Novel tree breeding strategies

Reporting

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Final Report Summary - NOVELTREE (Novel tree breeding strategies)

Executive Summary:

The challenges facing forest geneticists and tree breeders include recognition of changing demands on forests for a wider range of high value forest products and increasing interest on sustainability of forest ecosystems under climate change. Following a systems biology approach and using cutting-edge genomics technologies, NOVELTREE succeeded in deepening our understanding of the nature and origin of genetic variation for important adaptive traits, increased growth and wood yield and in leading to more efficient management of breeding stocks designed to exploit genetic variation whilst maintaining diversity in the long term.

During the project, huge experimental efforts were devoted to the evaluation of phenotypic responses of forest tree individuals and populations in both natural conditions covering European afforestation areas and in controlled environments mimicking latitudinal transfer (photoperiod, temperature) and biotic (pathogen attacks) or abiotic (high/low temperatures, water limitation) constraints. For the first time, significant genetic variation for phenotypic plasticity under partial additive control has been observed for both adaptive, yield- and wood-related traits in different model tree species. Superior genotypes expressing broad adaptability to contrasted environments could be identified among the top ranking performers at all environments. For traits with adaptive thresholds like water use efficiency or bud phenology, the specific pattern of phenotypic plasticity over tested environments has a high predictive interest. Specific high throughput methodologies to assess performances under water limitation and wood
chemical composition have been developed. The genetic variation detected for wood properties associated to transformation of the cellulosic biomass in bioenergy could result in increased competitive advantage for Europe’s forest industry.

For all species of interest, deoxyribonucleic acid (DNA) marker discovery efforts have been transitioned to next generation sequencing (NGS) technologies. By allowing the generation of millions of short reads sequences for given sets of candidate genes or for complete genomes, several thousands to three millions of single nucleotide polymorphism (SNP) are today available to ‘tag’ alleles conferring desirable traits and explore more efficiently natural diversity. Specific high throughput genotyping arrays have been developed for all species and provided a more precise description of genetic structure in both natural and breeding populations.

A suite of new decision support tools (Popsim and Metagene, see http://www.igv.fi.cnr.it/noveltree/) have been developed and made available online to European tree breeders. They can assist in the evaluation of alternative breeding strategies allowing reduction of recombination costs, management of antagonistic selection objectives and genetic gain/genetic diversity compromises. The large size of breeding populations of forest trees represents a challenge for the implementation of marker-assisted selection and genomic selection. There is, therefore, an effort to be made in the redefinition of elite populations with a narrower census while keeping genetic representativeness. Moreover, such compact elite populations could allow a concentration of phenotyping efforts to explore variation of new traits and provide training populations for either detection of relevant polymorphisms or genomic prediction.

An operational bioeconomical model which includes a module for enhanced growth and stem quality due to genetic gain demonstrated economic returns of the genetically-improved material (Motti, see http://www.metla.fi/metinfo/motti/index-en.htm). Thanks to integration of new production functions from more than 140 genetic experiments and climatic modelling, a web-based decision tool provides a user friendly interface for deployment recommendations of Scots pine seed orchards in northern Sweden and Finland. New insights on the genetics of the plant-pathogen relationships in the context of cultivated plantations have provided elaboration of revised breeding and deployment guidelines of improved hybrid poplar clones.

Dissemination and communication is an important part of NOVELTREE. Annual newsletters provided updates on the research and outputs. Training workshops, open conferences, breeding and deployment web-based decision tools, species breeding guidelines and a web-based book all inform a large audience about the new concepts and technologies available to help tree breeding meet the new challenges of climate change and participate to a more sustainable management of Europe’s forests. In total, the project has lead to the publication of 25 peer-reviewed scientific publications and to a book chapter on genomics and forestry. Many other publications are under preparation and will be published after the end of the project.

Project Context and Objectives:

The challenges facing forest geneticists and tree breeders today include recognition of changing demands on forests for a wider range of high value forest products and increasing interest on sustainability of forest
ecosystems under climate change.

NOVELTREE was designed to enable significant genetic improvement of tree characteristics and forest products properties to face climate change constraints and satisfy the needs (quality, quantity, sustainability, vulnerability) of the forest-based sector and consumers. This project provided an improved understanding of response of forest tree species to a wide range of climatic conditions which were of major help for a revision of selection criteria and the successful achievement of multiple selection objectives. New high throughput phenotyping tools allowed higher selection intensity, increased accuracy in genetic prediction and more innovative integration of physiological responses to biotic and abiotic constraints. Important focus was placed on deciphering adaptive significance of phenotypic plasticity and evaluating its genetic variation in forest tree populations. A second important objective of the project was to lean on the rapid pace of genomic research to pinpoint and label genomic regions involved in the genetic control of both adaptive and productivity traits. Large efforts were put on NGS technologies which provided a more complete coverage of tree genomes. Innovative breeding tools combining phenotypic and molecular information were adapted from animal breeding to allow: earlier genetic evaluation, higher selection intensity, increased accuracy in genetic selection and better monitoring of genetic diversity along generations. New generalised decision-support tools were made available to tree breeders and forest owners to compare efficiency of simulated multi-generation breeding plans and to optimise spatial and temporal deployment of improved genetic stocks in both prevailing and future climate. Demonstration of the efficiency of these novel breeding strategies focused on model tree species of high economic importance in different European regions: maritime pine, Scots pine, spruce and poplar.

By adopting a coordinated and collaborative approach, NOVELTREE project had unified the efforts and resources developed at the European level in forest tree breeding, reinforced the leadership of Europe in forest tree breeding and produced a competitive advantage to the European forest-based sector in the global market. Knowledge and technologies developed through research were transferred to academic and non-academic beneficiaries.

