Introduction

Plant biotechnology is a field of scientific research in which rapid advances have been made in recent years, and which appears to have much potential for further development. Numerous opportunities for using biotechnology in plant breeding have been identified, some of which might be appropriate for the improvement of crops in developing countries. In this conference we will focus on forest trees and discuss currently available biotechnologies and their application in the forestry sector with reference to their potential use in developing countries today. Please note that for the purposes of this conference, the term “forestry sector” specifically excludes fruit orchards.

Most forest tree species are characterised by inherently high levels of variation and extensive natural ranges. This high level of genetic variation needs to be maintained to ensure present-day and future adaptability to changing environmental conditions. It is also needed to maintain options and potential for improvement to meet changing end-use requirements. Forests provide a wide range of goods and services such as timber, fibre, fuelwood, food, fodder, gum, resins, medicines, pharmaceutical products and environmental stabilisation. Similar goods and services are often provided by a wide range of genera and tree species. Despite the availability of a large number of forest tree species, less than 500 have been systematically tested for their present-day utility for human beings and less than 40 species are included in intensive selection and breeding programmes.

Selection in breeding populations with a broad genetic base is the most common approach to forest tree improvement. Although demand for wood is the driving force in the development of large-scale forest plantations, several selection and breeding programmes aim at enhancing other goods and environmental services provided by forest trees and shrubs.

Since most forest tree species are characterised by long generation intervals and a generally long juvenile phase before flowering, much time is needed before assessment of important traits can be carried out. For example, if wood quality is of interest in breeding for timber or fuelwood, selection can only be carried out after trees have reached a certain size which, in some cases, can require decades. The above factors are limitations to rapid improvement and only a maximum of three or four generations of breeding have been completed in a few forest tree species to date (*Eucalyptus grandis* and some pine species).

Description of Biotechnologies in the Forestry Sector

This section provides a summary of recently developed biotechnologies that could be used, or more widely used, for forest trees in developing countries today [1].

Biotechnologies based on molecular markers

Reliable information on the distribution of genetic variation is a prerequisite for sound selection, breeding and conservation programmes in forest trees. Genetic variation of a species or population can be assessed by measuring morphological and quantitative characters in the field or by studying molecular markers in the laboratory. A combination of the two methods is required for reliable results.

Molecular markers can be used for:

- **Quantification of genetic diversity.** The use of molecular markers for the determination of the extent of variation at the genetic level, within and between populations, is of value in guiding genetic conservation activities, which are aimed at maintaining genetic diversity with respect to traits of both known and unknown importance, and in the development of breeding populations for specific end uses. It should be noted that studies on genetic diversity based on molecular markers must be interpreted with caution, due to frequently low correlations with patterns of variation for adaptive traits, which are of major importance in forestry.

- **Genotype verification.** Molecular markers have been widely used for identification of genotypes and applied in taxonomic studies, biological studies and “genetic fingerprinting”. Good taxonomy is fundamental to conservation and tree improvement programmes and to programmes involving hybridisation between species. The use of molecular markers has revolutionised studies of mating systems, pollen movement and seed dispersal. Results of such biological studies are of considerable practical significance to advanced tree improvement programmes, specifically in population sampling, design and management of seed orchards (i.e. orchards consisting of clones or seedlings from selected trees, and cultured for early and abundant production of seeds for reforestation), estimation of pollen contamination and development of controlled pollination methods. Germplasm identification, through “genetic fingerprinting”, has been used in advanced breeding programmes which rely on controlled crosses or in which the correct identification of clones for large-scale propagation programmes is essential.

- **Gene mapping and marker-assisted selection (MAS).** Genetic linkage maps can be used to locate genes affecting quantitative traits of economic importance. Quantitative traits, such as wood yield, wood quality or pulp yield, are usually controlled by many genes, termed quantitative trait loci (QTL). By using molecular markers closely linked to, or located within, one or more QTL, information at the DNA-level can be used for early selection. The potential benefits of MAS are greatest for traits that are difficult, time-consuming or expensive to measure (for

example, wood quality traits or pulp yield). Mapping and MAS tend to be used only in species of high economic value and have most potential in clonal breeding programmes, where additional genetic gains can be rapidly multiplied.

