GENETIC ENGINEERING AND TRADITIONAL BREEDING: WHERE IS THE INTERFACE?

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Abstract. -- Propagation of genetically superior stock a cornerstone of any genetic improvement program. For clonal forestry and genetic engineering to be useful to a tree improvement program, highly efficient methods of vegetative propagation need to be developed. It is well established in crop plants that genotype is an important determining factor in the develop of successful propagation methods. Preliminary data from tree species shows that similar genotypic effects exist in these species. To maximally exploit superior genotypes in ongoing sexual breeding programs, responsiveness to vegetative propagation needs to be selected to insure continuing flow of genotypes among sexual breeding, clonal forestry and genetic engineering efforts. Future research should be undertaken to determine the nature and degree of heritability of vegetative propagation in valuable tree species. In addition a highly regenerable select nuclear population should be created as the common pool through which genetically engineered genotypes could be moved into clonal forestry or sexual breeding programs.

Keywords: Clonal forestry, genetic engineering, sexual breeding, genotypic effects, vegetative propagation

INTRODUCTION

One of the cornerstones upon which any crop improvement program rests is the ability to move individuals with improved genotypes into operational field plantings. There are two methods for producing commercial quantities of selected genotypes, either through seedlings or via some form of vegetative propagation. The ideal propagation approach should allow exploitation of both the additive genetic gain achieved through sexual breeding and the non-additive gain identified during testing for superior phenotypes. In addition, the propagation method should produce plants at very low costs and in the large numbers required for commercial cropping. It is also important that the propagation method be highly efficient for all selected

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genotypes, otherwise valuable genotypes will be lost to commercial growers. Finally, with the advent of biotechnology, it is important that the propagation method interface with both sexual breeding programs and gene transfer methods employed with genetic engineering approaches to crop improvement.

In forest tree improvement programs, the traditional method of propagating improved genotypes for operational plantings is through seedlings. The advantages of this method are that the additive genetic gain is captured, and production from mature trees of tested superiority is highly efficient and inexpensive. Although there is variability among families, all genotypes within the select breeding population can be exploited at reasonable cost. However, sexual breeding does not allow capture of the non-additive genetic gain identified during progeny testing. Also, the long breeding times for tree species greatly slows the rate of genetic improvement. Another disadvantage is that the sexual breeding process can interface with genetic engineering only indirectly, after a select genotype has been produced using vegetative propagation methods.

Asexual, vegetative propagation methods have been explored for their usefulness with forest tree species. These methods include rooted cuttings, micropropagation (the induced growth of pre-existing, undeveloped buds), and various tissue culture methods (including adventitious shoot formation and somatic embryo formation). These methods have the advantage of capturing both the additive and non-additive genetic gain of select individuals. Also these methods can be used to propagate superior individuals produced via sexual breeding and are used to produce transgenic plants (plants carrying a newly inserted gene) created through genetic engineering. However, vegetative propagation methods have a number of disadvantages. Propagules, even those produced by rooted cuttings, are more expensive than seedlings. Vegetative propagation methods lose much of their efficiency when the source of material is from old, mature trees. In addition, an increasing number of studies with a wide variety of plants has shown that vegetative propagation efficiency is influenced by genotype. The forester's ability to move select genotypes to the field using vegetative propagation will be determined by the degree of genetic correlation of tissue culturability with economic traits of interest.

For clonal forestry to become reality and to be usefully integrated into long-term genetic improvement programs, vegetative propagation methods must be developed which can multiply all select genotypes inexpensively and with high efficiency. Therefore, it is timely to ask if the genotypic dependence of vegetative propagation will present a major obstacle to the development and long-term potential of clonal forestry.

THE NATURE OF THE CORRELATION

Crop Plants

The existence of genotypic effects on vegetative propagation is well documented in crop plants including common bean, rice, tobacco, tomato, sweet clover, alfalfa, potato, petunia, corn, wheat (Keyes et al., 1980, and literature cited therein) and sweet potato (Templeton-Somers and Collins, 1986). Genotypic effects have been observed using a variety of culture methods, including callus growth and shoot regeneration (Templeton-Somers and Collins, 1986; Mok and Mok, 1977), root formation (Keyes et al., 1980), anther culture (Phillips and Collins, 1977), and somatic embryogenesis (Chen et al., 1987; Brown and Atanassov, 1985). Observation in red clover (Keyes et al., 1980), alfalfa (Chen et al, 1987), corn (Tabata and Motoyoshi, 1965), and sweet potato (Templeton-Somers and Collins, 1986) showed statistically significant additive genetic effects for both tissue growth and differentiation. Such heritable variability indicates that the frequency of genotypes more responsive to tissue culture techniques can be raised by genetic selection.