To achieve these goals, NOVELTREE brought together an interdisciplinary group of 15 public and private partners from seven European countries with expertise in quantitative genetics and breeding, population genetics, genomics, pathology, tree physiology, forestry and economics. NOVELTREE was structured in four research and technology work packages (WPs), a fifth one focusing on dissemination, technology transfer and training and a sixth being devoted to the management of the project and relationships with other on-going projects in and outside Europe.

WP1. The objectives of WP1 were to define integrative selection criteria relevant to tree plasticity, adaptation and production of high added value wood products over a wide range of environments in Europe. WP1 focused on the tree phenotype that needs to be understood in order to predict and improve both short and long term tree responses to already shifting environmental and market conditions. For the four model species or group of species, investigations concerned wood production as main integrative selection objective. In a context of climate change and evolution of land uses, other related traits such as adaptive responses to biotic and abiotic constraints and quality of wood products were included in forest breeding programmes. Moreover, specific emphasis was put on quantification and comprehension of phenotypic plasticity of performances under a wide range of environments. Emphasis was put on
development of high throughput phenotyping methodologies in order to increase the possibility of high selection intensity and therefore increased genetic gains. WP1 also provided WP3 and WP4 with a range of genetic parameter estimations for model tree species to compare efficiencies of different breeding strategies. Phenotyping of genetic material included in association studies and mapping allowed identification of relevant molecular polymorphisms for traits of interest in WP2.

WP2. One of the primary aims of this WP was to extend the genomic resources available for poplar and conifer species to yield a more precise picture of genetic variation at genome level and to allow identification of polymorphisms at genes related to the breeding-target-trait, the so called 'candidate genes'. The adoption in most species of NGS technologies placed NOVELTREE at the cutting edge of research on development of SNP markers. Another important objective of WP2 was to develop high throughput methods to genotype large sets of individuals at candidate genes in association and QTL mapping populations and help future implementation of marker-assisted prediction in tree breeding programmes.

WP3. This WP prepared the integration of these genomic resources through the building and adaptation of the most efficient methods of genetic prediction and multi-trait/multi-generation selection strategies and by demonstrating their application through well-chosen case studies in model species. Designing of improved/novel tree breeding strategies benefited from the participation of experts in methodology developments for animal breeding. The novelty for forest tree species was to incorporate relevant information acquired from enhanced knowledge in both phenotyping (WP1) and genotyping (WP2). The benefit of this novelty was a significant increase in selection accuracy, selection at a younger age and an improved control of genetic diversity. A parameter driven simulation model was developed to evaluate and optimise breeding strategies over generations, considering the above determinants and with possibilities for species-specific inputs. More specific genetic phenomena (genetic correlations, major genes, phenotypic plasticity) were approached by allele based simulation models. All relevant outcomes of WP3 were made available to the European tree genetics RandD community through guidelines and expert tools for optimal design of breeding strategies.

WP4. A first objective of this WP was to assess efficiency of novel/improved breeding strategies identified in WP3 for increasing competitiveness of the European forest-based sector. Prediction of the yield and economic benefits of genetically improved planting stocks on timber supply and sustainable management of forest resources required the integrated knowledge of geneticists, silviculturists, biometricians and economists. Successful incorporation of genetic gains in forest management, biomass production and timber supply also required improved tools to update optimal deployment guidelines. WP4 developed a decision-support tool for dissemination of optimal seed transfer rules for Scots pine in Sweden and Finland. For a given seed source, the user will be informed about geographic restrictions on its utilisation; effects of site transfer on its survival and productivity and expected effects of climate change. The development put emphasis on user friendliness and visualisation of effects in maps and graphs and system will be freely accessible for forest owners. A long-term sustainable approach to pest management with poplar plantations as case-study used latest knowledge on durability of pest genetic resistance to revise guidelines for spatial and temporal deployment of improved cultivars.

WP5. The objectives of this WP were to:
1. disseminate knowledge and results to different audiences (research, forest tree breeders and professionals of the forestry wood chain, policy makers and public)
2. transfer technology to research and tree breeding companies and
3. provide training in emerging technological approaches in and outside the project.

This WP ensured that the science and results of research carried out in the project were adequately transferred to the relevant scientific and forestry community within the European Union (EU). It is essential that budget holders, policy makers and stakeholders are aware of the progress being made in the area of novel tree breeding, its application and benefits. It is also essential that the science is applied as fully as possible by up-dating the knowledge of scientists.

Project Results:

