzed the physiological, biochemical, genetic structure, and conservative protein motifs of 42 *JrWD40* family genes, whose expression to abnormal temperature were tested to predict the potential biological function. The results showed that the open reading frame (ORF) of these WD40s were 807~2460 bp, encoding peptides were 29610.55~90387.98 Da covering 268~819 amino acids, as well as 12~112 phosphorylation sites. JrWD40 proteins were highly conserved with 4~5 WD40 domains and shared certain similarity to WD40s from *Arabidopsis thaliana*. *JrWD40* genes can be induced to varying degrees by low and high temperature treatments. *JrWD40-32*, *JrWD40-27*, *JrWD40-35*, and *JrWD40-21* are affected by high temperature more seriously and their expression levels are higher; while *JrWD40-37*, *JrWD40-26*, *JrWD40-20*, *JrWD40-24* and other genes are inhibited under low temperature stress. *JrWD40-40*, *JrWD40-28* and *JrWD40-18* were first suppressed with low expression, while as the treatment time prolonging, the expression level was increased under cold condition. *JrWD40-14*, *JrWD40-18*, *JrWD40-34* and *JrWD40-3* displayed strong transcriptions response to both heat and cold stress. These results indicated that *JrWD40* family genes can participate in walnut adaptation to adversity, and can be used as important candidates for walnut resistance molecular breeding.

Keywords: *Juglans regia*; Abiotic stress; *WD40*; Expression

NACs: transcription factors involved in the response to abiotic stress in *Eucalyptus globulus*

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The NAC transcription factors (TFs) family is present in a large variety of species and is one of the most numerous TF families specific to plants. The NAC family has described to be involved in multiple biological processes especially in the transcriptional regulation associated with plant abiotic stress response, where it has been seen that the response of these TFs is strong and rapidly induced. The abiotic stress affects the establishment, plant survival and productivity of several species including *Eucalyptus globulus*, one of the most cultivated forest tree species in the world. Freezing temperatures and long periods of drought is currently a major issue for the forest industry. Based on RNA-seq data from *E. globulus* plants, subjected to cold and drought stress, reported by Aguayo et., (2019), a total of 103 *EgloNAC* genes with a complete CDS were identified. According to their putative orthologous genes in *E. grandis*, a phylogenetic analysis allowed to classify these genes into nine main groups and nineteen subgroups. The expression patterns of 103 *EgloNAC* genes under abiotic stress were determined by in silico DEG analysis from RNA collected from leaves of plants under abiotic stress. Transcriptome profiling indicated that the expression of multiple *EgloNAC* genes is activated by cold and drought stress.

Keywords: cold stress, drought stress

Proteomics reveals changes in differentially expressed protein accumulation duri ng seed germination in *Cupressus gigantea* W. C. Cheng et L. K. Fu

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Seed germination is a significant and critical period in the early stages of plant growth and development. Seed germination begins with water absorption and ends with embryonic germination, which is divided into four main stages including to swelling absorption, germination, germination and embryonic elongation. In the present study, we firstly applied exogenous GA3 (1.0 mg/mL) with a light/dark adjustment of 16h/8h and a culture temperature setting of 20°C to improve the germination of *C. gigantean* seeds. The proteomics was used jointly to analyse protein changes during four pivotal periods in the seeds, which was also the first application in *C. gigantean* seeds. In total, 34 differentially expressed protein (DEP) spots representing unique proteins were identified, of which 13 appeared at the germination stage and 17 appeared at the radicle elongation stage. These identified DEP spots were clustered into eight main functional groups, mainly comprising cellular architecture proteins, energy metabolism, transport, stress response, molecular chaperones, amino acid metabolism, oxidoreductases, involvement in ABA signaling pathway. Among these proteins, most of them were identified as being closely related to amino acid metabolism, such as glutamate metabolism proteins (Spot 41 and Spot 47), glycolysis-related proteins (Spot 18, Spot 30 and Spot.68) and pentose phosphate pathway proteins (Spot 36 and Spot 0). Thus, these results provide reliable proteomic data of *C. gigantean* seeds during the seed germination.

