Spanish approach to Forest Tree Genomics: coordinated multidisciplinarity at **work in a nation-wide coordinated initiative**

M.T.Cervera[1] and C. Díaz-Sala[2]
[1]INIA-CIFOR. Ctra. de La Coruña Km 7. 28040, MADRID, Spain
[2]Department of Plant Biology. University of Alcalá. 28871, Alcalá de Henares. MADRID, Spain

Background

We can not overstate the ecologic and economic importance of trees. They are essential components of the natural landscape, and play a crucial role in global carbon maintenance, response to global climate change and conservation of biodiversity. Trees also are the raw material-base of a multibillion forest product industry including the conversion of biomass to energy. Our livelihood is linked to the sustainable production of goods and services derived from forests. In Spain forest land accounts for about half the land area, where conifers are the main species of growing stock volume. Soil protection, provision of wood and non-wood products -including cork, resin, pine nuts etc.- are important forest functions as well as are tourism and recreation, which give other value to landscape.

Despite their importance from a social, ecological, environmental and economic perspective, little is know about the mechanisms that underpin growth and survival of trees. This is surprising, given that an understanding of these mechanisms may help to develop efforts aimed at ensuring the long-term maintenance of forest health, and the forest productivity.

Nowdays, extreme weather conditions, such as long drought periods, as well as habitat destruction due to human activities are depleting forest populations. They also cause forest stress and disturbances that shape forest systems by influencing their composition, structure, diversity, productivity or functional processes. The depletion of forest populations by the effects of long-term impacts of changes in temperature, precipitation etc. or human activities are enhancing the greenhouse effect and global warming. It is imperative that long-term survival of forest trees is guaranteed so as to ensure that biodiversity, carbon management, and the livelihoods of millions of people world-wide are sustained and enhanced.

Unlike animals, plants cannot avoid unfavourable habitats, sudden changes in the climate or environmental stresses such as freezing, drought, or pollutants by moving over long distances to more suitable environments. So if they are to survive, they have to be able to adapt rapidly to new conditions. Perennial plants such as trees are even more confronted to environment changes through their life span. Genetic diversity is the basis of the ability of organisms to adapt to changes in their environment through natural selection. The knowledge of the genetic basis for adaptation, the identification of loci associated to the genetic control of adaptive variation (candidate genes) and the characterization of phenotypic effects of alleles and genotypes at such loci will allow selection of the most adaptive individuals to specific environments, which is the foundation for forest sustainability and conservation. In addition, advances through micropropagation, somatic embryogenesis and improved rooting techniques will also make it easier to get larger numbers of selected elite genotypes into the forest.

Just as genomics tools are recognised as a crucial component of strategies aimed at improving human health and the quality of life, genomic tools will be crucial to ensure the long-term sustainability of forest health and productivity. Recently,

remarkable progress has been made in the understanding of the mechanisms that control growth and survival in trees through the applications of genomics. Through the application of cutting-edge tools of genome analysis, a comprehensive picture of the genes and cellular processes involved in many aspects of tree growth and development is emerging, and a suite of tools are being developed that will be critical in the sustainable management of forests. Knowledge obtained in these studies points out to the way forward for improving quantity and quality of trees for desired end-uses or enhancing the ability of trees to adapt to environmental stresses such as pollution and climate change.

In Europe, several large programs have recently been initiated that are aimed at large-scale analysis of tree genomes. These efforts will create invaluable publicly accessible databases on tree genomes, and will constitute a unique platform which will help tree biologists to understand growth, development and adaptation of forest trees. Due to the substantial lag time between seed germination and sexual maturity, trees have not been as amenable to traditional breeding approaches that have been so useful in the improvement of short-lived crops. The availability of the genomics tools will enhance the opportunities for increasing the rate of tree improvement. This can be achieved both by using molecular markers as early selection criteria in traditional breeding, and through genetic engineering. These same tools can be used to device precise diagnostics to monitor forest productivity and health. Genomics programmes will contribute to identify quality related traits as early as possible in tree development which will be of value in speeding up breeding cycles, using specific genes or genes from model species, and reducing the need to plant out and grow on all of the progeny from crosses. Proteomics and metabolomics development will enhance this trend, linking gene discovery with function. The challenge is to ensure that the investment that has been made in basic research truly adds value to economically important tree species in order to maintain forest diversity, productivity and the continued survival of forest ecosystems in response to environmental change.

