Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Review

Integrating genomics into *Eucalyptus* breeding

Dario Grattapaglia

Plant Genetics Laboratory, Embrapa Recursos Genéticos e Biotecnologia, Caixa Postal 02372, 70770-900 Brasília, DF, Brasil, and Graduate Program in Genomic Sciences, Universidade Católica de Brasília, UCB, SGAN 916 Módulo B, 70790-160 Brasília, DF, Brasil
Corresponding author: Dario Grattapaglia
E-mail: dario@cenargen.embrapa.br

Genet. Mol. Res. 3 (3): 369-379 (2004)
Received April 30, 2004
Accepted August 25, 2004
Published September 30, 2004

ABSTRACT. The advent of high throughput genomic technologies has opened new perspectives in the speed, scale and detail with which one can investigate genes, genomes and complex traits in *Eucalyptus* species. A genomic approach to a more detailed understanding of important metabolic and physiological processes, which affect tree growth and stress resistance, and the identification of genes and their allelic variants, which determine the major chemical and physical features of wood properties, should eventually lead to new opportunities for directed genetic modifications of far-reaching economic impact in forest industry. It should be kept in mind, however, that basic breeding strategies, coupled with sophisticated quantitative methods, breeder's experience and breeder's intuition, will continue to generate significant genetic gains and have a clear measurable impact on production forestry. Even with a much more global view of genetic processes, genomics will only succeed in contributing to the development of improved industrial forests if it is strongly interconnected with intensive fieldwork and creative breeding. Integrated genomic projects involving multi-species expressed sequence tag sequencing and quantitative trait locus detection, single nucleotide polymorphism discovery for association mapping, and the development of a gene-rich physical map for the *Eucalyptus* genome will quickly move toward linking phenotypes to genes that control the wood formation processes that define industrial-level traits. Exploiting the full power of the superior natural phenotypic variation in wood properties

found in *Eucalyptus* genetic resources will undoubtedly be a key factor to reach this goal.

Key words: *Eucalyptus*, Genomics, QTL, Association mapping, Genolyptus

INTRODUCTION

Wood is a raw material used globally in several forest-based industries that generate millions of jobs and billions of dollars in revenues involving pulp and paper, steel, and solid wood products, among others. It is in this remarkable world industry, where increased forest productivities and refinements in the quality of wood products have become increasingly important, that genomics expects to provide additional tools to help breeders increase and accelerate gains. Prospects of fast and accurate methods for early marker-based selection in trees have been announced during the last 10 years. Although significant progress has been made in the accumulation of knowledge of tree genomes and concerning the function of a still relatively small group of genes, mainly involved in lignin biosynthesis, it has become clear that various challenges still remain before more refined and higher impact applications can be implemented so as to fully realize the promises of genomics for forest trees, and more specifically, for *Eucalyptus*.

A number of groups throughout the world are developing dynamic molecular biology research projects with forest trees, mainly involving Pines, Poplars and Eucalypts. Reviews of the status of some of these efforts were published recently (Jain and Minocha, 2000). Now in 2004, further significant advances have been made, including the completion of an 8X draft sequence of the Poplar genome, the first tree to have its full genomic sequence made available to the public (Bradshaw et al., 2000; Tuskan, 2004). In Brazil, two large-scale eucalypt genomic research initiative projects were started in 2001 and 2002, respectively: the ForEst project, an EST sequencing initiative in the State of São Paulo, and the Genolyptus Project - Brazilian Network of Eucalyptus Genome Research, which involves a nationwide network of labs and forestry companies devoted to an integrated molecular breeding approach (Grattapaglia, 2003). It is our understanding that the eventual success of such initiatives, and in fact of any other forest tree genomic research project, will depend on a few prerequisites:

a) A multidisciplinary approach, involving scientists from the very diverse areas of genetics, biochemistry, molecular biology, breeding, statistics, phytopathology, and wood technology among others;

b) The systematic construction of a full array of genomic resources, including a comprehensive expressed sequence tag (EST) database, large sets of transportable hypervariable co-dominant molecular markers, high resolution genetic maps, physical maps of large genomic fragments, bioinformatics, and statistical genomic tools;

c) The generation of experimental biological resources specifically targeting genomic experiments, i.e., segregating populations for the most relevant traits, collections of clones with contrasting phenotypes, specific populations with different degrees of linkage disequilibrium for association mapping studies, and eventually for positional cloning of genes;

d) Intensive phenotyping efforts, involving the establishment of a large network of field

experiments for silvicultural trait measurements, bioassays for disease resistance screening and the optimization of high-throughput wood quality trait determination methods that allow the assessment of large numbers of samples;

e) Integrative experimental approaches between genetics and genomics that can reap the full benefit and resolution power from quantitative genetics methods to sequencing and mapping technologies.