Keywords: *Cupressus gigantea* W. C. Cheng et L. K. Fu, Seed germination, Two-dimensional electrophoresis (2-DE).

QTL mapping and candidate genes analysis for nitrogen use efficiency in a poplar F_1 population

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Poplar trees have an important economic value and ecology function, and are often planted on nitrogen-deficient land and their growth is severely affected. *Populus deltoides* 'Danhong' has high nitrogen use efficiency (NUE) traits, and *P. simonii* 'Tongliao1' show strong tolerance to stressful environments. We performed high- and low-nitrogen treatments with their F_1 hybrid population in the field. Based on high-density genetic mapping for the QTL mapping of NUE-related traits, 448 QTLs and 682 candidate genes were identified. Four traits related to NUE under high and low N environment were used to detect respective 333 and 46 QTLs with the logarithm of odds (LOD) values 3-5.53 and phenotypic variance explanation (PVE) rates 3.3-11%. Sixty-nine QTLs related to the nitrogen response index were detected with LOD values 3-5.82 and PVE rates 3.3-12.1%. Bulked segregant analysis (BSA) was performed with extreme differences in NUE-related traits (stem biomass and nitrogen response index), and 121 SNP locus, 65 Indel locus, and 541 candidate genes were identified. We identified two main QTLs related to nitrogen use efficiency and their molecular markers. A combination of QTL mapping, BSA, and transcriptome analysis was used to screen key NUE-related candidate genes. The Gene coexpression and amino acid metabolism network were together established, providing new insights into NUE-related gene regulatory relationships. This study will offer gene resources for the genetic improvement of poplar nitrogen use efficiency.

Keywords: Nitrogen use efficiency, *Populus*, QTL mapping, BSA, coexpression network

GWAS and RNA-seq mining candidate genes of *Populus cathayana* under nitrogen treatment

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Poplar growth and wood properties have a high degree of response to nitrogen, and nitrogen is an important factor limiting poplar wood yield. Revealing the regulation mechanism of poplar growth traits under nitrogen treatment is significant for improving wood yield and nitrogen use efficiency. Here, we used a GWAS association population consisting of 410 genotypes to identify genetic loci that regulate growth traits in *Populus cathayana* under nitrogen treatment. The developing xylem samples of 13 genotypes from 5 regions were taken for RNA-seq. We combine RNA-seq and WGCNA to analyze the xylem gene regulation mechanism under nitrogen treatment and mine candidate genes for important traits. Integrating the results of GWAS, RNA-seq and WGCNA analysis for the regulatory mechanism of poplar growth under nitrogen treatment, three important regulatory genes (*PtrMYB059*、*PtrNAC123*、*PtrNAC025*) in the process of poplar wood formation were identified. In addition, *PtrNAC123* and *PtrNAC025* genes may affect tree growth and development by regulating the nitrogen metabolism of *P. cathayana*. This study provides strong evidence for molecular genetics and gene pleiotropy of *P. cathayana* and new genetic resources for poplar growth and development and nitrogen response research.

Keywords: *Populus cathayana*; nitrogen; growth traits; RNA-seq; GWAS;

Evaluation of hairy root transformation in *Eucalyptus grandis* clones and development of hydroponics and soil-based hairy root biomass culture systems

