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Advances in Biotechnology: Powerful Tools for Tree Breeding and Genetic Conservation

INFORMATION NOTE

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SUMMARY

A range of biotechnological tools has been used by Forest Research to enhance the efficiencies of research and development programmes associated with tree breeding and genetic conservation. This Information Note presents some examples where such tools have been applied. These include enhancement of flowering in conifers, assessment of the efficiency of seed orchards, characterisation of populations, and efforts to make clonal forestry of Sitka spruce a reality. Work remains to be done to reduce the length of generation turnover, maximise gain within a generation, reduce the cost of the selection process, provide more data on which to base conservation strategies and develop markers to identify stages of physiological development.

INTRODUCTION

Tree breeding can take over 30 years to progress from initial pollination to the stage where improved propagules become commercially available (see Lee, 2004 for a brief summary of the first generation of conifer breeding in Britain). Any cost-effective tool which can improve the efficiency of this process has to be considered. Biotechnological tools in tree breeding can be applied in two key areas: increasing the efficiency with which tree breeders select the best genotypes in a given generation and increasing the efficiency with which the best genotypes are planted in the forest.

Biotechnological tools can also be used to provide a sound foundation on which to develop genetic conservation strategies. Whereas foresters may have traditionally used morphological traits to distinguish between populations of a given species, biotechnological tools can identify genetic differences between populations and even provide information about how they colonised the UK following the last glacial period. Using this information conservationists can identify how closely related populations are, give guidance on preferred seed sources and help delineate the different origins and provenances within a species.

DEFINITIONS

The UN Convention on Biological Diversity (1992) has defined biotechnology as *'any technological application that uses biological systems, living organisms or derivatives thereof to make or modify products or processes for specific use'*. This is a broad definition that ranges from low-technology processes, such as the production of rooted cuttings from young stockplants in the vegetative propagation of Sitka spruce family mixtures, to state of the art processes which could enable the selection of very young trees in the laboratory using molecular markers to replace the current method of costly field trials.

RECENT APPLICATIONS OF BIOTECHNOLOGY

Biotechnological tools have been used in forest research in the UK for over 30 years and have been applied in a number of areas:

Flowering research

In the early 1980s a project was initiated to make the Sitka spruce and hybrid larch breeding programmes more efficient by manipulating the physiology of mature trees so that they flowered more frequently and with a greater profusion. Work by Philipson (1987) showed how flowering could be induced in potted grafts of both species by stem injections of a mixture of gibberellic acids ($GA_{4/7}$) in combination with drought and high temperature treatments. This was a highly successful project and is now an established technique in breeding work for both the production of seed for mass vegetative propagation and for increasing the harvest of seed from seed orchards.

Biochemical and molecular studies

Studies of variation at the biochemical level were started in 1971. The original aims of the research were to

investigate the variation which existed within and between populations, and to discover markers which enabled the seed origin to be determined. Over the years, studies have involved a range of different types of biochemical markers including polyphenols, terpenes and isozymes and DNA.

Terpene analysis was first used to partition the natural distribution of lodgepole pine in northwest America into 15 biochemically distinct regions (Forrest, 1980). A similar range-wide study carried out on Sitka spruce found a gradual biochemical change over its long and very narrow natural distribution (Forrest, 1989).

Other biochemical studies of relict native Scots pine woodlands resulted in the definition of the regional boundaries now used in the Forestry Commission's Scottish Forestry Grants Scheme (Kinloch, Westfall and Forrest, 1986). The study also found high levels of genetic variation, even within the smallest and most depleted stands.

Further work involved the use of isozyme markers to study the mating system in seed orchards. Outcrossing rates in a young Sitka spruce seed orchard were found to be high, suggesting little self-pollination in the orchard (Cottrell and White, 1995). The same study revealed that individual clones did not make an equal contribution to the total pollen cloud. A similar study in hybrid larch seed orchards found that the proportion of hybrid seed harvested could be lower than 20% when harvested from Japanese larch mothers and rarely higher than 50% when harvested from European larch mothers (Ennos and Tang Qian, 1994).

More recently, the work has progressed to encompass a range of DNA based molecular techniques. The polymerase chain reaction (PCR), a means of amplifying minute quantities of DNA, has been used to generate genetic fingerprints of commercial poplar clones (Cottrell *et al.*, 1997a). The same technique was used to assess the critically low level of genetic diversity present in black poplar in the UK and provided evidence that most of the surviving trees consisted of just a few clones (Cottrell *et al.*, 1997b).

Ancient oakwoods in Britain have been subjected to a number of recent molecular studies. DNA markers have been used to determine postglacial colonisation routes and to describe the distribution of variation within and between oakwoods (Cottrell *et al.*, 2002a,b). These techniques have now also been used to look at the recolonisation routes of black poplar.