GENETIC RESOURCES FOR *EUCALYPTUS* GENOMICS

While genomic resources, such as EST databases, bacterial artificial chromosome (BAC) libraries, molecular markers, and microarray supplies, should become increasingly more easily available and should not be a limiting factor in the near future, biological resources and precise phenotyping represent the real limitation of many genomic projects in many instances (Morgante and Salamini, 2003). Especially in forest trees, where generation times and phenotype assessment can take years, the availability of ideal experimental populations should be one of the main targets in any genomic level project. In this context, the driving principle of eucalypt genome initiatives should be that there is ample genetic variation within the genus *Eucalyptus*, and more specifically within the subgenus *Symphyomyrtus*, to allow profound genetic modification of the current planting stock in Brazil. For example, *Eucalyptus globulus* contrasts with commonly used tropical species, such as *E. grandis*, *E. urophylla* and *E. camaldulensis*, as it has a number of wood properties that are extremely interesting for industry. *Eucalyptus globulus* germplasm stands out as a very rich source of genetic variation for all the target wood traits, and therefore it is a key resource for eucalypt genomic research, especially for the pulp and paper industries. It is now well known by breeders and wood technologists that *E. globulus* has the best combination of wood properties for pulp and paper among the commercially planted *Eucalyptus* species, resulting in a high pulp yield, requiring approximately 25% less wood to produce a ton of cellulose. While only 2.98 cubic meters of *E. globulus* wood are required per ton of pulp, 3.89 cubic meters are needed from *E. grandis. Eucalyptus globulus* has a very adequate wood density, in the range of 550 kg/m^3, the longest fiber length and the greatest content of holocellulose and pentosans among all intensively planted *Eucalyptus species* (Sanchez, 2002). Therefore, even though it has slow growth rates, due to its temperate origin, *E. globulus* wood is currently the preferred raw material for mills, generating a pulp that has increasingly been seen as a distinct and superior product by the market.

Considerable experimental data from hybridization experiments in Brazil are already available to clearly demonstrate that the introgression of temperate *E. globulus* alleles into tropical hybrid breeding programs, coupled with clonal propagation of selected individuals, will result in significant reductions in wood consumption (T. Assis, personal communication). Most Brazilian breeders are currently investing heavily, based on the potential impact that the use of *E. globulus* could have in their programs. It is now just a matter of time and systematic investment for such gains to reach industry. This same trend was also transferred to the ongoing construction of the biological resources for genomic research in the Genolyptus project.

QUANTITATIVE TRAIT LOCUS MAPPING

The construction of a comprehensive microsatellite-based linkage map for commercial

species of *Eucalyptus* is now well advanced (Brondani et al., 1998; Brondani and Grattapaglia, 2002). In the context of Genolyptus, a target was established to develop and map 1,000 microsatellite markers. Sources for this large number of microsatellites are now becoming available, not only from the enriched library approach, but also from a shotgun genomic library, a large set of ESTs, and from BAC end sequences. The availability of transportable, multiallelic, PCR-based co-dominant microsatellite loci provides a fundamental tool to carry out linkage and quantitative trait locus (QTL) analysis in eucalypts and allows researchers to move from phenotypes to target genomic regions controlling traits of interest. Transportability of marker loci among pedigrees across species of the same subgenus (Marques et al., 2002) should facilitate the information exchange and comparison of QTL mapping data. The determination of interspecific QTL synteny will allow a directed search for new allelic variation at known QTLs within and among species, expanding the opportunities for marker-assisted introgression and marker-assisted selection (MAS) in hybrid breeding. Molecular marker maps have been successfully used to detect major effect QTLs for wood properties in rotation age traits such as volume growth, wood specific gravity, bark thickness, and stem form in *Eucalyptus* (Grattapaglia et al., 1996; Verhaegen et al., 1997) and for several wood traits such as pulp yield at kappa 18, alkali consumption at kappa 18, basic density, oven-dry lignin content, extractives-free lignin content, extractives content, cellulose content, heat content, fiber length, and fiber coarseness using near-infrared analysis of wood core samples (Myburg et al., 2001).

