INCORPORATION OF BIOTECHNOLOGY INTO TREE IMPROVEMENT PROGRAMS

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Abstract.--This paper examines the techniques collectively referred to as biotechnology, the breeding strategies used by the Western Gulf Forest Tree Improvement Program (WGFTIP) and various ways of incorporating the new techniques into the overall program.

The main techniques of interests are new methods of vegetative propagation, gene transfer and expression, regeneration of viable plants from transformed tissue and the use of restriction fragment polymorphism (RFLP's). The main breeding strategies of interest are sublining of breeding populations and step-wise screening procedures. The incorporation of most techniques is rather straightforward and driven both by the availability of the necessary technology and economics. The issue of population improvement is more complex. Some of the issues that need to be resolved are discussed, although the best strategy is not obvious at this time.

Keywords: Breedign strategy, step-wise screening, sublines, vegetative propagation, molecular genetics, tissue culture.

INTRODUCTION

Recently a number of presentations have been given that discussed this topic, but most fail to address some of the important issues. An exception is the article by Riemenschneider et al. (1988), which gives a very thoughtful discussion of the topic. For the purpose of this discussion, the concept of biotechnology is not a very useful one. Biotechnology is not a discipline, but rather a fairly specific set of tools, which can be used for a variety of purposes. In the following discussion, we will not try to make a detailed description or exhaustive list of these techniques, but rather try to organize the available techniques into categories with application to tree improvement.

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BIOTECHNOLOGIES

The first broad category is the introduction and maintenance of genetic variation. Currently two very powerful techniques are available that contribute to this. One is the production of somaclonal variation. Somaclonal variation occurs in tissue culture, particularly callus culture, with the result that plants derived from the same ortet may show considerable variation. This variation can actually be transmitted sexually. The other technique is genetic transformation, also known as genetic engineering or gene splicing, in which a specific piece of DNA is inserted into a recipient cell. This technique allows the introduction of new genetic variations by inserting a desirable gene into a population. For instance, a gene for rust resistance could be inserted into a rust susceptible species such as slash pine *Pinus elliottii* Engelm. var. *elliottii*).

The next major category is selection. Here too biotechnology can contribute a couple of techniques. One example is in *vitro* screening procedures. Newton and van Buijtenen (1984), for instance, reported on procedures to screen loblolly pine genotypes for drought tolerance in *vitro*. The other technique involves the use of restriction fragment length polymorphisms (RFLP's). These are genetic markers, which can be used the same way as isozymes or morphological traits. They are different only because they can be produced in very large numbers, which makes it possible to develop very detailed chromosome maps. This, in theory makes it possible to do very early evaluations of genotypes. A number of things ne3d to be known to accomplish this. First, one has to establish that a particular marker is closely linked to a locus controlling a desired trait. Second, one needs to determine whether the desired allele and the marker are in the coupling or repulsion phase. In the coupling phase the marker and the desirable allele are adjacent to each other, while in the repulsion phase the marker and the undesirable allele are adjacent. Once this is known, one can select progenies on the basis of presence (coupling) or absence (repulsion) of the marker.

Another major area of importance to tree improvers is genetic recombination. Again, biotechnology offers a technique here, although it is perhaps further from application than those mentioned previously. This is the technique of cell fusion. In theory, this technique enables the production of hybrid nuclei that could not be achieved by sexual means.

The final category is propagation techniques. Here, biotechnology offers plantlet regeneration via tissue culture (organogenesis) and plantlet regeneration via embryogenesis (Cheliak 1987). The success of these techniques is highly species dependent, but both techniques have been successful for some forest tree species.

Let us now look at the same situation for operational tree improvement techniques using the same broad categories.

OPERATIONAL TREE IMPROVEMENT TECHNIQUES

Introduction and Maintenance of Genetic Variation

The best opportunity to introduce genetic variation is at the beginning of the breeding program by establishing a broad genetic base. During the course of selection variation will be gradually diminished and the best one can do is what the politicians call damage control. By maintaining a large minimum population size and subdividing the breeding population, the probability of losing genetic variation is reduced, but not eliminated. One can also introduce new material into the breeding population, but after several generations of selection this will partially offset the progress already made.

Selection

Here we have a number of well established techniques, such as first generation selection, progeny testing and second generation selection. New techniques being developed now include early testing and step-wise screening. In early tasting a trait is selected at the seedling stage which is reasonably well corrElated with future performance. Total above ground dry weight is looking vEry promising (Lowe and van Buijtenen 1989). Other traits, such as drought resistance or rust resistance can be naturally tested at an early age. Step-wise screening is another procedure that we have used successfully. It consists of evaluating a number of families in a series of tests, each eliminating part of the families. By using the more economical tests first, a great reduction of progeny testing costs can be achieved. We have, for instance, selected longleaf (Pinus palustris Mill.) in short-term tests (three years) for their ability to survive and emerge from the grass stage quickly. The best families from the short-term tests are then established in a long-term test to observe their growth and form.

