Guest Editorial

Forest Biotechnology: A South African perspective

INTRODUCTION

While it is a pleasure to have the opportunity to present a commentary on biotechnology as it applies to South African forestry, this is a somewhat daunting task. The problem one immediately faces pertains to the word biotechnology, which has a host of different interpretations. Broadly, biotechnology might be considered as the application of living organisms to produce or modify products for particular purposes. As such, many forestry activities and especially those linked to exotic plantation forestry could be considered as "early phase" biotechnology. The more widely recognized forms of so-called first generation biotechnology include the utilisation of yeasts to produce for example bread or to ferment alcoholic beverages. Second generation biotechnology includes higher levels of technological input and thus includes activities such as large-scale vitamin production and in the plant environment, tissue culture. Third generation biotechnology has captured the imagination of the world and is generally considered to be the use of recombinant DNA technology to produce or modify biological products. Thus, at the outset, offering a brief editorial on forest biotechnology in South Africa, by definition, implies that the outcome will be somewhat superficial and biased towards our own views.

HISTORICAL PERSPECTIVE

South African forestry companies and foresters have been actively applying biotechnology since the first plantations of exotic species were established more than one hundred years ago. Certainly in the early days of forestry in this country, exciting opportunities arose from the selection of species in trials and interestingly, from the natural hybridisation of *Eucalyptus* species. Forestry research was given high priority in the country and the influences of early investments are clearly seen today. South Africa is seen as one of the world leaders in plantation forestry and often times, looked upon with some envy by groups in other parts of the world, who desire equivalent highly productive plantations. What is often forgotten is that the success of these plantations is based on more than one hundred years of research and development. Development of tree crops is by its very nature a slow process. There is no question that the successes that we enjoy in South African forestry today are the legacy of great vision and insight on the part of the "fathers" of South African forestry.

The great variation in the South African environment has had a substantial influence on the development of forestry in the country. Selection of species suited to areas with distinctly different climates and soil types has involved great effort. This fundamental phase of forest biotechnology is an ongoing process and will most likely continue far into the future. For some species such as those of *Eucalyptus*, natural hybridisation has given rise to local land races with outstanding properties. The early 1980's marked the first steps towards clonal forestry and particularly the opportunity to capture the genetic gains emerging from natural and later artificially produced hybrids of both pines and eucalypts. This has given rise to a wide range of different technologies linked to propagation including the use of macro cuttings, micro cuttings, hydroponics, tissue culture, somatic embryogenesis to mention but a few. These various approaches have involved a wide spectrum of associated activities pertaining to selection of improved planting media, maintenance of genetic stock, as well as mechanical and electrical engineering to mechanise and upscale the planting process.

Certainly one of most dramatic changes in plantation establishment in recent years has been introduction of vegetative production of planting stock and clonal forestry. Although this approach has been pursued for about twenty years in South Africa, it might still be considered to be in its infancy. Clonal eucalypt forestry is by far most advanced but much has yet to be achieved. There remain genotypes and even species that are difficult to root in sufficiently high numbers to be commercially viable. The same is true for the pines as well as for species of *Acacia*. While much outstanding research has been done in the fields of cell and tissue culture to expand opportunities linked to vegetative propagation, there is a great deal that might yet be achieved.

MODERN BIOTECHNOLOGY

Recombinant DNA technology and the opportunity to produce plants and animals carrying foreign genes has captured the imagination of agricultural and forestry scientists. Discussions regarding forest biotechnology are commonly restricted to this field and indeed it is here that the greatest controversy lies. While we do not consider that forest biotechnology is restricted to methods associated with recombinant DNA and genetic modification, this field is perhaps most controversial and will form the bulk of our further discussion.

While it might not be widely recognised, recombinant DNA technology has already had some impact on forestry in South Africa. However, involvement has been relatively limited. This is not due a lack of appreciation for the value of this technology by forestry companies, but high research costs and other immediate priorities have meant that there has not yet been substantial investment in this area. This is in contrast to other countries especially in North America and Europe where there are a number of large research groups focused on DNA-based methods, including genetic modification to improve forest trees. One argument commonly invoked is, therefore, that South Africa can simply import products linked to this technology and that there is no strong justification to develop these locally. The obvious flaw in this argument is that, while some of these DNA-based tools may be applicable to South Africa, the tree species and genotypes of interest to us and planted commercially are unlikely to be the specific focus of studies in other countries. Thus at the very least, there is a good argument for developing expanded national competence to apply recombinant DNA technology to the species that are planted here. In addition, we would argue that the research environment in South Africa is sufficiently advanced and has potential to allow for active participation in consortia developing recombinant DNA based products.

