THE PRODUCTION OF HOMOZYGOUS TREE MATERIAL

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Homozygous trees will never be the desired ultimate step in a forest tree improvement program. However, they will serve many purposes in forest genetics research: (1) in the detection of genetic markers; (2) in the isolation of traits under simple genetic control for the study of growth and differentiation phenomena; (3) as a tool as well as reference material in the study of breeding mechanisms in natural populations; (4) as reference material in a study of the efficiency of different selection schemes; and (5) in the study of sex control mechanisms in dioecious species. Furthermore, homozygous trees could serve as an intermediate step for the production of heterotic intra- and interspecific hybrids.

The inbreeding approach to the production of homozygotes is inefficient in trees. An alternate approach is the induction of haploid parthenogensis followed by colchicine duplication of the haploid chromosome set. In fact, recent evidence by Muntzing (1963) indicates that this approach may be the only way to produce true homozygosis in an entire genome. He found segregation at a conspicuous locus in rye still after 30 generations of selfing and suggested that heterozygosity may have been preserved in many additional, more concealed traits.

The first successful attempt to apply haploid induction to forest-tree material was reported by Kopecky (1960), who recovered 11 haploid plants after pollinating *Populus alba* L. with pollen from *P. tremula L.* and *P. nigra* L. Meanwhile, several researchers have indicated they are engaged in studies of this kind, and we can expect additional information on the subject.[2]

Choice of Organism

For a number of reasons, *Populus trichocarpa* Torr. & Gray, the native black cottonwood, seemed best suited as experimental material for our studies. The genus *Populus,* a member of the *Salicaceae,* has a uniform chromosome number of 2n = 38. Even though this number indicates that polyploidy may have played a role in the past evolution of the genus, chromosome behavior suggests that the present-day material can be considered diploid (Dillewijn 1940). More gain in efficiency

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can be expected from applying the haploid method to a diploid than to a polyploid organism.

All poplars, with the exception of P. *lasiocarpa,* Oliv. are dioecious wind pollinators. However, occasional hermaphroditic individuals have been reported in several poplar species and interspecific hybrids (reviewed by Lester 1963). A higher degree of heterozygosity is to be expected in natural populations of dioecious than of monoecious organisms. Artificially produced homozygotes in a dioecious organism would allow a wider range of comparison regarding the relative effects of homo- vs. heterozygosity. Furthermore, the production of homozygous stock in poplars may give insight into the genetic control of sex expression in this genus. It is realized that this advantage may be offset by a correlated disadvantage of imbalanced sex distribution among artificially produced homozygotes.

The mechanics of poplar breeding are simple. Flowering branches can be removed from the trees and kept in water culture in the greenhouse from pollination to seed harvest. This makes it possible to keep large numbers of potential embryos under experimental conditions in a relatively small area: approximately a half-million ovules can be grown in an area of 150 square feet. The period from pollination to mature seed is short enough to keep environmental variability at a minimum. Once the seed is harvested it germinates readily (no dormancy), thus allowing early scoring of progenies. Dormant branches can be induced to flower as early as January; alternatively they can be kept dormant in cold storage until May. Both methods help to extend the period during which haploid induction experiments can be conducted.

Many poplars, including P. *trichocarpa,* are ideally suited for cloning. Once haploids have been recovered, they can be propagated vegetatively, thus increasing the probability that some of them will be diploidized successfully. Black cottonwood occurs both in small, isolated, as well as in large, continuous populations covering a wide range of distribution. Much genetic diversity can be expected that may have a bearing on its response to haploid induction.

Lastly, *Populus* is one of the few tree genera in which haploids have been reported (Kopecky 1960; Tralau 1957). If we are to gain insight into those aspects of reproductive physiology that are associated with haploid parthenogenesis, it seems advantageous to study an organism in which the phenomenon can be expected to occur.

Experimental Considerations

While our long-term program aims at the mass production of haploids for subsequent establishment of homozygous stock, our immediate objective is to develop a repeatable technique of stimulating haploid parthenogenesis in female tissue of black cottonwood. This technique may ultimately consist of using pollen of a particular poplar species on black cottonwood; it may possibly involve the use of irradiated pollen, chemical agents, environmental shocks, or any combination thereof. Regardless of the specific nature of the ultimate technique, it is safe to predict that it will be more successful in certain genotypes than in others (Chase 1952).