Biotechnologies based on vegetative propagation

Strategies supporting large-scale utilisation of genetic material with a narrow genetic base must be appropriately integrated into tree improvement programmes. Vegetative propagation within such programmes allows for a fast release of new materials and for appropriate matching of clones to different local conditions. It also allows continued cultivation of given clones and to efficiently change the mixture of clones used in a given programme. Vegetative propagation also supports other currently available biotechnologies (in vitro storage and cryopreservation: in vitro selection).

- **In vitro storage and cryopreservation.** In vitro storage refers to the storage of germplasm in aseptic culture under laboratory conditions, while cryopreservation refers to the storage of cells, tissues, seeds, etc. at temperatures of liquid nitrogen (-196 ºC). The two techniques do not seem to be widely used in genetic conservation activities for forest trees, but they may serve as back-up strategies for species with seed storage problems.

- **In vitro selection.** In vitro selection refers to the selection of germplasm based on test results using tissue culture under laboratory conditions. Many recent publications for crop plants have reported useful correlations between in vitro responses and the expression of desirable field traits, most commonly disease resistance. Positive results are available also for tolerance to herbicides, metals, salt and low temperatures. For the selection criteria of major general importance in forest trees (in particular vigour, stem form and wood quality), poor correlations with field responses will limit the usefulness of in vitro selection. However, in vitro selection may be of possible interest in forestry programmes for screening disease resistance and tolerance to salt, frost and drought.

- **Micropropagation.** For crop and horticultural species, micropropagation (in vitro vegetative propagation of plants) is now the basis of a large commercial industry involving hundreds of laboratories around the world. Successful protocols now exist for a large number of forest tree species, and the number of species for which successful use of somatic embryogenesis has been reported is increasing (somatic embryogenesis is a step in micropropagation where somatic cells are differentiated into somatic embryos). So, in the future, it is likely that micropropagation in the forestry sector will become commercially more important. Compared to vegetative propagation through cuttings, the higher multiplication rates available through micropropagation seem to offer a quicker capture of genetic gains obtained in clonal forestry strategies.

One major factor impeding early application of micropropagation in many large-scale forest plantation programmes, is that breeding and selection of desired clones are not sufficiently advanced for clonal forestry to be contemplated. Current high costs will also be an impediment to the direct use of micropropagation in many programmes. Technologies resembling those used commercially in horticulture are most likely to be affordable for a limited number of high-value forest tree species, particularly those for which propagation by cuttings is difficult. Micropropagation is unlikely to be used for the production of planting stock of non-industrial forest tree species.

Genetic modification of forest trees

Genetically modified organisms (GMOs) are defined as organisms that have been modified by the application of recombinant DNA technology (where DNA from one organism is transferred to another organism). The term “transgenic trees” is also used for genetically modified trees, where a foreign gene (a transgene) is incorporated into the tree genome.

One of the first reported trials with genetically modified forest trees was initiated in Belgium in 1988 using poplars. Since then, there have been more than 100 reported trials, involving at least 24 tree species—most of these are timber-producing species. The majority of the field trials were carried out in the USA and Canada. Whereas it is estimated that roughly 40 million hectares of transgenic agricultural crops were grown commercially in 1999, there is no reported commercial-scale production of transgenic trees. Information on field trials of genetically modified trees has been published by the Organisation for Economic Co-operation and Development (OECD) [2] and the World Wide Fund for Nature (1999) [3].

Traits for which genetic modification can realistically be contemplated in the near future include insect and virus resistance, herbicide tolerance and lignin content. However, insertion of any gene into a tree species with expected functional results will be a substantial undertaking, and insertion of enough genes to confer e.g. long term insect resistance in a perennial species even more so. Virus and insect resistance, in particular, are of major significance for crop plants. By contrast, these traits are not the most important in breeding programmes of forest tree species (poplars being an exception). Reduction of lignin is a valuable objective for species producing pulp for the paper industry; work on this aspect is underway in aspen.

A major technical factor limiting the application of genetic modification to forest trees, is the current low level of knowledge regarding the molecular control of traits which are of most interest, notably those relating to growth and stem and wood quality. Genetic modification of these traits remains a distant prospect. Investments in genetic modification technologies should be weighed against the possibilities of exploiting the large amounts of genetic variation, which are generally untapped, available within any single species in nature.

Biosafety aspects of genetically modified trees need careful consideration because of the long generation time of trees, their important role in ecosystem functioning and the potential for long distance dispersal of pollen and seed.

Forestry in Developing Countries

Forests cover approximately 26% of the world’s total land area [4]. They are the source of vital commodi-