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Hairy root transformation is a biotechnological tool implemented in many plant species to study various aspects of plant biology such as root development and root-microbial interactions among others. The technique encompasses infecting plants with *Agrobacterium rhizogenes*, a gram-negative bacterium that induces root formation because of its inherent root-inducing plasmid. Previously, a *Eucalyptus* hairy root transformation protocol was developed which showed higher rates of transformation efficiency for seedling hypocotyl inoculation than that of stem inoculations of micropropagated clones. In this study we evaluated the efficiency of hairy root transformation of different *E. grandis* clones, analysed their growth in hairy root cultivation in hydroponics and optimized a soil culture system. Cuttings from four *E. grandis* clones (TAG14, TAG5, ZG14 and CG96) and seedlings from an *E. grandis* family were inoculated with the *A. rhizogenes* strain A4RS transformed with a pGWAY-0 empty vector harboring the DsRED fluorescent marker. The fluorescent roots were visualized at three weeks post-inoculation and the transformation efficiencies calculated. We observed transformation efficiencies ranging from 0-47%. To allow the transformed plants to harden and develop more secondary xylem, we developed a hydroponics-based hairy root biomass culture system using a commercially available nutrient kit. The plants from the clonal lines were grown in the hydroponics system for sixteen weeks after which the shoot and root length were measured. ZG14 and CG96 performed better in the hydroponics system than TAG14 and TAG5. To further optimize transgenic plants' growth, we optimized a soil biomass culture system by testing six different substrates comprising different ratios of fine silica sand/plaster sand and vermiculite. The 1:1 ratio of fine silica sand: vermiculite proved to be the best substrate for the growth of *Eucalyptus*, with 100% survival of plants after six weeks. Our data demonstrates that clonal cuttings show genotype-specific variation in *A. rhizogenes*-mediated hairy root transformation efficiency as well as hydroponic hairy root culture growth rates, while soil composition has a significant effect on rooted cutting survival. The optimized hairy root transformation protocol and biomass culture systems will aid in efforts aimed at studying biological functions of genes involved in various processes in *Eucalyptus* growth and development.

Keywords: hairy roots, hydroponics, transformation, Eucalyptus

Genetic basis of tree architecture

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The aim of our research is to understand molecular mechanisms controlling tree development. We will explore natural variation in forest trees to identify novel genetic regulators of tree architecture, cambial activity and wood quality. Our model organism is an important forestry tree, silver birch (*Betula pendula*), whose small genome is currently being sequenced. This tree is monoecious, and already very young seedlings can be induced to flower under greenhouse conditions. Birch will bring the power of inbreeding and short generation times into tree genetics, enabling exploitation of advanced crossing schemes for genomic mapping.

The focus of our study is a collection of naturally occurring birch mutants: a diverse set of trees with atypical cambial activity, branching pattern or leaf phenotype. We are currently most advantaged in characterisation of highly branching, bush-type trees, together with sweeping variants, which have strikingly downwards hanging branches. We aim, through a whole genome sequencing approach, to map the causative genes behind these phenotypes, and study their function in transgenic trees.

Besides its fascinating basic science aspect (what makes a tree a tree?), understanding molecular mechanisms regulating tree development has also significant applied value. Historically, due to their large size and long generation times, trees have not been easily accessible for traditional breeding. Detailed knowledge about the regulatory mechanisms controlling tree traits will provide us tools for the domestication of forest trees. Boosting efficient production of wood in commercial forests is essential for sustainable management of natural resources. With birch as our model, this project represents a novel approach of tree genetics, with potential for ground-breaking insights into tree development and breeding.

Keywords: Tree architecture, genetics, genomics, birch

Where are We in Recombinant Forest Biotech? Some Lessons about Science and Society in a Fractious and Changing World

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My goal is to reflect on the state of recombinant biotech, meaning gene edited and genetically engineered trees (both termed GE), and why biotech has largely failed to live up to its initial promise to help improve productivity, sustainability, and pest resistance of plantation forests.

Despite amazing advances in gene and genome science in recent years, progress in bringing biotech trees to the forest has largely stalled. This is due to a confluence of big forces, both social and biological. One the social side key factors have been 1) "green" certification systems that are a major controlling force in forestry in much of the world, and have almost no allowance for the use of biotech trees in forests—including for developmental and breeding studies. 2) Method-based regulations that make normal forest genetic research and breeding costly and risky, and greatly constrain essential field-based trait and breeding studies beyond small "boutique" analyses. And 3) ethical unease among the public and companies about several factors, that together hamper investment. These include trust in large forestry and biotech corporations and their transparency, the often abusive roles of patents, and the use of "monoculture" plantations—all exacerbating GE concerns, especially of gene flow. On the biological side, key issues are 4) gene transfer and editing systems for most major plantation species are very inefficient, and often nearly impossible for many genotypes or species, 5) a lack of reliable tools for precise gene control and editing, and a paucity of clear demonstrations of beneficial trait modifications that address major issues like stress tolerance and wood properties in the field. And, 6) a lack of ability to effectively integrate GE trees into conventional breeding, a growing concern with expanding pest and climate stress. Priorities should include transformation innovations, field studies, and aggressive outreach to the public.

