POPLAR PLANTS THROUGH ANTHER CULTURE

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Abstract.-- Twenty-three clones of several poplar species and interspecific hybrids were subjected to anther culture for production of haploids in 1981. The calli were induced on MS basal medium supplemented with 1 mg/1 kinetin + 2 mg/1 2,4-D. Leafy structure was regenerated from calli in P. angulata x P. simonii, and (P. trichocarpa x P. angulata) x (P. balsamifera x P. deltoides) on the basal medium plus 1 mg/1 BAP and 0.2 mg/1 NAA while roots were produced from those in P. balsamifera x P. deltoides, P. x euramericana cv. eugenei and P. deltoides. P. maximowiczii x P. deltoides was the only clone whose calli produced adventitious shoots. The shoots were rooted in the basal medium plus 0.02 mg/1 NAA in 10 days.

In 1982, 12 clones representing a variety of poplar species and interspecific hybrids were tested on 2 different kinds of cytokinins in combination with two different kinds of auxins to evaluate their efficiencies on callus induction. All but one responded to the treatments, but Leuce poplars were less responsive than Aigeiros and Tacamahaca poplars.

Haploid plants are sporophytes with gametophytic chromosome numbers which is half of the normal diploid complement. Normally the gametophyte in higher plants is nursed by the sporophyte but it can be completely autonomous. This ability of autonomy could be used under artificial environmental conditions to induce haploid plants.

Haploids were first successfully induced from pollen through anther culture in Datura (Guha and Maheshwari 1964). The investigations were soon extended to many crop and tree species. To date, anther and pollen cultures have been successful in 53 genera (Maheshwari et al. 1980, Huhtinen 1978) including the following genera in tree species: Populus (Wang et al. 1975, Zhu et al. 1980), Aesculus (Radojevic 1978), Betula (Huhtinen 1978), and Hevea (Chen et al. 1979).

Sommer and Brown (1979) stated that production of haploids presented manifold advantages in tree improvement and the immediate value is the diploidization of the haploids to produce isogenic pure lines for breeding and isolation of genetic variants. The procedures also contribute to genetic, mutational, physiological, and biochemical studies based upon haploid genetic constitution.

In the present study, research on haploid induction from pollen through anther culture is described in several poplar (Populus) species and interspecific hybrids.

Materials and Methods

Poplar species and interspecific hybrids, and their clone number used in the study are listed in Table I. In 1981, 23 clones were investigated for the potential of callus formation and production of haploids. In 1982, 12 clones representing 6 species and 6 hybrids were selected for the study. The trees were growing at the Maple, Ontario poplar arboreta and their ages ranged from 17 to 25 years.

Branches bearing catkin buds were collected in the spring and forced to flower by water-culturing in a laboratory at room temperature. Development of the catkins was examined daily with the acetocarmine staining technique to determine the stage of microsporogenesis.

Catkins were sampled when pollen reached the microspore (mononucleate) stage prior to mitosis. Discs bearing stamens were removed from the catkins and surface-sterilized with 70% alcohol for a few seconds followed by 10% Javex bleach for 10 minutes. They were rinsed with sterile double-distilled water 3 to 4 times and individual anthers were then incubated on culture media.

The MS basal medium (Murashige and Skoog 1962) was used and supplemented with different kinds of plant growth regulators. The callus-inducing medium contained the basal medium with 2 mg/1 of 2,4-D (2,4-dichlorophenoxyacetic acid) and 1 mg/1 of kinetin and the organ-inducing medium with 1 mg/1 of BAP (benzylaminopurine) and 0.2 mg/1 of NAA (naphthaleneacetic acid). The rooting medium included the basal medium and 0.02 mg/1 of NAA but the macronutrients were one half of the original concentration and sucrose was lowered from 3% to 2%. In addition, different combinations of cytokinins and auxins were tested for the induction frequency in 6 clones each of species and hybrids in 1982 and the combinations were 1) MS+1 mg/1 BAP+2 mg/1 2,4-D; 2) MS+ 1 mg/1 BAP+1 mg/1 NAA; 3) MS+1 mg/1 kinetin+2 mg/1 2,4-D, and 4) MS+1 mg/1 kinetin+1 mg/1 NAA. All the media were autoclaved at 1 kg cm^{-2} and 120 C for 15 minutes.

The anther 'cultures were kept in an incubator at 25 C in darkness. When calli were formed in the anther cavities, they were transferred to the organ-inducing medium and incubated in an incubator at 25 C during the day and 15 C at night with a 16-hour photoperiod and an irradiance of about 60 µ m^{-2} sec^{-1}. The adventitious shoots were planted in a rooting medium when they were about 3 cm in height.