Arguably, South Africa has the best infrastructure in the continent for the development and deployment of genetically modified trees. Three genetically modified agricultural crops (soybean, cotton and maize) are already being propagated both commercially and by small-scale farmers (cotton and maize) in South Africa. Therefore, regulatory systems have already been established by the South African Government and can easily be applied to the production of transgenic trees. Clearly, trials to evaluate the expression of transgenes in the different tree genotypes would be required before transgenic trees could be deployed commercially.

FOREST CERTIFICATION

Most forestry groups in South Africa have been granted certification by the Forestry Stewardship Council (FSC), and thus have impressive standards of environmental management and sustainability of forest production systems. FSC currently prohibits the deployment of genetically modified trees. This is certainly an impediment to expanding activities relating to recombinant DNA technology in forestry. Thus, forestry companies both in South Africa and elsewhere in the world must rely on independent research organisations to develop the technologies that they will most likely require in the future.

TRANSGENIC TREES

Contrary to the views of many foresters, the introduction of genes into selected clones or improved seed is not particularly complicated. This process does, however, require appropriately equipped laboratory facilities and especially staff having competence in plant tissue culture and recombinant DNA techniques. We contend that this competence exists in South Africa and the country has some outstanding laboratory centres where the appropriate research and applications can be undertaken. Yet we see two main primary hurdles standing in the way of the development and deployment of genetically modified trees in South Africa. The first and arguably the most important of these is access to appropriate genes for transformation. In some cases these genes are available but they come at a high price. Royalty agreements will also greatly increase the cost of large-scale deployment in plantations. In other cases desired genes have yet to be discovered and this process demands substantial and long-term research. In our view, this presents a major research opportunity for the South African forestry industry as well as for researchers in this country.

A second important hurdle hindering the development of genetically modified trees is linked to the relatively long rotations associated with tree farming. After new genes have been introduced into trees, considerable time is needed for growth, before the properties of the introduced genes can be assessed. The situation is rather different in the case of agronomic crops where numerous generations of plants can be grown in a single year and the results of genetic modification can be assessed rapidly. More so is the case with microbes such as yeasts where recombinant strains, for example for wine or beer production are known to already be available for use when required or desired. Our argument would be that forestry has always been a long-term investment and many successes that we enjoy today are due to investments made decades ago. We would thus contend that our forestry industry would benefit greatly by substantially increased involvement in the development of transgenic plantation trees. Thus already available genes, although perhaps bearing some cost, can be tested in the improved genotypes that are so important to our industry. This would also allow for the development of the human resource capacity to ensure that the appropriate research will be available to forestry in South Africa in the future.

TRANSGENIC MICRO-ORGANISMS

The production of transgenic trees is by no means the only application for genetic modification that is in the interests of forestry. It is well recognised that pests and diseases pose one of the greatest threats to exotic plantation forestry both South Africa and elsewhere in the world. The tremendous growth of the trees providing the outstanding fibre for pulp and paper mills as well as for saw timber is closely linked to the fact that they have been separated from their natural enemies. This is a situation that is changing and severe impacts are already being felt. Contending with damaging pest and pathogen invasions is also becoming increasingly difficult. In the past, changing species and more recently introducing disease resistant hybrid clones have provided useful lines of defence. These approaches will remain important in the future but are likely to be gradually saturated. Thus, new and innovative technologies will be increasingly needed to avoid losses. In this regard there are many opportunities linked to genetic modification that might be used to contain pests and pathogens. One exciting example that we have pursued is to consider the use of fungal viruses for pathogen control. The genomes of these viruses which result in reduced virulence in pathogens can be integrated into the fungal genomes. These genetically modified fungi then become effective vectors of the viruses, with the potential to control the target fungal pathogens. Research at our institute has already shown great promise for using genetically modified or transfected forms of the Eucalypt canker pathogen *Cryphonectria cubensis*, to reduce the impact of the disease that it causes in plantations.