Keywords: Gene editing, regulations, certification, plantation, biosafety

Development and Regulatory Review of Transgenic American Chestnuts for Restoration

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American chestnuts were among the most culturally and environmentally important trees in eastern U.S. forests, until they were decimated by an invasive fungal pathogen which causes chestnut blight. Restoration of these majestic icons has been an important societal and scientific goal for more than a century, but until recently, enhancing blight resistance while maintaining American chestnut traits has been an elusive goal. The American Chestnut Research and Restoration Project at the SUNY College of Environmental Science and Forestry has developed a transgenic tree known as Darling 58, which was engineered with a gene called oxalate oxidase (OxO). This gene comes most directly from wheat, but it is also found in many other foods and wild plants. The enzyme made by this gene breaks down oxalic acid, which is a toxin used by the blight fungus to attack American chestnut stems. Darling 58 can tolerate blight infections better than comparable non-transgenic chestnuts. This could help rescue remnant populations of American chestnut which persist in the wild, and also potentially enhance blight resistance in hybrid American chestnut orchards. But before these transgenic trees can be distributed or used for restoration, they must be evaluated by three federal agencies in the U.S. This regulatory review process is typically applied to annual crop plants produced by large companies, so there are many challenges in applying it to a wild restoration tree produced by a university research group. This talk will focus on the science and history of transgenic chestnut trees, and also consider legal and ethical implications of the use of biotechnology for conservation.

Keywords: Conservation, genetic engineering, transgenic, chestnut blight, *Castanea dentata*

Engineering Sterilty in Hybrid Poplar for Genetic Containment

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A trait of great interest for transgenic forest trees is genetic sterility, a result of common long distance gene flow and presence of wild relatives, making regulatory and market approval challenging. We are targeting the key floral development genes *LEAFY (LFY)* or *AGAMOUS (AG)* to engineer stable and complete sterility in hybrid poplar grown in field conditions. We will first describe our work using RNA interference (RNAi) to suppress these target genes, where we observed a range of phenotypes, from normal flowers to strong sterility, under field conditions. The phenotypes were, fortunately, highly stable across growing seasons and after vegetative propagation. We will then describe our current results from gene editing, using CRISPR-Cas9 to specifically mutate *LFY* or *AG*. We observed a high frequency of knock-out mutations for all the alleles of both target genes in wild type and FLOWING LOCUS T (FT)-induced early flowering backgrounds. We observed great variation in floral and vegetative form in the early flowering trees, at least partly a result of variation in heat-induced FT overexpression required to elicit flowering. We have also produced a field trial of edited, naturally flowering trees—some of which should begin to flower in 2023. We intend to study the vegetative and floral phenotypes for these trees, including possible chimerism, over the coming years.

Keywords: CRISPR, RNAi, poplar, containment, sterility

Genetically modified eucalyptus tolerant to glyphosate - an effective product for weed control

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Eucalyptus is the main forest species cultivated in Brazil, encompassing 7,5 million hectares. Weed competition is one of the main challenges in eucalyptus plantation management, reducing yield and increasing operational costs. In addition to direct yield losses caused by weed competition, herbicide applications can lead to yield losses due to herbicide drift, as well as the risk of operator contamination during manual application. To overcome these issues, FuturaGene developed a GM eucalyptus variety, 751K032, which carries the *cp4 epsps* gene that encodes the EPSPS enzyme conferring tolerance to glyphosate herbicide, which has been inserted into other GM crops for over 30 years. Biosafety studies were conducted according to CTNBio requirements for commercial approval. Studies demonstrated a stable T-DNA insertion in the genome, with two functional copies of the gene *cp4 epsps* and one functional copy of the gene *nptII*, as well as absence of unwanted DNA sequences and a Mendelian inheritance pattern. No meaningful homologies to known or putative allergens and toxins were observed, and safety assessment studies indicated that it is unlikely to cause allergenic or toxic effects to humans/animals. The GM variety is similar to the conventional clone in silvicultural, morphological, and reproductive characteristics. Chemical composition studies revealed no differences between the GM variety and the conventional. There were no adverse effects on non-target organisms, no change in the soil microbiota and no differences on decomposition in the soil. Studies with *A. mellifera* and *S. bipunctata* larvae and adults with GM and non-GM pollen, revealed no differences in mortality and survival rates. Therefore, cultivation of 751K032 was judged to be as safe as the conventional clone for human/animal health and for the environment. In 2021, CTNBio evaluated and granted the approval of the GM eucalyptus 751K032 for commercial uses (DOU 214, Nov 16th, 2021 – Section 1, page 8).