The genetic architecture of quantitative traits will eventually determine the impact on breeding of knowing the genes underlying a given trait. Even if all the genes are known and mapped, the efficiency of MAS will always be less than phenotypic selection, if they follow the infinitesimal model (Bernardo, 2001). However, the evidence available until now indicates that major-effect genes do exist. A realistic strategy for the implementation of MAS in *Eucalyptus* might be to tackle only a few major QTLs for a quality trait of significant added value. With the advent of more efficient marker technologies, such as co-dominant microsatellites, the continued mapping of major QTLs of trees, and the revision of breeding and deployment strategies to better exploit within-family variation, the prospects for using MAS in advanced generation breeding of eucalypts are promising (Grattapaglia, 2000).

ASSOCIATION MAPPING

With the rapid advancement of genome projects generating a large amount of sequence information and single nucleotide polymorphism (SNP) (one-letter variations in the DNA sequence that contribute to differences among individuals) data, plant genomics has experienced a growing interest in an alternative approach for the identification of genes underlying quantitative traits. The new model is based on the possibility of investigating sequence variation directly in genes and not at anonymous linked markers. This approach exploits candidate gene sequence variation, and it relies on the existence of linkage disequilibrium (non-random association between alleles at linked loci) between detectable sequence polymorphism, SNPs and quantitative trait nucleotide, which ultimately determines the patterns of phenotypic variation.

From an operational point of view, the candidate gene approach has the advantage that once a major effect gene is determined and validated, MAS could then be practiced directly on the gene and, therefore, would not rely on the need for strong association (linkage disequilibrium) between the marker allele and the favorable allele of the gene of interest.

One of the key issues when embarking in an association mapping experiment is the selection of candidate genes. This is not an easy task, and every effort should be made to maximize the probability of choosing the proper genes. The choice of candidate genes is an elusive target for the majority of phenotypes relevant to forest trees. It requires knowledge of biochemistry, physiology, and development, which is generally not available even for well-defined phenotypes and/or known metabolic pathways. The criteria generally used in the choice of candidate genes include: 1) knowledge of the biochemical role of enzymes, such as those involved in lignin biosynthesis; 2) inference of function based on significant similarities to other sequences in databases; 3) existence of loss of function of mutants in model plants, such as those available in *Arabidopsis*; 4) co-localization of gene and QTL for the relevant trait on linkage maps, always with the understanding that the probability of the gene being in fact the correct candidate depends heavily on the precision of the QTL localization; 5) differential gene expression patterns assuming that differentially expressed genes are the causal agents of the observed phenotypic variation, which is many times not the case, and 6) differential expression at the protein level between contrasting phenotypes.

Testing the role of a candidate gene can be carried out by a conventional co-segregation analysis in structured segregating populations, in which the gene is used as a marker in the attempt to relate the sequence polymorphism in the gene with variation in the quantitative trait. Allelic variation at the gene is defined by haplotypes, comprising a number of SNPs. The majority of SNPs have no effect, but some cause subtle differences in the final effect of the gene and hence the phenotype. Allelic variations are revealed by DNA sequencing of fragments of candidate genes. Significant differences in phenotypic means among candidate gene haplotype classes should identify candidate gene alleles with the greatest effect on the trait of interest. Another approach to test and validate candidate genes is to look for SNP-phenotype associations in germplasm collections or in natural populations with contrasting phenotypes. The objective again is to correlate the distribution of candidate gene genotypes in the form of DNA sequences with relevant phenotypes.

Some laboratories have started association mapping work for wood traits, both in Pines (Brown et al., 2001) and in *Eucalyptus* (Moran et al., 2001; Thamarus et al., 2002), by sampling trees in the wild or from breeding programs that display contrasting phenotypes for wood quality traits. We have recently started such an effort in the context of the Genolyptus project, exploiting a large constructed EST database and the BAC library resource. We are also interested in estimating the extent of linkage disequilibrium, both in genes and in random genomic sequences, in different types of populations and species, so as to have a better idea of the prospects and difficulty of establishing useful associations. Care has to be taken, however, in properly choosing populations or germplasm collections so as to avoid or minimize spurious false positive associations that are not in *cis*, i.e., due to linkage, but rather due to historical events, such as recent hybridizations. A number of genes, mainly involved in lignin and cell wall biosynthesis, and in floral development, as well as those coding for transcription factors, will most likely be the first choice for candidates.