Since the female tissue offers more diversity for experimental manipulation than the male, a screening for responsive genotypes seems more justified in the female than in the male sex. The first step in the experimental procedure should therefore be a screening for responsive females with the aid of the most generally successful induction method. Once responsive females have been singled out, a variety of induction methods could be tested on them.

Since the majority of experimentally induced haploids in angiosperms, including those in Popu*lus alba,* have been recovered after hybridization (reviewed in Kimber and Riley 1963), it was decided to screen for responsive females in P. *chocarpa* after they had been exposed to pollen of a number of other poplar species (*P. nigra, P. deltoides* Bartr., P. *canescens* (Ait.) Sm., P. *grandidentata* Michx., and P. *tremuloides* Michx.).

For lack of information it is assumed that the probability of recovering haploid seedlings following hybridization in non-selected female genotypes of P. *trichocarpa* is of the same order of magnitude as in other angiosperms, i.e. approximately 0.1 percent of the number of ovules involved (Kimber and Riley 1963). In the absence of known seedling markers, it is further assumed that 50 percent of the viable haploids are not recognized. On the assumption that the frequency of ovules giving rise to observable haploid seedlings follows a Poisson, distribution, it can be calculated that it takes 6,000 ovules to detect one or more haploids with 0.95 probability (Burington and May 1953). This figure serves as the basis for calculating the required number of branches per genotype to be tested with each pollen species.

Last, it seems reasonable to suspect that the past breeding history of a population will be reflected in the response by its members to haploid induction. *A priori,* one would expect a higher probability of haploid survival in small, isolated populations than in large, continuous populations. A comparison of females derived from these two extreme conditions would therefore be desirable.

Pilot Studies

Pilot studies were required to familiarize ourselves with culture and breeding techniques and to solve some physiological problems associated with our induction method. One phenomenon, in particular, posed a serious problem, namely, the

premature abscission of female inflorescences after treatment with either no pollen or foreign species pollen, especially in the case of wide crosses.

In 1964 we carried out experiments to study the effects of IAA treatment in preventing premature inflorescence abscission. Branches from three different female trees were treated one day after pollination with different concentrations of IAA in two different media (water, lanolin). While the auxin treatment successfully delayed inflorescence abscission in one genotype, it did not allow the inflorescences to reach mature-seed stage. No effects were observed in the other two genotypes.

In 1965 we tried another approach by making use of observations reported by Brewbaker (1962), Stairs and Mergen (1964), and a number of other researchers, that genetically inactive pollen may still have sufficient physiological activity to perform some stimulatory function. Their observations had been based on the study of pollen having received high doses of acute ionizing radiation.

For this purpose, we exposed P. *trichocarpa* pollen to a Co^{60} source for a dose of 100,000 r. This pollen was then tested as *agens stimulans (a)* singly, and in combination with pollen of (b) P. *trichocarpa, (c) P. deltoides,* and (d) *P. canescens*. Parameters studied included the percentage of inflorescences reaching mature-seed stage; the number and size of ovules as well as the number of embryos per pistil at different stages during development; nuclear size and chromosome number in embryos and seedlings. The results have been very encouraging and can be summarized as follows:

1. Irradiated pollen, singly or in combination, increased the percentage of inflorescence reaching maturity. In fact, 48 percent of the inflorescences treated with only irradiated pollen were retained to the mature stage.

2. Irradiated pollen stimulated the growth of pistils and of many of the ovules contained therein.

3. In mixture with normal P. *trichocarpa* pollen, the irradiated pollen was sufficiently competitive to significantly decrease the number of diploid embryos per pistil. This suggests that the tubes of the irradiated pollen successfully grew to many of the receptive ovules and prevented them from being fertilized by normal pollen.

4. No mature haploid embryos were found among the ovules treated with irradiated pollen only. This supports the findings of Ehrensberger (1948) that the survival of haploids in angiosperms depends on the presence of a functional endosperm.

At the University of Washington, studies are in progress to further investigate the behavior of irradiated poplar pollen, singly and in mixture with normal or foreign species pollen, to better understand its physiology. This will help us to evaluate its usefulness not only as a tool in our haploid induction program but also as a tool in intra- and interspecific hybridization.

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