WP1 - Choice of selection criteria adapted to sustainable forestry. Phenotypic and genetic decomposition of complex traits, such as increased growth and yield under environmental constraints, has revealed significant genetic variation for growth strategy involving key phenological stages such as budflush, transition phase, growth cessation and budset. Efforts were concentrated on definition of optimal environmental conditions for early tests on one year old seedlings in large-scale greenhouse experiments or in nursery tests on two-year-old cuttings. When the genetic material is covering large latitudinal or altitudinal clines, evaluation of genetic variation requires experiments using several temperature/photoperiod regimes (Annex I, Figure 1). New indirect methods of evaluation of frost tolerance based on seasonal changes of mitotic activity in buds have been tested in Scots pine and Norway spruce species but did not respond to initial expectancy and remained too work-demanding for practical tree breeding. Genetic variation of potential use in breeding programs has also been detected for physiological responses to water limitation of the photosynthetic and hydraulic systems. If direct evaluation of transpiration efficiency is highly time consuming, bulk wood 13C composition proved to be a reliable predictor of water use efficiency in maritime pine and poplar with relatively high through-put. Implementation of direct selection on water use efficiency will be possible once the up-scaling of intrinsic water use efficiency from leaf to canopy levels will be confirmed. Carbon isotopic discrimination could however be used as interesting covariate to control potential negative effect on transpiration efficiency of direct selection on productivity. A special emphasis has been placed on the development and test of a new robotised phenotyping method for assessing tree transpiration and water-use efficiency under controlled irrigation. This new infra-structure is now accessible to European research projects in the 'Tree4Future' infrastructure project (see http://www.trees4future.eu/transnational-accesses/inra-platform-for-phenotyping-tree-water-relations.html). Significant genetic variation for hydraulic properties such as vulnerability to cavitation was observed in maritime pine. The 'cavitron' screening approach developed up to now still shows limitation in the number of genotypes evaluated per unit time and requires still some additional research before being applicable routinely in tree breeding programs. Concerning biotic constraints, relevant criteria associated to avoidance, resistance and tolerance against diseases were explored in the Melampsora-poplar rust pathosystem. Limited genetic variation for escaping pathogen epidemics has been observed from the analysis of the relationships between bud flush phenology, early growth, rust susceptibility and impact of rust infection on growth potential. No significant clonal variation could either be observed for leaf wettability, a constitutive resistance which do not exert selective
pressures on pathogen populations. Resistance mechanisms, involving interactions between the pathogen and the host are by far the most important available defence strategy. The analysis of relationships between rust susceptibility evaluated in field tests and laboratory assessments with controlled strains helped to identify the best predictors of high quantitative resistance level under natural infection: presence of qualitative resistances even overcome today by the pathogen, long latent period and reduced sporulation intensity. Protection from rust infection of part of the field experiment can provide more precise estimation of rust tolerance (relative decrease of growth potential) for which significant genetic variation seems to exist independently from genetic variation for rust resistance. In order to reduce evaluation costs, the efforts need now to focus on the identification of relevant indirect physiological predictors of tolerance. Specific higher throughput methodologies to assess performances for wood mechanical and chemical properties and energy content with a non-destructive way on micro-samples have been developed and validated on Populus sp., Salix sp. and maritime pine. This tool chain developed by Ugent wood laboratory is now accessible to European research projects in the Tree4Future infrastructure project (see http://www.trees4future.eu/transnational-accesses/dsc-tga.html). High predictive efficiency at low cost was achieved for wood chemical contents such as lignin and cellulose contents with near infrared spectroscopy (NIRS) applied after appropriate calibration on a reference sample. Huge experimental efforts have been devoted to the evaluation of phenotypic responses of forest tree individuals and populations in both natural conditions covering European afforestation areas and in controlled environments mimicking latitudinal transfer (photoperiod, temperature), biotic (pathogen attacks) or abiotic (high/low temperatures, water limitation) constraints. An initial selection of 25 different experimental series including the six model species was used as support of specific investigation of two complementary but distinct responses:

1. phenotypic plasticity - the direct influence of environment on the development of each tree - and
2. environmental sensitivity to natural selection -the change in the optimum population composition with the environment.

Different norms of reaction linking phenotypic performances with different descriptors of environmental conditions combining site or climatic year effects were developed and implemented. Examples of time-related phenotypic plasticity from maritime pine experiments showed how either short-term change in wood density or repeated assessments of the same trait over two climatically contrasted years can provide a very interesting tool for obtaining individual plasticity estimates. For the first time, significant genetic variation for phenotypic plasticity under partial additive control has been observed for both adaptive performances, yield- and wood-related traits in different model tree species. These results offered new opportunities to fine-tune the range of tree response to more uncertain environmental conditions. Although significant in all species and for all traits of adaptive and economical interest, genotype by environment interaction explained a smaller part of phenotypic variation than genetic variation itself. Moreover, superior genotypes expressing broad adaptability to contrasted environments could be identified among the top ranking performers for yield at all environments. However, when the focus is in other traits with adaptive thresholds like water use efficiency or bud phenology, the specific pattern of phenotypic plasticity over tested environments could have a predictive interest and could be used in the selection process in complement to stability parameters.

WP2 - Identification of DNA polymorphisms for monitoring genetic diversity and marker assisted selection (MAS). WP2 was the high-tech WP which used the latest tools and DNA-marker knowledge. DNA-marker
resources were extended in two directions:

1. by the increase of the number of neutral or ‘background’ markers such as microsatellites or SNP which can be used to monitor genetic diversity and control population structure in breeding programmes especially for Scots pine and maritime pine for which such resources were expected for long time by the scientific community;
2. by the generation of new markers associated to the functional polymorphism of genes related to the breeding-target-traits, the so-called candidate genes.