Vegetative propagation techniques

The large-scale rooting of Sitka spruce cuttings taken from juvenile, genetically improved stockplants (macropropagation) is a recognised commercial success which originated with basic biotechnology research carried out by Forest Research (John and Mason, 1987). Work has subsequently been carried out into the tissue culture (micropropagation) of Sitka spruce and, although useful in a number of research applications, it is not yet a commercially viable technique.

One of the major limitations to the practical application of clonal forestry (defined as the mass replication of tested genotypes) is our current inability to multiply tested genotypes before age-related physiological changes alter the growth performance of cuttings. It takes around nine years to select clones based on their field performance, and yet cuttings taken from a stockplant more than six years from seed can demonstrate poor rooting success and slow growth. Techniques are needed which both arrest physiological changes related to age and accelerate the multiplication of selected genotypes. Good progress is now being made in both of these areas. Firstly, cryopreservation (storage of tissue at –196°C) is being developed to prevent physiological changes taking place while field evaluation is under way. Secondly, somatic embryogenesis (SE) is a new technique being developed that allows a callus to form numerous clonal embryos non-sexually in the laboratory. These embryos can then be germinated to form clonally derived plants (John *et al.*, 1995). Recent advances in each of these techniques lead Forest Research scientists to believe that clonal forestry of Sitka spruce will be a practical reality for commercial nurseries in the near future.

POSSIBLE FURTHER ADVANCES IN THE FUTURE

Advances in biotechnology are taking place very quickly, and familiarisation can convert a technically difficult method into a simple and routine procedure over a relatively short period of time. Forest Research is involved in pioneering several such techniques:

Molecular markers

A new and large biotechnology project has just started known as Marker Aided Selection (MAS). This is a technique whereby scientists extract DNA from young

plants and screen for certain economic traits such as high wood density, good stem straightness or disease resistance using molecular markers. The goal is to reduce the cost of genetic testing and, as far as possible, transfer activities from costly, forest-based comparative field trials to laboratory-based screening after the development of a database of suitable molecular markers.

This is another area which is receiving considerable international interest with research teams generally offering their findings freely within the public domain. Forest Research is part of this new drive and is contributing by establishing a series of definitive and very large clonal tests, allowing accurate correlation between field-based performance and laboratory-based markers. Whether continued field-based verification trails will be required will ultimately depend on cost and confidence in the new technology.

The use of clones in forestry

Advances in propagation and cryostorage techniques make it probable that commercial Sitka spruce planting stock will ultimately be based exclusively on clonally produced material. Research establishments elsewhere in the world are claiming some success in encapsulating somatic embryos to make artificial seed which could then be managed in traditional nurseries in the same manner as conventional seed. Clonal forestry at no more than the cost of conventional seedling production remains a goal.

Genomics

The area of biotechnology relating to genomics or gene expression is relatively new but is generating a great deal of interest. Some of the genes present in an individual are not active throughout its lifetime. The environment and the phase of tree development influence their activity. It may be possible to use the activity of particular genes as indicators of the physiological state of the plant. For example, Forest Research is currently involved in a project which aims to find genomic markers which will act as a tool for nursery growers to assess when their plants are truly dormant and in an appropriate state to be lifted. Furthermore, markers for cold-hardiness are being investigated to provide information on the suitability of stock for cold storage. Other markers of practical benefit are those that could identify whether a plant is still sufficiently juvenile to provide healthy rooted cuttings. An improved understanding of the genes involved in the flowering process might provide the ability to induce flowering at a younger age which would allow researchers to reduce breeding generation intervals and to increase the productivity of seed orchards.

Genetically modified organisms

None of the biotechnology work described above involves genetic modification (GM) techniques. Although there is currently no Forestry Commission sponsored GM work being carried out on conifers in Great Britain, the feasibility of tackling the problem of Dutch elm disease through GM is being explored (Gartland *et al*., 2000). No GM elms have reached the point of planting outside the laboratory. No material will be released until the benefits to society and industry have been clearly demonstrated to exceed the risks.

CONCLUSIONS

Biotechnological tools are important in a number of ways. They help the breeder to make more accurate selections of the most suitable genotypes at an earlier age and at reduced costs relative to traditional field-based systems. They enable rapid multiplication of these superior genotypes. They are of value to the conservationist in characterising the different populations that exist across the distribution of a species so that management guidelines can be based on sound scientific information. They can also help scientists to understand tree physiology through knowledge of gene function, and this in turn benefits the nurseryman and growers to operate more efficiently.

Biotechnology is now an established tool in forestry research and its application promises to become central to future developments in forestry and forest science.

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