Genetic Recombination

This is, of course, accomplished by inter-crossing. It is reasonably safe to consider the partial diallel as the ultimate mating design, primarily because it is virtually impossible to successfully complete a full diallel and anything less is a partial diallel.

To control inbreeding it has proved advantageous to sub-divide the population into breeding groups or sublines (Lowe and van Buijtenen 1986). All crossing for breeding purposes is carried out within the breeding groups, while in the seed orchards, the design is such that crossing is almost exclusively among breeding groups. This implies the other major consideration which is the separation of the breeding population and the production population. The breeding population is contained in the scion banks and progeny tests and the production population is the seed orchard. Generally speaking, only the very best trees out of the breeding population are included in the seed orchards.

Propagation Techniques

Many good techniques are available, the success of which is highly species dependent. Sexual propagation in scion banks and seed orchards is usually the most economical way to mass produce desired genotypes. Grafting techniques have been developed for many species, but the cost is usually too high for mass production. Rooted cuttings can be produced readily for some species such as poplars, spruce, and eucalyptus, but with much greater difficulty (or not at all) for other species such as pines and oaks.

INTEGRATION OF THE TWO SETS OF TOOLS

We just developed two separate lists of tools serving the same four major purposes. So now, how do we integrate the two? The answer, of course, is very simple, combine the two lists, as shown in Table 1. On the surface, this seems

Table 1. Combined list of currently operational tree improvement techniques and biotechnologies useful for breeding.

<u>Introduction and Maintenance of Genetic Variation</u>

- Use of exotics
- Selection in variable species
- Crosses among diverse parents
- Somaclonal variation
- Genetic transformation

<u>Selection</u>

- First generation selection
- Progeny testing
- Advanced generation selection
- Early testing
  - Drought resistance
  - Rust resistance
  - Shoot dry weight
  - In *vitro* screening procedures
  - RFLP's

<u>Genetic Recombination</u>

- Partial diallel mating
- Cell fusion
- Breeding group (sublines)
- Separation of breeding and production populations

<u>Propagation Techniques</u>

- Rooted cuttings
- Tissue culture plantlets
  - Organogenesis
  - Embryogenesis
- Grafting
- Seed orchards

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too easy and some nasty minds might accuse me of having been an administrator too long and learned how to play these slick paper tricks. So, let us consider this in more depth. Figure 1 shows the breeding cycle as we currently practice it. Initially a base population was established of first generation selections. New materials can still be introduced from outside the cycle, although this becomes increasingly difficult as the selected material increases in quality. The initial or introduced material then goes through the selection and testing phase, including step-wise screening. From there, the selected material is mated and the new materials are propagated for another round of selection and testing. The breeding population may contain anywhere from 300 to 3000 genotypes. Out of these the best ones will be selected to go into the seed orchards, typically, 10 to 50 genotypes. The seed orchard produces the improved seed which goes to the nursery and is then planted on a large scale. As you all know, this amounts to about a million acres annually in the south. Under some circumstances it is possible to take the best few genotypes and mass produce them using rooted cuttings.