As one important challenge for NOVELTREE was to incorporate genomic technologies into forest tree improvement programmes and to demonstrate the efficiency of MAS large efforts were devoted to identify SNP resources covering the most interesting genomic regions for four species of major economical and ecological interest: Scots pine, maritime pine, Norway spruce and black poplar. Large sizes of conifer genomes have led to the adoption of a candidate gene approach for a set of important target traits (cold tolerance, growth phenology, resistances to abiotic and biotic stress, wood properties). For populus nigra, availability of a reference genome sequence and access to new sequencing technologies allowed genome scans for a set of 50 different genotypes. All species exceeded expectations, with more than 1 300 SNP for Scots pine up to more than 3 300 000 for black poplar. The use of new sequencing technology and fruitful collaboration with other on-going EU-projects or north-American partners participated to this high success beyond initial objectives. The development of high throughput genotyping tools is undoubtedly a prerequisite to the identification of genomic regions (QTL) involved in observed phenotypic variation and to the implementation of marker assisted breeding. For four forest tree species, NOVELTREE project has explored benefits of the most recent SNP discovery studies to customise ten genotyping tools ranging from 48 to more than 10 500 SNP. Workflows of SNP selection were designed according to scientific objectives and array design requirements. Thanks to the pooling of resources across different EC-funded projects in some species (maritime pine, poplars) or through the cooperation with Canadian projects in others (spruces), larger genotyping assays than envisioned have been obtained in all species. The genotypic information collected on different tree populations with these ten SNP arrays will be of great value for the molecular genetics and forest tree breeding community. As example, the identification thanks to SNP of unique gene pools for major productive regions of maritime pine (e.g. Aquitaine, north-western Spain) provided a new, fast and cheap genotyping tool for identification of forest reproductive material. Important progress has been accomplished in development of SNP-based genetic maps in four conifer species. In maritime pine, a consensus map comprising 357 SNP from 292 different genes is now available and could be aligned on p. taeda linkage map thanks to the sequence homology of the mapped markers and their orthologs in this other pine species. In Scots pine, two genetic maps have been produced with respectively 183 and 166 new SNP identified in genes involved in cold tolerance and photoperiodic pathway. In Norway spruce, a 270-F1 progeny population is genotyped with a 3 072 SNP illumina GoldenGate array designed jointly with candidate gene-based SNP originated from circadian clock and photoperiodic pathway and background SNP. Finally, in Sitka spruce, a new genetic map with 799 SNP has been produced and further enriched with the alternative SNP discovery approach known as Reduced-representation sequencing. Thanks to the phenotypic evaluation of these mapping pedigrees for different traits of interest in WP1, important progress has also been accomplished in detection of genomic regions involved in the target traits. A strong emphasis was put on phenology in all species but otherwise other target traits varied among species, reflecting differences in adaptive constraints and economic goals
among species. The other target traits correspond to responses to biotic constraints in poplar and Norway spruce, to abiotic constraints (temperature, water limitation) in Scots pine, poplar and maritime pine and finally, to wood related-traits in poplar and maritime pine. Initial efforts yielded contrasted results according to target trait and species. Some major QTLs explaining more than 20% of the phenotypic variance have been discovered in maritime pine and Scots pine for cold tolerance, water-use efficiency and wood-related traits. They should be considered as genomic regions of high priority in implementation of MAS. However, the large number of QTLs with small additive effects, large confidence intervals, site or year specific effects detected in most situations highlighted the complex nature of the target traits studied. QTL detection is known as a robust method to detect relevant polymorphism but its resolution is limited, especially when possibilities of phenotyping are limited. Association mapping which used linkage disequilibrium created through the history of the population should provide higher resolution and allow screening of a larger number of alleles per locus than QTL mapping. The NOVELTREE project contributed to this effort by mapping sets of simple sequence repeat (SSR) and/or SNP markers in breeding and association mapping populations of four conifer species and one poplar species. Various strategies were adopted in the different species, reflecting availability of both phenotypic and genotypic data. In maritime pine, a strategy based on the use of small genotyping arrays in a suite of populations and crosses was favoured and a few promising SNP associated to growth potential and wood composition were identified. In both poplars and Norway spruce, a single large, range-wide and composite association mapping population was genotyped with a single and large genotyping array. In contrast, genotyping in Scots pine was concentrated on a single and large population from central Finland. In all these species, the analysis of the association between the mapped markers and phenotypic traits of interest has been initiated and has started to deliver preliminary results.

WP3 - Design and implementation of improved/novel tree breeding strategies This WP served to identify and demonstrate strategies for breeding that benefit from genomic techniques, revision of selection criteria and integration of successful methodologies from livestock breeding. The approach was to evaluate proposed strategies by simulation and other quantitative methods, to demonstrate efficiency on a few well-chosen case studies and to present resulting tools and guidelines to the European tree breeding community. Popsim and Metagene, two complementary simulation tools were greatly enhanced through the project allowing greater scope to study and compare breeding strategies for a better management of genetic diversity and a significant increase of selection efficiency. Popsim is an example of a parametric model and is particularly flexible for implementing complex population management and selection schemes over multiple generations. With it, we can breed founders according to a plan, generate offspring for progeny testing and for recruitment populations, evaluate field test data (BLUP breeding values), make selections according to specified criteria, generate deployment populations and evaluate their genetic gain, effective size and diversity. Metagene is based on an allelic model and so has the ability to integrate new insights on the nature of quantitative genetic variation and to simulate behaviours influenced by the interaction among specific genes, such as genetic correlation or marker-assisted selection. These tools were posted on the NOVELTREE public website (see http://www.igv.fi.cnr.it/noveltree/) and a registered user base receives periodic updates to the software and documentation. Two other software tools were added to the breeder's tool chest, providing ability of:

1. estimation of relatedness over multiple generations from molecular markers and
2. optimum selection to maximise gain in seed orchards with a constraint on diversity (Opsel). All these
tools were applied to forest case studies in poplar, Norway spruce, maritime pine and Scots pine. Individual tree merit prediction which referred to the aggregation of breeding values of all traits under selection and individual contribution to the genetic diversity of the population benefited of integration of methodologies already in practice in other plant models and in advanced livestock programmes but to a more limit extend in forest trees. The most innovative and efficient prediction methods were:

1. merits that use information from resource competition models. Forest production relies on co-existence of neighbours in light and root space and therefore there is potential to increase biomass production even in limited resource conditions and to increase homogeneity of the products. Fitting competition at both the genetic and residual level provided a significantly better prediction than models which either ignored competition or fitted it at just the genetic or just the residual level. Biological validation was based on a 19-year old Sitka spruce clonal trial growing in Scotland.
2. merits which allow management of identified trade-offs including phenotypic plasticity and adverse correlations between breeding objectives. Simulation studies gave insight on how genetic correlations react to directional selection and identified the consequences of selecting against adverse genetic correlations under contrasting selection regimes.
3. merits that maximise multi-generation selection accuracy through the integration of all available molecular and phenotypic information; the so-called marker- and gene- assisted selection. An extensive bibliographic review was carried out to explore the most favourable conditions for development of MAS in forest trees (genetic architecture of traits, number and quality of relevant molecular markers, size and diversity of training and candidate populations, etc.).
4. merits that balance short-term and long-term genetic gains thanks to control of pedigree information and/or DNA fingerprints.
5. merits based on genome-wide evaluation that use dense SNP technologies under rapid development.