Microbes and especially fungi and bacteria are becoming increasingly interesting to paper and pulp industries worldwide. These organisms are already applied in fermentation processes to produce enzymes used in pulping. There are also situations where the organisms themselves are used *in vivo* to treat wood and paper products. These approaches can offer environmentally friendly alternatives to the use of toxic chemicals. We believe that they will be increasingly used in the future. Here again, there is great potential to use genetically modified fungi and bacteria to either enhance their efficacy and to expand their potential applications. Some interesting research in this field has already been undertaken in South Africa and there are great opportunities for expansion in the future. In this regard, the country has a widely envied microbial biodiversity and products from locally occurring organisms could provide unique products of great value to forestry.

DISRUPTION OF GENE EXPRESSION

The most common use of recombinant DNA techniques in the production of improved genotypes has been by inserting genes into the genome of elite lines. Another process, which has evolved from the use of this technology, is that of RNAi (RNA interference). Double stranded RNA fragments of a specific sequence can interfere with the activity of genes having the same sequence. Double stranded RNA can be used to target a specific gene or suite of genes and at the molecular level, switch them off. Utilising RNAi is already having a significant impact on the development of new medicines. RNAi offers forestry and pulping processes the same potential benefits and there are many examples on which one could speculate. An obvious application, would for example, be linked to fungi used in biopulping. Huge effort is typically necessary to select fungal strains producing enzymes, which for example degrade lignin but not cellulose. Often the best lignin degrading organisms also degrade some cellulose and are not suitable for biological pulping. However, using RNAi technology, it would be possible to target the cellulase enzymes and inhibit their activity without affecting the production of the lignin degrading enzymes.

MARKER AIDED SELECTION

Not all modern forest biotechnology necessarily involves inserting genes into trees or microbes of interest to forestry. The products of this same technology offer the potential to identify genes or fragments of DNA linked to genes that can be used in tree breeding through so-called marker aided selection (MAS). This technology has been very successfully applied in agricultural crops and it is likely to offer equivalent opportunities in forestry. Identifying genes linked to desired traits requires a firm foundation in classical breeding and the availability of breeding lines. Forestry companies face the problem of relying on plants with relatively long rotation ages, which hinders the development of MAS. Identifying genes linked to traits important to forestry is likely to be slower than it has been for agricultural crops. However, some genes useful to forestry will emerge from research on agricultural crops and model plants such as *Arabidopsis thaliana*. Other traits of interest to forestry such as fibre quality are not of concern in agricultural crops and have thus not been studied in these plants. Such traits need to be studied in trees and then verified in model systems. Thus, a great deal of basic research is still necessary before MAS can be applied actively in forestry.

DNA FINGERPRINTING

Marker aided selection should not be confused with DNA fingerprinting, although the two approaches make use of similar technologies. DNA fingerprinting of humans and domestic animals is widely accepted as the most sophisticated means for identification and it is commonly applied in legal contests. The DNA of trees and animals is the same and thus, similar DNA fingerprinting tests used for animals are easily applied to forestry species. However, while DNA-based testing is relatively well established in forestry, it is important to understand that in order for these fingerprints to stand the test of litigation, a significant amount of research is necessary. In humans, much of this research was accomplished during the latter two decades of the last century in labs around the world. Because forest trees represent many different species, a great deal more effort will be needed to establish DNA fingerprinting services, than has been true for humans.

The development of fingerprinting markers has become relatively routine and the techniques including RAPDS (Random Amplified Polymorphic DNA's), AFLP's (Amplified Fragment Length Polymorphisms) and microsatellites are well-established in many labs including our own. In this regard, it is necessary to ascertain allelic frequencies of markers within each species and thus to be able to calculate the possibility of a specific genotype occurring more than once within the population. Thus in the forestry context, it is necessary to be able to calculate the probability of two breeding programmes developing an identical genotype by chance. If this probability is considered to be sufficiently small then fingerprints are considered to be relatively sound. In humans, problem cases arise in communities that are known to be relatively inbred and where many of the alleles or markers are identical in many individuals. Intermarriage between relatives is discouraged in human societies and usually occurs only in geographically isolated communities. The situation in forestry is different in that it is common to back cross trees to one or other parent, in an attempt to produce offspring that have specific characteristics of the original parent. Similarly, while clonal reproduction is virtually unknown in humans (only documented in identical twins), this process is important in forestry. Thus trees in some breeding programmes may be genetically much more similar to each other than would be the case in the natural population. These dynamics must be clearly understood in breeding programmes and knowledge pertaining to the genetic diversity in native and introduced populations of tree species are necessary if DNA fingerprints are to be used in legal suits.