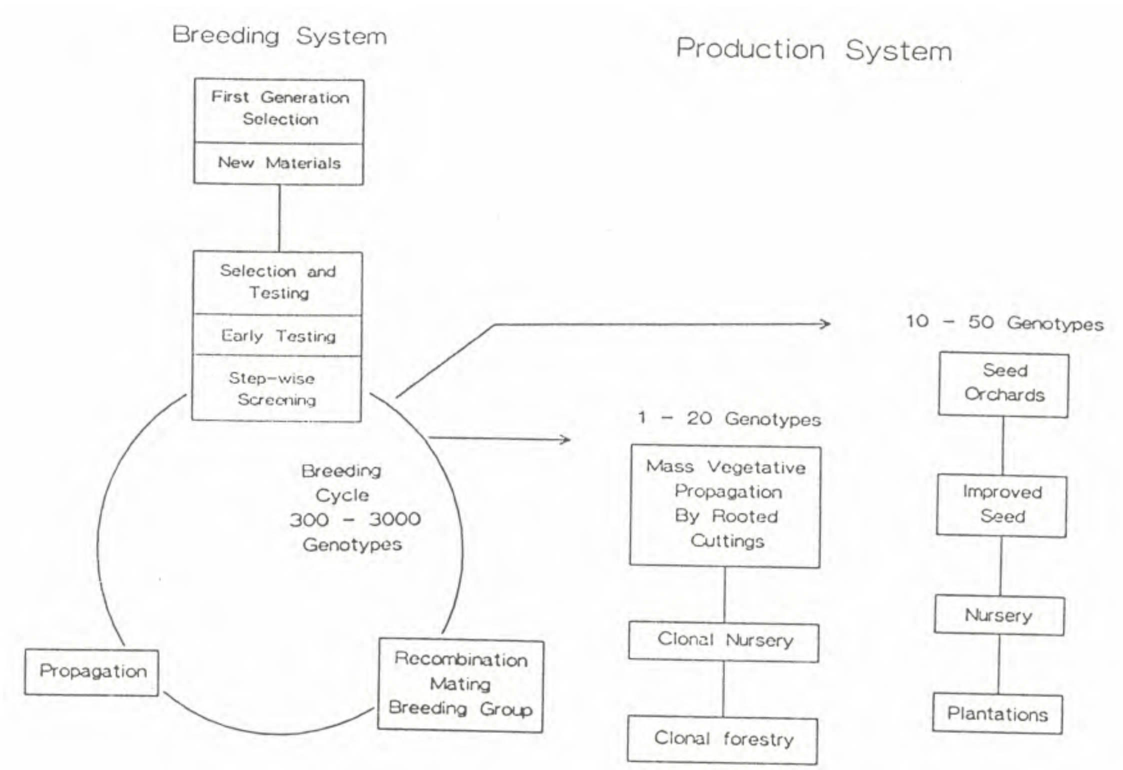

Figure 1. Tree Improvement Using Current Technology

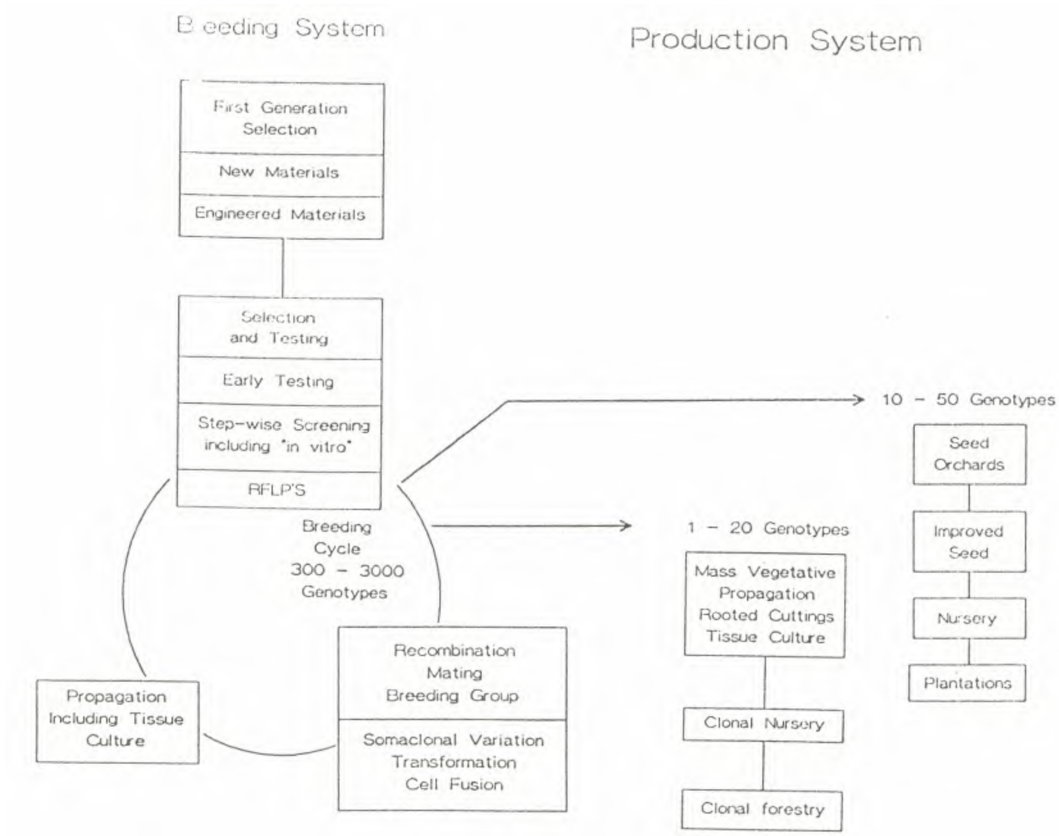

Figure 2. Tree Improvement Including Biotechnologies

Now, let us look what is changed when biotechnologies are added (Figure 2). First of all, at the introductory step, other materials, such as engineered materials can be added and fed into the system. Materials can also be introduced during the recombination phase. At the introductory phase, the materials are essentially unrelated to the existing population. They should go through the selection and testing steps. This might include in vitro early tests and RFLP's to assist early screening. In the recombination phase, somaclonal variation, transformation, and cell fusion might be used to introduce new variation. This is a powerful new addition to the breeding cycle. Previously we could only minimize the loss of genetic variation. Now we have added some promising tools to maintain or actually increase genetic variation. This material then goes through a propagation phase, which might include tissue culture techniques. Next it must be evaluated in the selection and screening phase, after which the cycle repeats itself. The number of genotypes used should stay about the same as before. The seed orchard phase is essentially unchanged, and material can be deployed in seed orchards as it was before.

The capabilities of vegetative propagation have also been expanded, since it now is possible to produce plantlets via tissue culture using techniques based on organogenesis or embryogenesis. These can be used alone or in combination with rooted cuttings, such as the system used in New Zealand with radiata pile.

A HYPOTHETICAL EXAMPLE

For illustration, let us use an example that is not too far fetched. Assume that someone introduces a gene for Roundup® resistance in Virginia pine. This would be of considerable benefit to the Christmas tree growers since weed control is extremely important in Christmas tree production. Christmas tree growers can also pay more for a seedling than a forester and the somewhat expensive techniques might be economical in Christmas trees before they would be economical in operational forestry. Let's further assume that the gene has been introduced in one of the best individuals available, in other words in a tree that is already part of the breeding cycle. What steps would follow?

1) First, the materials should be put in a short-term test to verify that it indeed possesses Roundup® resistance.
2) It needs to be put into a long-term test to verify field performance.
3) It could be mass propagated vegetatively for sale to Christmas tree growers,
4) It could be introduced into the breeding population by crossing it to other outstanding parents, and
5) The clone could be included in a seed orchard.

There remain number of questions to be answered. One is the question of economics. Currently, Christmas tree seedlings sell for $50 to $60 per thousand. However, a grower could afford perhaps up to $1 per seedling ($1000 per thousand) if a tree was really exceptional, which means it had good quality and shortened the rotation age by one year. This technology would seem fairly realistic. For instance, one organization currently is selling rooted loblolly pine cuttings at a cost of $150 per thousand.