Theory was combined with simulation to show how the outcomes from differing techniques for genomic evaluation depended on the species genome. Validation was provided by applying genome-wide evaluation to a large full-sib family of Sitka spruce. Other improved breeding designs focussed on relevant constraints in forest tree breeding such as:

1. sustainable management of genetic diversity up to seed production which could benefit from optimised breeding population structures explored by Popsim-Opsel, an improved multi-generation breeding strategy evaluation tool.
2. cost-efficiency of the breeding value evaluation step where polymix breeding with paternity analyses was evaluated as an alternative to classical full-sib mating allowing simultaneous evaluation of breeding values from half-sib/full-sib individuals and production of the next-generation recruitment population.
3. time-efficiency which could be significantly improved by integration of clonal replication in genetic tests for tree species with late sexual maturity.
4. performance stability over time which could be better addressed in selection thanks to the identification of genetic mechanisms underlying forest tree phenotypic plasticity, especially molecular regulation of major candidate genes involved in control of growth rhythm.

Bringing together findings from across the different WPs and indeed across the entire project, a major work was conceived to present best practices for tree breeding in Europe. A team of collaborators
covering expertise on forest genetics and breeding drafted the outline for the document, which includes an introduction on principles for evaluation of strategy alternatives, chapters on best practice for three major conifer species in Europe (Norway spruce, Scots pine and maritime pine) and concluding with a wrap-up chapter on extension to other species and situations. The document has been edited for publication as a general report to be widely distributed to a European audience.

WP4 - Benefits and optimal deployment of improved forest material under a global change context

Genetic improvement through breeding, selection and testing has had a significant impact on the productivity and quality of plantations established worldwide. Growth and yield models offer useful tools for estimating genetic gain at rotation age and at stand level. In NOVELTREE project, a more integrative bioeconomical model was being built as an extension of the existing simulator Motti originally developed by Metla and allowed the assessment of effects of forest management practices on stand dynamics and the profitability of forest management. Simulations done for Scots pine stand management in Finnish conditions suggested that use of genetically improved material gives considerable economic gains in most geographic regions. Furthermore, it would be more beneficial in financial terms to focus breeding activities on improving growth than stem quality. With respect to regional- or national-level analyses, deploying genetically improved Scots pine seed material on only 25% of suitable sites would maintain the production of industrial and biomass wood observed for this species in 2010 while compensate the protected forest area on Southern Finland. Thanks to integration of new production functions from more than 140 genetic experiments and climatic modelling, Planter’s Guide Two, a web-based decision tool provides a user friendly interface for deployment recommendations of Scots pine seed orchards in northern Sweden and Finland. Together with climate researchers from Swedish and Finnish Meteorological Institutes, 24 climate indices considered important for forest tree performance in northern Scandinavia have been identified and correlated with performances collected in the genetic field experiments. Height growth and survival at a given site in northern Sweden or Finland were modelled as a function of the climate indices at the site and of the origin of the deployed genetic material. The best models identified for both height growth and survival provide good explanatory power and are valid for both countries, making a joint recommendation structure possible. Planter’s Guide Two was presented for the first time in October 2012 at a workshop organised in Helsinki for the forest sector and end-users. During the last decades, European poplar breeders learned the hard way that Melampsora larici-populina (Mlp) has an impressive adaptive potential. This past experience on poplar breeding underlines the need for more durable disease resistance that withstands mutations and genetic shifts in pathogens. New insights on the genetics of the plant-pathogen relationships in the context of cultivated plantations have provided elaboration of revised breeding and deployment guidelines of improved hybrid poplar clones. Breeding options should thus rely on a more careful exploration of the genetic variability available in the parent species for several features limiting pathogen dynamics and adaptation: constitutive resistance associated to leaf anatomy, combination of defeated complete resistance and different partial resistance sources, phenological avoidance and physiological tolerance (i.e. the ability to maintain growth despite being susceptible to the disease). Local adaptation of Mlp to poplar host genotype confirmed to be high and rapid, even in clonal mixtures composed of a wide range of different resistance sources. However, new clones combining several sources of resistance and tolerance cited above demonstrated a more stable performance over a longer period in experimental conditions both favouring early pathogen infection and inter-annual recombination on the alternate host. Revised deployment guidelines should generate more host diversity at regional scale through simultaneous release of several unrelated cultivars and regular revision of the
recommended elite material. An on-line book has been produced by the NOVELTREE consortium in order to disseminate easy-to-understand objectives and results of the project to the forest tree breeding community and students in forest genetics. This e-book, also published in 2013 as hard copy in INIA monography series brings together a discussion on the techniques, advantages, disadvantages, technological barriers and other challenges which face tree breeders as they consider the use of genomic selection, climate change, competition and phenotyping as part of their breeding programme.

Potential Impact:

The progress on adaptation and competitiveness of European forests provided by decades of conventional tree breeding for tree species of high economical importance are commonly recognised and implemented. As claimed in the Strategic Research and Innovation Agenda of the forest-based sector technology platform, genetic improvement is one of the most efficient ways of changing the genetic makeup of domesticated and wild populations for increased economic value and for adaptability to new environments. It is also a powerful tool for the understanding of the associations between phenotype, genotype and environment, as genetic improvement is an experimental process that is based on assessing and predicting those associations under controlled or monitored conditions.