Key Words: eucalyptus, GM, glyphosate, tolerance and weed

Gene editing approaches to modifying reproduction in Eucalyptus

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Eucalypts are the most widely planted hardwood tree in the world. They are cultivated for pulp and wood products, as well as for production of essential oils, energy, and honey for local communities. Genetic engineering (GE) of premium clones can enhance or add new traits to already vigorously growing trees, especially for discrete traits controlled by one or a few genes. However, gene flow from GE trees to non-GE or wild trees remains a major socio-ecological concern and a regulatory barrier to the use of these trees. In addition, hybrids are commonly preferred, whose production by seed can be facilitated by using male-sterile clones. To minimize concerns of gene flow and provide new options for hybrid breeding, we have been studying the effects of CRISPR mutation of several floral regulatory genes. These genes include *LEAFY* (*LFY*), *TAPETAL DEVELOPMENT AND FUNCTION 1* (*TDF1*), *SYNAPTIC 1* (*SYN1,* also known as *REC8*), and *HECATE 3-like* (*HEC3-like*). The knock-out events were generated in three hybrid eucalypt genotypes: one *Eucalyptus grandis x urophylla* WT hybrid (WT SP7) for evaluating vegetative growth, morphology, and physiology, and two SP7 genotypes that were previously transformed with the *Flowering Locus T* (*FT*) gene to cause rapid flowering. Given the differing target gene functions, our knock-out early flowering plants showed an array of sterile phenotypes, including complete prevention of flower development to production of normal (or nearly normal) but pollen-less flowers. The flowers from the *SYN1* knock-outs appeared normal, produced nectar, and had pollen that was morphologically abnormal and inviable; these trees may provide a means for inducing sterility while maintaining nutritive tissues to support pollinators. All the non-FT knock-out trees had normal vegetative growth rates and morphology, suggesting their use to modify flowering could be done without impairing wood or oil production.

Keywords: Eucalyptus, CRISPR, biodiversity, sexual containment, genetic engineering

HIGS for control of *Sphaerulina musiva* poplar leaf spot and stem canker disease: Efficacy, Stability, and Non-Target Impacts

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Productivity in hybrid poplar is threatened by the fungal pathogen *Sphaerulina musiva*—a major cause of leaf spot and stem canker (formerly *Septoria musiva*). Host induced gene silencing (HIGS) is a transgenic method whereby a host makes its own RNA-based "pesticides" that confer precise protection against pests and pathogens. It provides a new means to develop specific heritable resistance when natural sources are not available or difficult to breed for. To explore the feasibility of HIGS in the *Populus–S. musiva* pathosystem, we first tested whether *S. musiva can* take up dsRNAs *in vitro* using fluorescein-labeled dsRNAs and confocal microscopy. We further tested for uptake with dsRNAs targeting a fluorescent protein in a marked strain of *S. musiva* and with dsRNAs targeting housekeeping genes to test for effects on growth. Constructs targeting housekeeping gene targets *cyp51* and *dcl*, both homologs of effective HIGS targets in published studies, were transformed into *Populus trichocarpa* to test for disease resistance in greenhouse trials. With carefully chosen dsRNA transgene sequences, HIGS is expected to be specific to the targeted pathogen because of the sequence complementarity required for RNAi. We tested this assumption by comparing the fungal microbiomes between HIGS trees targeting *S. musiva* and control trees in a field trial to look for non-target effects of HIGS technology.