Tree Species

The existence of a correlation between genotype and vegetative propagation has also been observed for tree species. Zobel et al (1983) have reported 64-92% rooting ability for cuttings from 25 select *Eucalyptus* clones at Aracruz, Brazil. Frampton (personal communication) has observed variation in rooting ability of loblolly pine cuttings. Similar variation has been seen with sweetgum (Cunningham, personal communication) and with *Eucalyptus* species in commercial propagation nurseries in South American (Kellison, personal communication). Variation at the species, family, and individual plant levels has been observed during the development of tissue culture methods as well.

At the species level, Bergmann (this meeting) reports that direct extrapolation of the highly efficient method developed for shoot regeneration in *Pinus radiata* does not work for several pine species, although good response was seen with Pinus eldarica. This is not surprising, as it is generally accepted that tissue culture methods need to be independently developed for each plant species of interest.

At the family level, Amerson (personal communication) has observed that shoot regeneration from loblolly pine cotyledons cultured on cytokinin-containing medium shows considerable family and seed-to-seed variation. Similar observations have been made by Aitken-Christie (personal communication) in Pious radiata. The Forestry Research Institute in New Zealand is currently working on methods to mechanize meristem culture to produce large numbers of plants at prices competitive with seedlings (Aitken-Christie et al, 1988). Genotypic variation already presents an obstacle to these efforts as only 40% of select genotypes will give meristematic cultures capable of the long-term culture necessary for mechanization (Aitken-Christie, personal communication). Similar family and seed-to-seed variation has been observed in research aimed at the development of somatic embryo culture in loblolly pine (Amerson, personal communication). Clock and Gregorius (1986) undertook a study specifically designed to determine if genotype or culture conditions more strongly influenced growth of birch callus in vitro. Their results showed that the variation in growth was more strongly determined by genotype than culturing environment.

The existence of genotypic effects on vegetative propagation is well documented, however the nature of the correlation is not. If a positive correlation exists between the ability to vegetatively propagate and traits of economic importance, positive selection for vegetative propagation is already occuring. Aitken-Christie (personal communication) has observed that several of the select families of P. radiata show high efficiency when tissue cultured. However, in vitro work with 23 select families of P. taeda showed shoot regeneration ranging between 10-60 shoots/embryo, with an average of 27.8 shoots/embryo (Amerson, personal communication). Rooting of tissue cultured shoots produced from the same families showed a similar variation. This data is consistent with the hypothesis that the ability to vegetatively propagate does not correlate with currently selected characteristics, such as tree form.

Data from physiological and tissue experiments suggests that the correlation between responsiveness to vegetative propagation and tree form may be negative. It has been observed in many species of plants that cultivars which have strong apical dominance and which do not sucker readily are often difficult to vegetatively propagate. This observation is so wide-spread that it has become a "rule-of-thumb" to nurserymen and tissue culturists. Yet, strong apical dominance is a highly desirable phenotype in commercial forestry. This raises the possibility that selection for strong apical dominance may, inadvertantly, select for poor responsiveness to vegetative propagation methods.

IMPLICATIONS FOR CLONAL FORESTRY AND GENETIC ENGINEERING

If clonal forestry is to be a useful tool in tree improvement programs, inexpensive mass propagation methods for all select genotypes need to be developed. Sexual breeding programs are continually creating new superior genotypes. If these individuals show random variation in their responsiveness to vegetative propagation methods, clonal propagation will continue to be sub-optimal, unless methods are continually fine-tuned for each new select genotype. The necessity to modify tissue culture methods for highest efficiency for each select genotype will greatly increase costs making it highly unlikely that vegetative propagules will be cost-competitive with seedlings. If a negative genetic correlation exists between desirable tree form and responsiveness to vegetative propagation methods, breeding for form will be breeding towards a technological bottleneck. The best trees will be increasingly difficult to propagate vegetatively. In the long-term, this could preclude the use of genetic engineering for modification of select genotypes from sexual breeding programs.

In addition, gene transfer methods currently in use and those envisaged for the foreseeable future, require tissue culture methods for creating transgenic plants expressing newly inserted genes. Ideally, a genetic engineering approach to tree improvement would utilize tissue from superior individuals in an ongoing breeding program. The efficiency at which these genotypes regenerate plants will determine, to a large extent, the expense of creating transgenic plants. These select transgenic plants must then be multiplied for use either as new individuals to be integrated into an ongoing breeding program, or as cloning stock for mass vegetative propagation for operational plantings. Both of these uses requires efficient tissue culture regeneration.

For sexual breeding and genetic engineering approaches to complement each other, they need to have efficient, technological access to the same base population of genetically select trees. The development of highly efficient, regenerable lines within existing tree breeding populations would enhance the usefulness of in *vitro* methods for clonal forestry and genetic engineering. Genetic studies should be undertaken to assess the nature and degree of genotypic effects on vegetative propagation. Exisiting select breeding populations could serve as the base for a nuclear breeding program whose goal is to create a population of trees with economically superior characteristics and high efficiencies of vegetative propagation. This population could serve as the common pool through which genetically engineered individuals could be moved into clonal forestry programs or into sexual breeding programs, and the best genotypes could be made accessible to improvement efforts through genetic engineering.

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