European advances in this field are being made through the large-scale genome analysis of tree species, particularly poplar, birch, eucalyptus, oak and pine and through EU funded programmes including the European Forest Genomics Network (http://www.genosilva.org), allied to national genomics initiatives, for example the Spanish Forest Functional Genomics Network (http://foto.difo.uah.es/forestgenomics) and trans-national projects.

Spanish Forest Functional Genomics Network

The Spanish Forest Functional Genomics started in 2003 and it is funded by the Spanish Ministry for Education and Science. The **main objective** of the network is to transfer knowledge and technology from the basic science of plant functional genomics to the forestry sector, so as to benefit forest productivity and forest health.

The **general goals** of this programme are as follows:

- Bring together Spanish scientists to work on large-scale genome analysis of trees of economic importance in Spain.
- Identification of research priorities, and develop genomic resources for the identification and function of genes related to adaptation and productivity of Spanish forests

- Stimulate international cooperation
- Exchange information between scientists and forest industries and practitioners
- Exchange methodologies among scientist and research students
- Organization of specialized courses
- Disseminate information

The **means to achieve these objectives** are:

1) To bring together scientists from a wide range of disciplines including:
   - Molecular genetics
   - Molecular biology
   - Tree and cell physiology and biochemistry
   - Tree Breeders
   - Forest Practitioners
   - Industrial end users

   They share experiences in methodology and this will encourage multi- and interdisciplinary co-operation to increase our knowledge of tree growth and health. Meetings will be a platform for dialogue between developers of new genomics-based tools and technologies with forest practitioners. This provides a forum where research 'pull', from forest practitioners to scientists, will interact to determine current and future research needs and how they may be incorporated into current programs.
2) To provide a platform for the public understanding of new genomics-based tools and technologies related to forestry. To provide a mechanism to engage the public and to improve the public understanding of the potential of new genetic tools and biotechnology

Efforts are focused on species that have a significant impact in the Spanish forests and forest industry. The main forest species are pine, mainly, *Pinus pinaster*, followed by oaks (*Quercus suber and Quercus ilex*), *Fagus sylvatica, Castanea sativa* and *Eucalyptus*.

Genomics objectives

The application of genomics to these species will help to understand the genetic and molecular basis of wood and cork production, maturation and environmental responses in trees. Studies of such fundamental problems will be greatly facilitated by current development in genomic technologies.

- **Genome analysis**
  - Development of molecular markers.
  - Development of reference maps and selection of informative markers to integrate all available genetic maps and speed up the construction of genetic linkage maps. Integration of physical and genetic maps.
  - Development of BAC libraries and cytogenetic maps
  - EST (expressed sequence tag) sequencing and development of EST unigene sets. EST sequences are important tools for identifying SNPs (single nucleotide polymorphisms) and INDELs (insertions-deletions), and producing

cDNA microarrays for expression profile analysis. They are also used as an invaluable resource for mapping purposes.

- **Functional analysis**

The research interests are focused on the identification and characterization of genes important in three key areas:

- wood and cork production, including wood and cork quantity and quality
- tree health, including adaptation to environmental change, disease resistance, and responses to biotic and abiotic stimuli
- Development and reproduction, including vegetative propagation and tree maturation, seed formation, nitrogen metabolism or dormancy

Molecular tools previously described will be used to approach forward and reverse genetics and to elucidate gene functions, regulations and interactions. These strategies consists of cDNA microarrays, optimised protocols for genetic transformation and gene knockout tools. Gene expression information will be complemented with the use of systematic proteome analyses. Correlation between gene expression and phenotype (QTL analysis and association mapping) will also be performed.

- **Bioinformatics**

Specific working groups and companies are developing a platform to process, storage and distribute the large amount of data.

- **Transference of technology and information**

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