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Keywords: HIGS, SIGS, RNAi, Poplar

Faster biosafety evaluation of genetic containment in forest tree species using early flowering systems

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Gene flow from non-native species represents a serious threat to gene pool integrity of tree species in natural forests. The introduction of Asiatic elms into European countries promoted gene flow with local elm populations. Gene flow was also reported between the fast-growing poplar hybrid *Populus* × *canadensis* (*Populus deltoides* x *P. nigra*) and *P. nigra* in Europe. Similar gene flow events can be expected with tree lines obtained using genetic modification or genome editing.

Different biotechnological strategies have been followed in many crop species to avoid undesired gene flow (genetic containment). However, the evaluation of gene containment in trees is very difficult due to the long time required by trees to reach the reproductive phase. Therefore, biotechnological early flowering systems can be helpful in this case.

Early flowering lines were obtained at the Thünen Institute of Forest Genetics (Grosshansdorf) with poplar (*Populus tremula* L.) and birch (*Betula pendula*) using genetic transformation with a heat-inducible *FLOWERING LOCUS T* gene (HSP::*AtFT*). This approach allowed flowering induction in less than one year for both tree species. This contrasts with the years or even decades required by most forest tree species to initiate the reproductive phase.

Genetic containment can be achieved using gene constructs that disturb normal development of reproductive structures. The *BARNASE* gene, a ribonuclease from *Bacillus amyloliquefaciens*, and a flower specific gene promoter, *ENDOTHECIUM 1* gene promoter from *Pisum sativum* (PsEND1), have been successfully used for gene containment in many plant species. Transgenic lines were obtained for poplar expressing both an early flowering (HSP::*AtFT*) and a containment (PsEND1::*barnase-barstar*) gene construct. This approach allowed the evaluation of a possible gene containment system within one year. RT-PCRs confirmed *BARNASE* gene activity in flowers, and pollen development was disturbed, leading to male sterile flowers (Briones et al., 2020).

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Keywords: precocious flowering, biosafety, gene containment

Regulation of modern biotechnology in Argentina

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The Environmental Risk Assessment (ERA) of genetically modified (GM) crops in Argentina is carried out by the National Advisory Commission on Agricultural Biotechnology (CONABIA) and the Coordination of Innovation and Biotechnology (CIyB) of the Ministry of Agriculture, Livestock and Fisheries. In recent years, a review process and some modifications were carried out such as the evaluation of transgenic crops with stacked genes analyzing the possible interactions between transgenes and expression products, the strengthening of the ERA taking into account the transportability of the data and conclusions of confined field trials, the adoption of the concepts of Familiarity and History of Safe Use on the risk assessment of expression products, special considerations for the unwanted effects of insertion sites, and the Insect Resistance Management Plan (IRMP) was reformulated. Regarding gene editing derived products, Argentina was the first country that developed specific regulation, in 2015. It consists in determine if the product is GM or not. To determine the regulatory status of these products, an instance of prior consultation (IPC) is allowed to developers. In 2021 the regulatory framework for NBT was updated, considering all organisms (animals, micro-organisms and plants) under the same NBT resolution, independently and without being linked to the commercial regulations on genetically modified organisms (GMOs). The final product-oriented analysis rather than the relative importance of the technology employed for its obtention is the key criteria to determine the GMO nature of the subject to regulation. To do so, the regulation system analyzes if a "novel combination of genetic material" (derived from the Cartagena Protocol on Biosafety) is inserted (or not) in the final genome of the crop. The intended product analyzed either can be real cases (already obtained) or hypothetical cases (a developing project). So far, several IPCs have been presented, to determine whether a new development will generate a transgenic or not but, there have been no consultations on genetic editing of trees.

Keywords: GMOs, genome editing; new breeding techniques; regulation, biosafety.