EST databases have been important sources of information for the selection of candidate genes for mapping and association studies. EST sequencing quickly generates a large index of partial genes for the organism of interest, making them available in organized collections of clusterized sequences for further molecular investigation. With such collections of genes, resources are then applied to their characterization rather than their isolation, significantly ac-

celerating the rate at which genetic research can be carried out. Moreover, such a random cDNAs sequencing approach can yield useful information about the relative expression levels of the corresponding genes in different tissue samples based on the number of times that a particular gene sequence is found in a particular tissue.

In the Genolyptus project, we have now completed an initial database of over 120,000 valid EST sequences derived from several different cDNA libraries, with increased focus on xylem transcripts. Several thousand cDNA clones were sequenced from *E. globulus*, *E. grandis*, *E. pellita*, and *E. urophylla* xylem and phloem libraries that were derived from a number of individuals for each species; thus, several alleles for candidate genes are now available. Opportunities now exist for SNP discovery both at the inter- and intraspecific levels. With this rich genomic resource in hand, experiments can now be carried out to test whether phenotypic differences in wood quality traits between *E. grandis* and *E. globulus* can be attributed to specific sequence differences in coding regions of candidate genes. However, we should not overlook the fact that sequence differences in regulatory regions of such genes could also play an important role in the definition of phenotypes. The search for those sequence polymorphisms is certainly a more challenging task that will depend, among other factors, on having an adequate genomic resource in the form of a physical map.

PHYSICAL MAPPING

Finding a single gene in a complex genome requires a set of powerful tools. Most relevant traits are controlled by unknown genes that can be genetically mapped but are not easily identified. Genetic maps based on recombination frequencies among markers provide only megabase level resolution. In *Eucalyptus*, for example, 1% recombination between two markers or between a marker and a gene corresponds on average to 500,000 base pairs of DNA, which can contain several tens of genes (Grattapaglia and Bradshaw Jr., 1994). Sequencing of ESTs provides expressed gene tags but not the full gene and its regulatory sequences.

To learn the gene's precise location and structure, so as to isolate it, map-based cloning strategies integrated with physical mapping technologies are necessary. A physical map is an intermediate level of resolution between genetic maps and the full genome sequence, and it represents the necessary framework for full genome sequencing efforts. The availability of a physical map that is tightly linked to the genetic map and the mapping onto it of ESTs representing a large number of candidate genes, could make positional cloning feasible, once the genetic mapping of QTL regions is performed.

By cloning large fragments of DNA of around 150,000 base pairs of DNA in special vectors, such as BAC, it is possible to maintain a complete genome for detailed studies. By sequencing the ends of such fragments, and by fingerprinting them with a combination of high throughput techniques (Luo et al., 2003), these can be arranged in overlapping sets of contiguous fragments (contigs), building physical maps. These maps can then be anchored to the genetic maps by the location of the shared molecular markers. This approach allows, for example, the construction of localized physical maps in genomic regions that genetic linkage mapping has revealed as containing a QTL for some important trait.

The construction of partial physical maps, localized in specific regions of the genome, with the objective of gene isolation, will become a common theme as genomic projects of forest

trees advance. Initially, this should be particularly interesting for qualitative traits, such as some disease resistance (Junghans et al., 2003) or flowering traits. In fact, once a genomic region identified by QTL mapping is narrowed down to a small contig of BAC clones, specific fragments could be used in transgenic experiments to prove function and eventually to generate a transgenic tree. The investigation of genes underlying quantitative traits would then be the next step. In this context, a complete physical map for *Eucalyptus* certainly is a valuable resource for all molecular studies in *Eucalyptus* for years to come. With this concept in mind, we have undertaken this task in the Genolyptus project. A *Eucalyptus grandis* BAC library with over 70% of the inserts with over 150 kb has been built in Brazil (S. Brommonschenkel, personal communication), and the fingerprinting phase is now beginning. Although *E. grandis* was the obvious choice, as it constitutes the genetic base of most planted Brazilian elite gemplasm, we are now in the process of building a second BAC library from *E. globulus*. Accumulated evidence indicates that genomic homology and locus ordering between *E. grandis* and *E. globulus* is very high (Marques et al., 2002; Myburg et al., 2003). It is, therefore, not in our immediate plans to also build a physical map for *E. globulus*, but rather to use the physical/genetic mapping information derived from *E. grandis* to identify and explore specific genomic regions in *E. globulus*, by using its BAC library. It will be possible, for example, based on the map information derived from *E. grandis*, to clone the full homolog genes from *E. globulus*, and thus compare in detail the regulatory regions that could be responsible for differential patterns of gene expression and resulting phenotypic variation.