In forestry programmes where only a limited number of genotypes are deployed, DNA based fingerprinting has been used very successfully. Here it is generally not necessary to determine genetic diversity or allelic frequencies. Questions that are usually considered relate to the identification of clones, where planting records have been confused or identifying the parents of particular clones. Where controlled crosses have been used, it is sometimes also necessary to confirm the identity of the pollen parent and this is easily achieved with DNA based fingerprinting. The most common method used is that of RAPD. AFLP's may provide a greater level of resolution but few commercial research labs can justify the expense of running these gels or covering the costs of patents held on this technology. Microsatellites are used most commonly in the case of human DNA fingerprinting and have many advantages, however, the overall cost is extremely high and rarely justified in the case of routine identification in commercial forestry programmes.

FINGERPRINTING PESTS AND PATHOGENS

Fingerprinting trees is not the only form of DNA fingerprinting that is important to the forestry. DNA fingerprints or diagnostics that enable rapid and accurate identification of pests and pathogens are powerful tools that are essential in disease management programmes. These fingerprinting tools can be used to identify species but they are also used to recognise individual genotypes of pests and pathogens. Genotype recognition is particularly important to assess genetic diversity of tree pathogens or pests in order to support breeding programmes. Some fungi reproduce clonally and in some cases disease outbreaks are caused by a single genotype. Breeding of trees that have resistance to a single pest or pathogen genotype has the significant disadvantage that such a trait is likely to be easily overcome. This is generally due to changes in the pathogen or through the introduction of new individuals of the pathogen.

NEW DEVELOPMENTS IN BIOTECHNOLOGY FUNDING

During the last year, the South African Government launched its Biotechnology Development Programme. This year marked the development of three regional Biotechnology Innovation Centres (BRICs). While this highlights a government commitment to promoting biotechnology, none of the programmes that have been funded to date, have had a plant focus. It is our understanding that a Plant Biotechnology Innovation Centre is in the process of being developed. However, the guaranteed funding for this programme is likely to be less than that of the other centres. We are disappointed at delays in the initiation of a national plant biotechnology strategy and also at the low levels of funding that are being considered for this. Our view is that plant biotechnology, and in our case forest biotechnology, is crucially important for the future of one of South Africa's most important industries. While forestry companies will clearly need to make increased investments, government support will also be most important.

BIOINFORMATICS

The era of genomics has clearly dawned and the number complete genome sequences is increasing rapidly. Currently plant genome sequences are lagging behind those of animals. The first tree genome sequence, that of poplar, has just recently been completed (http://genome.jgi-psf.org/poplar0/poplar0.home.html) and is in the process of being annotated using a framework of microsatellite markers (http://www.ornl.gov/ipgc/Links.htm). Poplar trees are not amongst the top ten most important commercial species in South Africa. However, even the sequence of this genome will be a tremendous resource for forest biotechnology in South Africa. To make full use of this resource, there will be a need to greatly expand capacity in the field of bioinformatics. The South African government has recognised the need for greater bioinformatics capacity in the country and there are already initiatives in place to achieve this goal. The National Biotechnology Network has already identified a number of "nodes" for bioinformatics. These nodes are at different stages of development and will become highly relevant to the South African forestry industry in the future and especially as it expands its reliance on forest biotechnology.

CONCLUSION

There is no doubt that the South African forestry industry can be substantially strengthened by investments in biotechnology and by developing increased capacity in this field. This is a rapidly evolving technology that requires research groups that collaborate across disciplines. The equipment and facilities required for advanced biotechnology research and development is generally extremely costly and certainly will not be within the reach of smaller groups. We are convinced that forestry companies will become increasingly dependant on biotechnology centres that have human "critical mass" and scientists that are able to sustain the technology. Vision and foresight will be required to ensure that such groups will be available to the industry in the future. The South African forestry industry has shown such vision in the past and its pre-eminent stature today, reflects this. A leading role for South African forestry will lean strongly on innovative applications of biotechnology in its broadest forms of application.

Brenda D. Wingfield, and Michael J. Wingfield
Department of Genetics, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria. Pretoria 0002. brenda.wingfield@fabi.up.ac.za and mike.wingfield@fabi.up.ac.za.