Functional evaluation of K^+/Na^+ symporter gene, *EcHKT1;1,* using composite transgenics of Eucalyptus

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In plants, high-affinity K^+ transporters (HKTs) are key determinants of Na^+ homeostasis under salt stress and contribute to reduce Na^+ specific toxicity. HKT transporters show functional diversity and have been implicated in Na^+ uptake from the external medium as in the case of *TaHKT2;1* or in the unloading of Na^+ from xylem like in *AtHKT1;1*. This study was taken up for functional evaluation of *HKT1;1* gene in eucalyptus. Relative expression profiling of *HKT1;1* in leaves of two contrasting non-transgenic Eucalyptus genotypes *viz.,* salt tolerant EC-7 and salt susceptible ET-88 clones, was analyzed. Ec-7 showed 9.61-fold downregulation of *HKT1;1* expression 24 h post salt stress, while salt susceptible clone ET-88 showed no such changes. To evaluate the function of *EcHKT1;1 in planta,* the gene was downregulated via RNA*i* in Eucalyptus composite transgenic roots that were generated from non-transgenic shoots using *Agrobacterium rhizogenes*. In these transgenic roots, *EcHKT1;1* downregulation ranged from 37 % to 74 %. About 33 % of the composite transgenic plantlets generated using the *EcHKT1;1* silencing construct were able to tolerate up to 400 mM NaCl, whereas control plantlets failed to survive at 350 mM NaCl. The average shoot to root ratio of sodium was 4.9 folds lower than the controls indicating restricted translocation of Na^+ to the shoots. This study suggests that *EcHKT1;1* may function like *TaHKT2;1* in uptake of Na^+ from the roots, and that *EcHKT1;1* may be a potential target for engineering salt tolerance in Eucalyptus.

Keywords: *gene silencing, RNAi, Na^+ transporters, composite transgenics, abiotic stresses*

Rapid evaluation of gRNAs for generating gene edits in Eucalyptus using GFP-tagged roots generated by *Agrobacterium rhizogenes* mediated transformation

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Genome editing is increasingly being used in gene function analysis and genetic modification of traits. CRISPR-Cas9 technology uses guide RNAs to target gene edits. Rapid evaluation of these gRNA's for engineering the desired gene-edits is important, especially in trees like Eucalyptus, in which the time taken to generate transformed plantlets using *Agrobacterium tumefaciens* is protracted. In Eucalyptus, the composite transgenic system generated using *A. rhizogenes* (Balasubramanian *et al*., 2011) has been used for gene editing to analyse lignin biosynthesis gene, *CCRI* (Dai *et al*., 2020). In this study, we used GFP-tagged roots of *Eucalyptus camaldulensis* generated using *A. rhizogenes* for rapid evaluation of gene deletions generated using gRNA constructs targeting the *EcHKT1;1* promoter and the first exon region. The two gRNAs were designed based on endogeneous tRNA processing system (Xie *et al*., 2015). The synthesized gRNAs driven by *MsPRP2* promoter was cloned in Cas9 and GFP based transformation vector *viz*., pHKN29::Cas9, and used for *Agrobacterium rhizogenes* mediated transformation of hypocotyl explants. The GFP-tagged roots, generated 36 days after co-cultivation, were used for DNA isolation and PCR analysis. When compared to the 1581 bp amplicon generated from the non-transgenic control seedlings, the pooled GFP-tagged roots showed a smaller 170 bp amplicon indicating the expected 1411 bp deletion between the promoter and first exon of *EcHKT1;1* due to the gRNAs used in the vector. This study, thus demonstrates the feasibility of using GFP-tagged roots rapidly generated by *A. rhizogenes* mediated transformation for quick evaluation of gRNA vectors for generating desired gene-edits.

Keywords: Gene Knock-out, CRISPR-Cas9, Na^+ transporter, composite transgenics, amplicon sequencing, gRNA screening.