GENOLYPTUS - "BRAZILIAN NETWORK OF EUCALYPT GENOME RESEARCH"

The Genolyptus project (http://genolyptus.ucb.br) was conceived to establish a foundation for a genome-wide understanding of the molecular basis of wood formation and disease resistance in *Eucalyptus*. This initiative is based on the generation of a suite of biological and information resources to discover, sequence, map, validate, and understand the underlying variation of genes and genomic regions of economic importance in *Eucalyptus*, with a focus on wood formation and disease resistance. The project is based on a strong partnership among the Brazilian federal government, through the MCT-Fundo Verde Amarelo, the academic/research sector represented by seven Universities and Embrapa, and industry, represented by 13 forestry companies. Both following and in parallel with this pre-competitive phase, forest companies and public institutions are expected to develop competitive projects in various organizational formats to translate the genomic platform into improved directional tree breeding technologies.

We are investing heavily in the generation and consolidation of the necessary biological resources for genomic research, with an intense effort devoted to field experiments to generate the diversity of phenotypes necessary to study gene function. QTL detection, the discovery of SNP haplotypes for association mapping, and physical mapping will link the phenotypes to genes that control processes of division, expansion, secondary wall formation, lignification, and programmed cell death, which together define industrial level traits, such as wood-specific consumption. The gene discovery/expression effort, based on EST sequencing and microarray profiling, is focused much more on looking transversally at the existing interspecific allelic variation in relevant genes involved in xylogenesis than longitudinally at the total number of genes in *Eucalyptus*.

The project is organized in nine subprojects, corresponding to nine themes, each one coordinated by specific scientists with experience in the area. Due to the interdisciplinary nature, however, the project displays a matrix format in terms of the action of the research teams, by which most experiments actually are built upon close collaborations amongst the subprojects. These subprojects are:

1. Generation and deployment of experimental populations in field experiments. This will be the backbone of the project and has contemplated the generation of several families of 1000+ individuals each, as well as the consolidation of association mapping populations.

2. Optimization of high throughput technologies for indirect wood quality determinations. A number of technologies such as near infrared spectroscopy were optimized and further investigated for the rapid assessment of wood physical and chemical traits in the large experiments deployed.

3. Genetic basis and identification of disease resistance genes. Building upon considerable existing knowledge of bioassays and segregating families, we are carrying out basic genetics studies, linkage mapping, and eventually, map-based cloning of resistance genes for a number of fungal/bacterial pathogens that have displayed growing importance in eucalypt plantations in Brazil.

4. Genetic mapping of QTLs for wood quality traits. With over 1000 microsatellites available from both genomic and EST sequences, and hundreds of candidate genes, reference linkage maps have been built using different segregating families. Another level of QTL detection precision and validation power for wood traits is expected with more markers and the large and clonally replicated experiments, leading to narrower genetic regions, to then move to physical mapping efforts.

5. BAC library construction and physical mapping. Efforts have been devoted to building a genomic resource in the form of two high-quality and deep coverage BAC libraries for *E. grandis* and *E. globulus*, to allow isolation of the complete genes, including promoter regions. A full physical map for *E. grandis* is under construction, using fluorescent fingerprinting technology. BAC contigs are being anchored to the genetic maps by a combination of approaches, and ESTs for candidate genes are being mapped to such BACs. Anchoring the physical map to the genetic map will allow focusing on genomic regions that linkage mapping reveals as containing QTLs for important traits.

6. Sequencing of the eucalypt transcriptome. A moderate-scale EST sequencing project with a 120,000 reads target was completed in November 2003. Several libraries were built, with a focus on xylem tissue from different species. This resource-building task has supplied sequence information for EST mapping, both physically and genetically, as well as biological reagents for microarray expression profiling.

7. Expression profiling using microarrays. Within the scope of the proposed project, pilot experiments are being carried out to investigate gene expression profiles in contrasting

states or phenotypes. The phenotypic diversity and possibility of clonal replication will be significant assets for the development of this technology for eucalypts.

8. Bioinformatics for the analysis, integration and accessibility of genomic data. This subproject is characterized by the development of a set of informatics tools to analyze and translate the data generated into a usable format by all the project participants. The challenge proposed is to integrate, not only sequence information, but also physical mapping data, QTL mapping data, expression data, and field experiment data, in an accessible form for annotation and use.