For forestry operations the economics look somewhat different. The current cost of seedlings is about $30 per thousand. One percent gain in volume growth is worth about $4 per acre. Assuming that gain by genetic engineering is as high as 50 percent and 500 trees are planted per acre, a seedling cost of about $400 per thousand could be justified. Of course this would not result in any profit for the grower so the cost should probably be no more than half of that, perhaps $200 a thousand. This is again in the feasible range, although the gain required to justify this cost may to difficult to achieve.

Another major question centers around the introduction of engineered genes into the breeding population. I'm not aware that anyone has addressed this question so far. Personally, I would like to introduce more than one copy of a desirable gene into the population, so how would one go about it? Would one introduce this gene in all 24 trees per breeding group? This might not be as difficult as it seems. If it can be done in one tree, it might not be that much

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[2] Mention of trade names is solely to identify material (or equipment) used and does not imply endorsement by the Texas Forest Service. If pesticides are mentioned, this does not imply recommendations for their use, nor does it imply that the discussed uses have been registered. All uses of pesticides must be registered by appropriate State or Federal agencies before they can be recommended.

more complicated to do it in 24. The most difficult part is usually getting the procedure to work the first time. If this is successful, however, current technology will insert the gene at 24 different loci. This may not necessarily be bad. After repeated mating, many trees will wind up with multiple copies of the introduced gene. Since most likely, there will be a dosage effect, this means that the resistance to Roundup might continue to increase after several cycles of selection. It also could mean that any unfavorable side effects would continue to accumulate. In the long run, selection would probably favor an optimal dosage.

From the foregoing it appears that, at least conceptually, there are no major problems integrating biotechnologies with the technologies that are currently operational. From a practical point of view it might, however, not be easy. Developing the technologies to the point where they can be applied routinely and economically will be a major challenge. There are also some organizational problems. Currently, biotechnologists and tree improvement specialists work at best on two different teams. On occasion I have received the impression they are working on competing teams. Neither situation is conducive to integration of the two technologies. As scientists we can alleviate the situation somewhat by being aware of what is happening in both worlds. The problem is, however, primarily an organizational one, and I expect it will soon be profitable to create tree improvement teams with biotechnologists, physiologists, geneticists and breeders as members.

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