Evaluation of *MsPRP2* promoter for root preferential and salt inducible expression in *Eucalyptus camaldulensis*

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Constitutive promoters are used in the genetic transformation to drive optimal expression of the transgene. The use of an appropriate promoter can facilitate transgene expression in response to environmental stresses like drought and salt, in the desired tissue or developmental stages. The use of such promoters can help to reduce yield drag due to the expression of transgenes. Root preferential and salt-inducible promoters have not been reported in Eucalyptus. A 652 bp region of the *MsPRP2* promoter has been shown to drive root preferential and salt-inducible expression in *Medicago sativa* (Winicov *et al.,* 2004). Similarly a 1062 bp of *GmPRP2* promoter has been reported to drive root preferential expression in Arabidopsis and Soybean (Chen *et al*., 2014). The 652 bp *MsPRP2* promoter and 346 bp *CaMV* promoter (Vickers *et al*., 2007) were analyzed using PLACE software. When compared to *CaMV* promoter, the *MsPRP2* promoter showed one additional salt-inducible motifs GT1GMSCAM4 (-382, -431 bp : *MsPRP2* and -154 bp: *CaMV*) and three additional root preferential motifs ROOTMOTIFTAPOX1 (-351, -416 , -458, -561 bp: *MsPRP2* and -336 bp: *CaMV*). The 652 bp *MsPRP2* promoter was used to develop *MsPRP2* driven *GFP* and *GUSPlus* gene based vectors *viz.*, pCAMBIA1305.1::*MsPRP2*:*GFP*:*HSP* and pCAMBIA1305.1::*MsPRP2*:*GUSPlus*:*HSP*. Callus generated from explants co-cultivated with AGL1 strain of *Agrobacterium tumefaciens* harboring the pCAMBIA1305.1::*MsPRP2*:*GFP* vector showed *MsPRP2* driven *GFP* expression. The transformation efficiency as assayed through *GFP* expression 85 days after co-cultivation ranged between 4.9 to 9.5 %. Hypocotyl explants were co-cultivated with *A. rhizogenes* strain, A4RS, harboring the pCAMBIA1305.1::*MsPRP2*:*GUSPlus.* Calli developed from hypocotyls cultured in 200 mM NaCl showed 14.5 percent *GUS* expression when compared to 1.6 percent in explants cultured in salt-free medium. These results show that the truncated promoter sequence of the *PRP2* gene from *Medicago sativa* can drive transgene expression in Eucalyptus callus and may also be salt-inducible.

Key Words: tissue specific promoter, salt tolerance, gene expression, transgenics, eucalyptus

Effects of long-term cultivation of transgenic birch and aspen with modified nitrogen metabolism on soil microbial activity and biomass

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The potential adverse effects of transgenic trees on soil processes get less attention than their other environmental risks. We investigated the effect of transgenic birch (*Betula pubescens*) and aspen (*Populus tremula*) plants expressing the marker *gus* gene or the cytosolic glutamine synthetase *GS1* gene from pine in a pot experiment under greenhouse (2 years) and natural conditions (2 years). Enzyme activity (11 enzymes from C, N, P, and S cycles) and microbial biomass (MBC, MBN, MBP) were analyzed at the end of each season, and physicochemical soil properties at the end of the experiment. Transgenic birch plants with the *GS1* gene differed in growth rate, habitus, and the content of C and N in leaf tissue. The effect of the GS1 gene overexpression in aspen plants was less noticeable. The nitrogen content in soil increased, while the content of potassium ions decreased, possibly to compensate for nitrogen reassimilated via glutamine synthetase. The observed statistically significant differences in enzyme activities and microbial biomass were temporary and inconsistent. The effects did not increase with plant age, but on the contrary disappeared by the end of the experiment. The birch and aspen plants with the neutral *gus* gene had a significantly weaker impact than those with the gene of nitrogen metabolism enzyme GS. In particular, GS plants showed the most stable (in 3 of 4 years) effect on N-cycle enzymes: nitrate reductase for birch and protease for aspen. Increase in the aboveground biomass and changes in the content of N in plant tissues can affect soil processes through litter. This is the first study assessing effects of transgenic plants with improved nutrient use efficiency on soil processes. The obtained results may be important for assessing potential environmental risks associated with commercial cultivation of transgenic forest trees.

Keywords: birch, aspen, glutamine synthetase, nitrogen use efficiency, soil microorganisms