Following a systems biology approach on four tree species of main economical importance in Europe and using cutting-edge genomics and modelling technologies, NOVELTREE succeeded in:

1. deepening our understanding of the nature and origin of genetic variation for adaptive traits, increased growth and wood quality to help better design of planting stock in the context of climate change and search for more competitiveness of wood-based products
2. integrating new knowledge from genomics and tree physiology with more complete description of phenotypic performances to predict accurately complex tree responses and adjust both breeding and deployment strategies to challenges of global change
3. developing genomic resources and decision tools which will help tree breeders, forest managers and the wood industry to meet the demands for a sustainable management of European forests and a competitive European forest-based sector
4. enhancing application of the most up-dates biotechnology- and modelling-based alternatives to speed up more efficient management of breeding stocks designed to exploit genetic variation whilst maintaining diversity in the long term
5. serving as a unified platform for exchange of ideas and knowledge with major counterparts across the world.

Significant progress in the understanding of the nature and origin of genetic variation for traits of interest in the context of climate change

During NOVELTREE project, the complexity of tree adaptive phenotype to various climatic and biotic constraints has been dissected in different field and controlled environments conditions. A very large range of traits of interest (growth, phenology, water use efficiency, drought tolerance, reproduction, wood properties) have been analysed in an integrative manner, in some cases for the first time in forest species.
Significant genetic variation for water use efficiency and hydraulic properties identified in poplar and maritime pine species offer new breeding perspectives for future climate in southern Europe. Moreover, significant genetic variation for specific wood properties such as lignin and cellulose contents or spiral grain in the same two species could boost industrial innovation and increase competitive advantage of wood in the bio-based economy. Several features limiting melampsora sp. pathogen dynamics and adaptation on poplar host demonstrated genetic variation and could open a new road map for more durable resistance to pest attacks of poplar plantations: constitutive resistance associated to leaf anatomy, phenological avoidance, complete non-native resistances and partial resistances and physiological tolerance (i.e. the ability to maintain growth despite being susceptible to the disease).

The application of genomic sciences to the description of this newly observed intraspecific genetic variation has accelerated the discovery of functional genes controlling both adaptive traits and wood properties. Genomic regions explaining more than 20% of the phenotypic variance have been discovered in maritime pine and Scots pine for cold tolerance, water-use efficiency and wood-related traits. Strong candidates for controlling annual growth rhythm in conifers have been identified in Norway spruce and Scots pine. Their polymorphisms in natural populations could explain the clinal variation observed for annual growth in northern Europe and will be of high interest to identify the most relevant gene pools. However, the large number of genomic regions with small additive effects, large confidence intervals, site- or year-specific effects detected in most situations highlighted the complex nature of the target traits studied and the need for more integrative research focusing on interactions between trait determinants.

Discussion about definition of new selection strategies for optimum short term responses of forests to climate change have identified two different approaches:

1. give priority to stability of yield and quality performances over environments without explaining specific responses to environmental conditions. This strategy revealed to be safe when large evaluation networks are available and could be achieved through selection in most forest tree species which benefited of large breeding populations
2. focus on individual plasticity estimates for well-chosen traits evaluated in environments well described by climatic or edaphic covariates.

This strategy proved to be specifically adapted to clonal selection. For the first time, significant genetic variation for phenotypic plasticity under partial additive control has been observed for both adaptive, yield- and wood-related traits in different model tree species. This new knowledge about genetically controlled plasticity and adaptation under various environmental conditions will improve performance predictions in both the short and long term, under current and predicted climate change scenarios, not only within but also across European countries.

Integration of genome information for a more accurate prediction of phenotypic performances

One important challenge for NOVELTREE was to take more advantage of recent advances in genomics in the implementation of forest tree breeding programmes. Marker and gene based breeding (MAS) has a great potential to boost selection efficiency and sustainable management of genetic resources but need to be demonstrated. An extensive bibliographic review with a specific focus on successes and failures of
MAS implementation in plants was carried out to explore parametric spaces of interest for forest trees and identify the readiness of MAS in these species. Some marker-based tools can already be used in the management of forest breeding populations and proof of concept has been demonstrated in two case-studies involving maritime pine and poplar. One of these utilities is fingerprinting where markers are used to assess or control the genetic identity of individuals. Another use of markers corresponds to that of parentage analyses which allow the estimation of the realised relationship matrix based on the molecular data instead of the relationship matrix derived from pedigree to perform a more accurate evaluation of breeding values. Efficient MAS methodology could also be recommended for identification of elite clones thanks to manipulation of very few genomic regions which have a major impact on crop value (i.e. disease resistance or specific wood property). Application of marker and gene-assisted selection in selection for wood quality related traits will significantly speed up breeding cycles and will allow a more precise targeting on specific properties.

The lessons learned on genomic selection during the NOVELTREE project will undoubtedly have an impact on the design and implementation of future Marker-assisted and genome-wide selection projects in forest trees. With genome wide approach, the burden of phenotypic evaluation can be limited to intensively managed core elite populations, with need of new phenotyping only every few generations to respond to new challenges and one step large genotyping effort. Most of forest tree species which benefit from active breeding programs, have wide distribution in Europe. Intensively managed core elites for these species, could be shared at European level to spread the costs and be readily monitored for new traits in a wider range of environments.

