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An attempt at generating haploid lines of *Poplar* species for genetic manipulation and breeding programs

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Introduction

The successful establishment of haploid-derived strains in herbaceous crop plants has proven that this procedure is both viable and extremely desirable. For example, over 80 new rice varieties have been established *via* this procedure (Siva Reddy *et al.,* 1985) and 20 000 ha of new tobacco varieties have been planted (Hu *et al.,* 1978). Production and use of haploids for tree breeding and biotechnological manipulation can be equally productive, yet only a relatively few attempts have been made to establish these lines (Wang *et al.,* 1975; Chen *et al.,* 1979; Zhu *et al.,* 1980; Karnosky *et al.,* 1981; Ho and Raj 1985; Hyun *et al.,* 1986; and, for oak and horsechestnut, Jorgensen, personal communication). The use of haploids has special significance for genetic improvement in forest trees and other woody species where traditional breeding procedures and genetic manipulation are more difficult owing to: 1) long generation times typical of many tree species; 2) high heterozygosity; 3) factors, such as parthenocarpy and self incompatibility, which are common; and 4) poor embryo viability which often occurs. The main objective is, therefore, to establish haploid cell lines of selected *Poplar* clones from anthers which will then be regenerated into shoots and roots for further manipulation *via* microculture procedures. Additional use of these tissues in protoplast fusion work will enable construction of new forest trees.

Materials and Methods

Poplar species were chosen as a model for the following reasons: 1) an extensive record of clonal lines exists with growth patterns and characteristics known for such genotypes; 2) availability of these clones as mature breeding stock; 3) some knowledge of poplar chromosome morphology; 4) viable procedures for poplar organogenesis and regeneration (Wolter, 1968; Russel and McCown, 1988); 5) transcription of desirable genes into poplar and increased recovery of transformed poplar shoots (Fillatti *et al.,* 1987).

Attempts to obtain haploid tissues were made from the following clones: 1) Eugenei (NC 5326, *P. deltoides* x *P. nigra*); 2) Androscoggin (NC11390, *P. maximowiczii* x *P. trichocarpa*); 3) Crandon (NC 5339) *P. alba* x *P. grandidentata;* 4) Wisconsin wild selection (Wis. W-5). Catkins isolated during dormancy were stored at −18°C (for a maximum of 3 mo), sterilized and allowed to elongate under ambient conditions. The scheme of Bajaj (1983) was followed with attempts at both pollen and anther cultures. Isolates were placed on different

Table I.

Breeding	*Biotechnology*
a. Recessive alleles expressed in one generation	a. Somaclonal hybridization
b. Homozygous diploids in one generation	b. Establish gene maps
c. Reduction of polyploid species	c. Somatic hybrids through fusion
d. Acceleration of superior homozygous lines for self pollination and crosses	d. Paternal cytoplasmic inheritance
	e. Detect haploid-associated metabolic pathways

media (Wolter and Skoog, 1966; Lloyd and McCown, 1980) to initiate viable cultures as well as differentiation medium (Russel and McCown, 1986). Ploidy levels were monitored microscopically using a modified 8-hydroxyquinone/acetocarmine procedure of Somego (1978).

Results

Successful viable isolates were established for all tested *Poplar* clones from anther microspores on the 2,4-D (2,4-dichlorophenoxy-acetic acid; 0.04 mg/l) medium of Wolter and Skoog (1966). Root differentiation was rapidly established for Eugenei with NAA (naphthalene acetic acid; 2 mg/l); shoot organogenesis was not achieved with this clone, though numerous levels of cytokinins were tested. The Crandon isolates were viable and callus cultures were established, but organ differentiation was not obtained. Wisconsin W-5 was the most amenable for the production of shoots on a medium supplemented with 0.01 μM *N*-phenyl-*N*-1,2,3-thiadiazol-5-ylurea (thiodiazuron). In all cases of successful shoot differentiation, ploidy levels were diploid. To date, differentiated roots have not been analyzed. Analysis of ploidy levels has been the largest technical problem of the investigation. *Poplar* species have the smallest amount of DNA of most tree species (7 pg/cell) thereby making monitoring extremely difficult.

Discussion and Conclusion

The conclusions from the results obtained to date are that production and maintenance of haploid tissue lines requires constant monitoring for ploidy levels. A rapid establishment of stable cultures (preferably shoot microculture) so as to avoid increasing ploidy levels through unstable callus subcultures is essential. If these objectives are maintained and haploid material is stabilized, the manipulations enumerated in Table I are possible (Bonga *et al.*, 1987).

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