9. Quantitative methods and statistical genetics tools. This subproject has the objective to coordinate all the efforts devoted to the development and application of quantitative methods to analytical tools throughout all the subprojects. Besides implementing methods and software to analyze and extract information from the genomic mapping data, we plan to develop and make available to the breeders some basic tools to initiate the integration of genomic data into their breeding procedures.

CONCLUSIONS AND PERSPECTIVES

As several genome projects in forest trees are advancing, further expectations will be generated in the forest tree breeder's community. The understanding should be, however, that genomic technologies will essentially provide information, not answers. The advent of genomics has nevertheless changed the way that we think about molecular genetics and has made it even harder to speculate on what can and cannot be done. Until a few years ago, molecular geneticists used to think of genes as acting linearly, i.e., gene A, once expressed, caused a particular reaction by gene B, and so on. The way that experiments were conducted was limited by the technology of studying one gene at a time. Nowadays, the ability to study the expression of thousands of genes in parallel has definitely changed that view.

Eucalypts are still in the earliest stages of breeding, and the major genetic changes that typically follow domestication have not yet been made. A genomic approach to a more detailed understanding of important metabolic and physiological processes that result, for example, in wood formation, and the identification of the genes that determine the major features of wood properties should eventually lead to new opportunities for directed genetic modifications of far-reaching economic impact. Furthermore, it is our understanding that even with powerful tools that allow a global and integrated view of genetic processes, genomics will only succeed in contributing to the development of improved eucalypts, if they are deeply interconnected with intensive fieldwork and creative breeding. Exploiting the full power of the superior natural phenotypic variation for wood properties found in *Eucalyptus* genetic resources will undoubtedly be a key factor to reach this goal.

ACKNOWLEDGMENTS

Research supported by the Genolyptus Project, a pre-competitive partnership between the Brazilian Ministry of Science and Technology through FINEP Fundo Verde Amarelo Competitive Grants and the following participating institutions: Aracruz Celulose S.A., Bahia Sul

Celulose S.A., Cenibra S.A., EMBRAPA, Ferro Gusa Carajás S.A., International Paper do Brasil Ltda., Jari Celulose S.A., Klabin S.A., Lwarcel Celulose e Papel Ltda., RAIZ Instituto de Investigação da Floresta e Papel de Portugal, Rigesa Celulose, Papel e Embalagens Ltda., Universidade Católica de Brasília, Universidade Estadual de Campinas, Universidade Estadual de Santa Cruz, Universidade Federal de Goiás, Universidade Federal de Lavras, Universidade Federal de Viçosa, Universidade Federal do Rio Grande do Sul, Veracel Celulose S.A., Votorantim Celulose e Papel S.A., and Zanini Florestal Ltda. D. Grattapaglia coordinates the Genolyptus Project and he received a research fellowship from the Brazilian National Research Council - CNPq.

REFERENCES

Bernardo, R. (2001). What if we knew all the genes for a quantitative trait in hybrid crops? *Crop Sci. 41*: 1-4.

Bradshaw, H.D., Ceulemans, R., Davis, J. and **Stettler, R.** (2000). Emerging model systems in plant biology: Poplar (*Populus*) as a model forest tree. *J. Plant Growth Regul. 19*: 306-313.

Brondani, R.P.V. and **Grattapaglia, D.** (2002). Towards the construction of a genus wide reference linkage map for *Eucalyptus* based on microsatellite markers. *Mol. Gen. Genomics 267*: 338-347.

Brondani, R.P.V., Brondani, C., Tarchini, R. and **Grattapaglia, D.** (1998). Development and mapping of microsatellite based markers in *Eucalyptus*. *Theor. App. Genet. 97*: 816-829.

Brown, G., Gill, G., Sewell, M., Wheeler, N., Megraw, R. and **Neale, D.** (2001). Towards association studies in forest trees: wood property QTL verification, candidate genes, and SNPs in Loblolly pine (*Pinus taeda*). *Abstracts of the 9th Joint Conifer Biotechnology Working Group and IUFRO Meeting*: "Wood, Breeding, Biotechnology and Industrial Expectations", Bordeaux, France, p. 104.

Grattapaglia, D. (2000). Molecular breeding of *Eucalyptus*: state of the art, applications and technical challenges. In: *Molecular Markers and Genome Mapping in Woody Plants* (Jain, S.M. and Minocha, S.C., eds.). Vol. 1. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 451-474.