Predicting phenotypes is mostly based on an additive paradigm and recent genome-wide approaches are not an exception to that rule, as genome-wide predictions result from the sum of independent effects. Current models, including genomic selection, are devised to predict an average performance of a genotype and seldom constructed to predict response curves over environmental clines. Evidence is rapidly accumulating that gene-to-gene interaction is a pervasive phenomenon, which hints at the complex genetic networking that causes genotype-by-environment interactions and plastic responses in functional traits, the ones that are relevant in a context of environmental changes as detailed above. The molecular and physiological approaches of phenotypic plasticity developed in NOVELTREE identified such interactive influences of environmental clues as photoperiod, temperature and drought index on phenology of Scots pine and Norway spruce, water-use efficiency and wood formation in maritime pine. New prediction models need to be devised to incorporate the environmental trends as co-variables, together with genomic predictors involving DNA polymorphisms and expressional and physiological profiles.

Developing genomic resources, breeding and decision tools

At the start of NOVELTREE project, the availability of molecular markers for most of forest tree species remained insufficient to allow a good coverage of these large genomes. Large efforts were devoted to extend the existing genomic resources in two directions: first with new neutral SSR markers for monitoring genetic diversity of pine species populations, second with SNP in order to identify genome regions associated with traits of interests and validate potential role of candidate genes in phenotypic variation. Both goals were attained with higher success than expected. All four tree species exceeded expectations, with over few thousand SNPs discovered for Scots pine and Norway spruce up to more than 3 300 000 for
poplar which benefited from a reference genome sequence.

Thanks to the pooling of resources across different EC-funded projects in some species (Maritime pine, poplars) or through the cooperation with Canadian projects in others (spruces) and the adoption of NGS projects for SNP discovery, larger genotyping assays than envisioned have been customised in all species. The scientific community but also tree breeders and managers of programmes of genetic resources conservation have at their disposal new molecular tools which could help them to identify polymorphisms of potential interest, characterise specific genetic pools or genetically improved material ready for deployment and more generally monitor genetic diversity in their management. In addition, high density gene based maps developed for four conifer species will provide opportunities for predicting breeding values and more comparative mapping to reveal the organisation and the evolution of these complex genomes.

Cutting-edge new technology has been deployed to solve some of the big bottlenecks in future phenotyping of complex adaptive traits and specific wood added values. Two phenomics platforms have been developed to measure respectively, tree responses under water limitation and wood properties with a high-throughput and non-destructive approach. Some of the methods still need further refinement, but altogether NOVELTREE has implied a big step ahead to foster phenotyping that can follow the vertiginous progress in genotyping. These tools have been included as infrastructures of the Tree4Future Seventh Framework Programme (FP7) project and can be used thanks to transnational access. They will surely offer unique possibilities for more rapid transfer of genetic progress on these new selection targets. To be able to predict performances of forest material in a larger range of environments than those used in the evaluation phase, it is useful to model the response of genotypes by norms of reaction, which can be defined as phenotypical functions of environmental gradients. Examples of time-related phenotypic plasticity from maritime pine and poplar experiments showed how either short-term change in wood density or repeated assessments of the same trait over several climatically contrasted years can provide a very interesting tool for obtaining individual plasticity estimates. These tools including the newly developed dendroplasticity approach open the gate for progressing in our adaptive interpretation of phenotypic plasticity for a number of traits and for integrating this information in the selection process.

Two major flexible simulation tools, Popsim and Metagene have been developed and distributed in order to help tree breeders to dovetail, genetic gain and diversity, time, financial and other resources, feasibility and robustness into coherent breeding strategies. Development of such marker-based simulation tool will help tree breeders and genomic researchers to get closer and pave the way for an easier integration of the huge amount of molecular data provided by new sequencing technologies into performance predictions. Specific emphasis has be put in seeding up the breeding process, increasing accuracy of genetic selection for past and new selection objectives and monitoring more precisely the genetic diversity.

Another contribution of NOVELTREE was to assess impacts of present and future genetically-improved forest trees on sustainability of forest management in the context of climate change and diversification of wood demand. Prediction of the yield and economic benefits of genetically improved stands has required the integrated knowledge of geneticists, climate specialists, silviculturists, biometricians and economists. A bioeconomical model has been built as an extension of the decision support system Motti (see http://www.metla.fi/metinfo/motti/index-en.htm) dedicated to the assessment of effects of alternative forest
management practices on stand development and the profitability of forest management. For the economical analysis, the user can enter stumpage prices by tree species and timber assortments, silvicultural costs and interest rate. Thanks to this updated stand-level simulator, the potential of present and future tree breeding can be analysed for:

1. giving economical benefits to forest owners and forest industry
2. assessing future forest growth under different scenarios of land and forest use and
3. giving tree breeders possibilities to compare economic outcomes of different breeding strategies.

Another important challenge for NOVELTREE was to provide tree breeders, forest owners and land managers with support decision tools for optimal spatial deployment of improved genetic stocks in both prevailing and future climate and under risk of pest and disease attacks. Recommendations based today on regional breeding zones need to evolve towards identification of high productive environments thanks to relevant parameters such as geographical coordinates, climatic variables, soil descriptors and biotic risk levels. Improved production functions for Scots pine in both Finland and Sweden have been produced through the combination of climatic index and yield performances collected on more than 140 genetic tests in Sweden and Finland. The main features of Planter's Guide Two, the newly developed web-based tool, are that:

1. the user can select his/her regeneration site from a map or by entering the coordinates;
2. the user can select an emission scenario to infer a future climate change;
3. the user can 'fine-tune' the micro-climatic conditions at the regeneration site by selecting a level of 'harshness';
4. the suitable seed orchards are presented in a table where they are sorted according to their performance at the selected site (the best on top);
5. seed orchard performance is calculated as the expected volume production over an entire rotation and given in relation to the estimated production from local origin stand seed;
6. the components of volume production (i.e. growth and survival) are given explicitly in the table;
7. the user can select specific orchards to find more detailed information about them and a map showing the suitable deployment area.

Main functionalities are today available and Planter's Guide Two has been presented to end-users at both national meetings and international workshop in Helsinki. It will be soon freely accessible to forest owners and will benefit of further improvement of the prediction models, the climate information and the friendly-user interface. Moreover, the experiences, developed structures and functions can also be used as a model system for other important species and productive forest areas in Europe. These new support decision tools will undoubtly reduce vulnerability of forests to climate change, secure deployment of genetically-improved material and make a better use of a wider relevant genetic diversity coming from different breeding programmes over much broad geographical areas.

Enhancing application of the most up-dates biotechnology- and modelling-based alternatives at the European scale.

Over the course of NOVELTREE, genome technologies have developed quickly with NGS offering the
possibility of more rapid and cost-effective SNP discovery on a large scale and 'genotyping-by-sequencing'. The transition made in collaboration with other on-going projects on forest trees provided an almost unlimited source of markers and genotyping capacity laying out the foundations of a new landscape genomic approach that will play an important role in predicting breeding values through genomic evaluation techniques i.e. genomic selection as well as in the optimal management of natural genetic resources. In the sequencing technology, cost can only be kept low by sequencing at low coverage per individual. Therefore, possibilities of restriction-site associates (RAD) DNA sequencing where only a targeted subset of sites in the genome is sequenced has been successfully explored in Sitka spruce as model species. More specifically, the genotyping assays developed during the duration of the project have already been used on newly designed association mapping populations or on parts of the breeding programs. In most cases this constitutes the first large scale genome wide association studies done in these species. The genotyping assays developed within NOVELTREE have also already been used by partners of the project in other European or locally funded projects. For example the genotyping assays developed in maritime pine and Norway spruce have already been instrumental in an array of projects (Linktree in the Biodiversa programme and ProCoGen in the FP7 programme) directly or indirectly related to breeding. These genotyping assays have also started to be used by groups outside the consortium. For example, in Norway spruce a newly designed association mapping population focusing on the Alpine region and managed by the Edmund Mach Foundation will be genotyped with SNP developed and validated during NOVELTREE project. A high-throughput certification test was developed from the NOVELTREE SNP arrays and used by policy makers to identify origin of reproductive materials deployed in the Iberian and French Atlantic regions, where major breeding programs are in place. These examples of direct applications of molecular breeding tools are a direct consequence of the efforts of dissemination paid by NOVELTREE consortium during the duration of the project.

The utility of all model-based simulation tools was demonstrated repeatedly throughout NOVELTREE as they offered valuable help to fine-tune breeding strategies of model species of high economic interest and prepare recommended 'Best Practice' for tree breeding in Europe. The document written for a broad audience presents the principles for design and evaluation of breeding strategies with updated state-of-the-art techniques, followed by descriptions of breeding strategies for the economically important conifer species, Norway spruce, Scots pine and maritime pine. The document concludes with a discussion of principles to extend best practice to other tree species in Europe and discuss the potential for geo-climatic cooperation in breeding and deployment models to enhance the returns from investment in tree breeding. While these guidelines reflect the state-of-the-art at the end of the project, all the developed expert breeding tools will open the door to regular updates.

Serving as a unified platform for exchange of ideas and knowledge

NOVELTREE was built partly upon previous networking efforts from European initiatives on forest ecosystems and had greatly contributed to strongly reinforce the forefront position of the European research on forest tree genomics and its application on tree breeding. The project benefited a lot from active collaboration of experts in methodology developments for animal breeding, of experts in tree physiology and of experts from the forest economy sector. The integrative strategies developed at the gene-allele level up to the forest landscape level provided new insights in challenging tasks of forest tree breeding such as:
1. identification of nucleotide polymorphisms that govern genetic variation of several traits of adaptive and economical interest;
2. genetic bases underlying plasticity and adaptation of tree genotypes and populations;
3. response of forest genetic resources to climate change and
4. compatibility between increased biomass production, quality of wood products and sustainable forest management.

Poplar, maritime pine, Scots pine, Norway and Sitka spruces have been chosen as model species in NOVELTREE because of their economic and ecological importance in different parts of Europe. The generic value of these different species in terms of biology, genetic structure, genetic history and responses to selection will offer soon broader applications to other conifer species of high importance in European forest economy. Moreover, environmental threats and hazards (climate change, new uses of wood-based products) are of cross-boundary nature and require joining forces for making comparable assessments of impacts on sustainability.

NOVELTREE has contributed to spread the scientific excellence developed during the project through efficient training and dissemination activities including:

1. an increasing number of oral and written communications in high ranking scientific journals and scientific meetings
2. four annual newsletters posted on the project website (see http://www.noveltree.eu) and distributed to an e-mailing list of over 500 people and 300 institutions
3. one training programme of four workshops to permit mutual awareness and transferability of expertise within and outside the consortium to both students, young and senior scientists
4. one workshop that concerned the European community of forest geneticists and tree breeders and where the newly developed breeding simulation tools have been be presented
5. one final international scientific conference complemented by a specific workshop for end-users and stakeholders
6. a web-based book targeted at students and a larger audience of users of genetic gains provided by forest tree breeding and
7. different breeding simulation tools to be uploaded from the project website.

After the end of the project, the public website (see http://www.noveltree.eu) which is offering access to the four annual newsletters, an updated list of published results, the two simulation tools (Metagene, Popsim) and the e-book will be maintained by the Institut national de la recherche agronomique (INRA) with the help of Istituto di Genetica Vegetale - Consiglio Nazionale delle Ricerche (IGV-CNR) for the specific page concerning the two softwares.

All the above forms of dissemination helped to move forward tree breeding science in Europe and to adjust forest management and production to regional specificities driven by climate change while enabling fulfilment of local and global needs.

List of Websites:
Project public website

The address of the public website is: http://www.noveltree.eu

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