Grattapaglia, D. (2003). Genolyptus. In: *Melhoramento Genômico (*Borém, A., Giudice, M. and Sediyama, T., eds.). Editora da UFV, Viçosa, MG, Brazil, pp. 51-72.

Grattapaglia, D. and **Bradshaw Jr., H.D.** (1994). Nuclear DNA content of commercially important *Eucalyptus* species and hybrids. *Can. J. For. Res. 24*: 1074-1078.

Grattapaglia, D., Bertolucci, F.L., Penchel, R. and **Sederoff, R.R.** (1996). Genetic mapping of quantitative trait loci controlling growth and wood quality traits in *Eucalyptus grandis* using a maternal half-sib family and RAPD markers. *Genetics 144*: 1205-1214.

Jain, S. and **Minocha, S.** (2000). *Molecular Biology of Woody Plants*. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Junghans, D., Alfenas, A.C., Brommonschenkel, S.H., Oda, S., Mello, E.J. and **Grattapaglia, D.** (2003). Resistance to Rust in *Eucalyptus*: mode of inheritance and mapping of a major gene with RAPD markers. *Theor. Appl. Genet. 108*: 175-180.

Luo, M.C., Thomas, C., You, F.M., Hsiao, J., Ouyang, S., Buell, C.R., Malandro, M., McGuire, P.E., Anderson, O.D. and **Dvorak, J.** (2003). High-throughput fingerprinting of bacterial artificial chromosomes using the snapshot labeling kit and sizing of restriction fragments by capillary electrophoresis. *Genomics 82*: 378-389.

Marques, C., Brondani, R.P.V., Grattapaglia, D. and **Sederoff, R.** (2002). Conservation of microsatellite loci and QTL for vegetative propagation in *Eucalyptus tereticornis*, *E. globulus*, *E. grandis* and *E. urophylla*. *Theor. Appl. Genet. 105*: 474-478.

Moran, G., Thamarus, K., Raymond, C., Qiu, D., Uren, T. and **Southerton, S.** (2001). Genomics of eucalypt wood. *Abstracts of the 9th Joint Conifer Biotechnology Working Group and IUFRO Meeting*: "Wood, Breeding, Biotechnology and Industrial Expectations", Bordeaux, France, p. 106.

Morgante, M. and **Salamini, F.** (2003). From plant genomics to breeding practice. *Curr. Opin. Biotechnol. 14*: 214-219.

Myburg, A., Griffin R., O'Malley, D., Sederoff, R.R. and **Whetten, R.** (2001). Genetic analysis of growth and wood quality traits in interspecific backcross families of *Eucalyptus grandis* and *Eucalyptus globulus*. *Plant and Animal Genome IX Conference*, San Diego, CA, USA, Abstract W82.

Myburg, A.A., Griffin, A.R., Sederoff, R.R. and **Whetten, R.W.** (2003). Comparative genetic linkage maps

of *Eucalyptus grandis*, *Eucalyptus globulus* and their F1 hybrid based on a double pseudo-backcross mapping approach. *Theor. Appl. Genet*. *107*: 1028-1042.

Sanchez, J.R. (2002). Panel de tecnologia, pasta y papel. In: Grupo de Investigacion AF-4 - Universidade de Vigo. *Socioeconomia*, *Patologia*, *Tecnologia y Sostenibilidade del Eucalipto*. Vol. 1. Ed. Universidad de Vigo, Graficas Diumaró, Vigo, Spain, pp. 187-192.

Thamarus, K., Groom, K., Murrell, J., Byrne, M. and **Moran, G.** (2002). A genetic linkage map for *Eucalyptus globulus* with candidate loci for wood, fibre and floral traits. *Theor. Appl. Genet*. *104*: 379-387.

Tuskan, G.A., DiFazio, S.P. and **Teichmann, T.** (2004). Poplar genomics is getting popular: The impact of the poplar genome project on tree research. *Plant Biol. 6*: 2-4.

Verhaegen, D., Plomion, C., Gion, J.M., Poitel, M., Costa, P. and **Kremer, A.** (1997). Quantitative trait dissection analysis in *Eucalyptus* using RAPD markers, 1. Detection of QTL in interspecific hybrid progeny, stability of QTL expression across different ages. *Theor. Appl. Genet*. *95*: 597-608.