The acetocarmine staining technique was used to determine the ploidy level of the plantlets after young leaves and root tips were hydrolyzed in 1N HCL for 12 minutes.

Results and Discussion

Anthers began to swell after a week of incubation and most of the anthers lost their red pigment and turned brown in color. After one more month's incubation, many calli were regenerated from anthers of all but 5 species and hybrids studied (Table II). However, most of the calli were derived from the connective tissue, anther wall and filament, and formed amorphous calli which were light brown or white

in color. The calli remained amorphous without any organogenesis throughout the culture period and were discarded. A similar observation was reported by Zhu et al. (1980) for many poplar species.

Some calli were evolved from the anther cavities where the abscission layers had split open. The calli were greenish or yellow in color and they reached about 5 mm in diameter in about 50 days from the anther incubation. These were the calli that were selected and transferred onto the organ-inducing medium.

The calli did not regenerate any adventitious shoots or roots until about one and a half months after the transfer. Root formation was observed in P. balsamifera x P. deltoides, P. euramericana cv. eugenei and P. deltoides while leafy structure was produced in P. angulata x P. simonii, and (P. trichocarpa x P. angulata) x (P. balsamifera x P. deltoides) (Table II). P. maximowiczii x P. deltoides was the only clone whose calli produced adventitious shoots.

Many adventitious shoots of this latter hybrid were regenerated from each callus and only a few reached 3 cm in height. The adventitious shoots were excised from the calli and planted in the rooting medium. They were rooted in 10 days and the roots were short and thick.

Many young adventitious shoots in P. maximowiczii x P. deltoides were sampled for karyotype analysis. The calli were found to be derived from pollen since most shoots from a callus had 19 chromosomes but others varied from 17 to 24 chromosomes. The deviation from the 19 haploid chromosomes may have resulted from the chromosomal non-disjunction in mitosis during the process of primordium initiation of the adventitious shoots. Lester and Berbee (1977) also reported the instability of chromosome numbers in roots of the poplar plants derived by tissue culture and the reduced growth vigor of some plants. However, the technique provides new options for obtaining increased genetic variability and establishing new variants for breeding (Larkin and Scowcroft 1981).

The results in callus formation from 4 different combinations of growth regulators are shown in Table III. All but one (P. alba) responded to the treatments and Leuce poplars were not as responsive as Aigeiros and Tacamahaca poplars. It appeared that different clones responded differently to the different combinations of cytokinins and auxins. Anthers of P. deltoides or its interspecific hybrids responded best to the medium containing kinetin along with either 2,4-D or NAA.

The results in this tudy as well as success in many other tree species and hybrids (Radojevic 1978, Huhtinen 1978, Chen et al. 1979, Zhu et al. 1980) indicated the potential of haploid plant induction through anther culture in woody species. The technique could pave the way for the fast production of isogenic pure lines which otherwise would require many generations of inbreeding or selfing.

TABLE I. List of clone number, species and their origin, used in the study.

CLONE	SPECIES	ORIGIN	YEAR USED 1981	1982
A57	P. alba	Germany		x
AG41	P. alba x P. grandidentata	Ontario, Canada	x	
AT12	P. alba x P. tremuloides	Ontario, Canada	x	
G8	P. grandidentata	Virginia, USA		x
E42	P. tremula	Scotland	x	
E155	P. tremula	Czechoslovakia		x
ET38	P. tremula x P. tremuloides	Finland x Ontario, Canada		x
T83	P. tremuloides	Ontario, Canada	x	
T84	P. tremuloides	W. Virginia, USA	x	x
TE67	P. tremuloides x P. tremula	Ont. x Czechoslovakia	x	
TE83	P. tremuloides x P. tremula	Ont. x Finland	x	
TE84	P. tremuloides x P. tremula	Ont. x Finland	x	x
TE86	P. tremuloides x P. tremula	Ont. x Finland	x	
Bacheleri	P. angulata x P. nigra	Oxford, England	x	
ANSI	P. angulata x P. simonii	Ontario, Canada	x	x
Jackii-1	P. balsamifera x P. deltoides	Massachusetts, USA	x	
D8	P. deltoides	Wisconsin, USA	x	x
D9	P. deltoides	Wisconsin, USA	x	
Carolina 1	P. x euramericana cv. eugenei	Ontario, Canada	x	x
V47	P. x euramericana cv. robusta	Michigan, USA	x	
Verni-rubens	P. x euramerican cv. vernirubens	Kew Garden, England	x	
MD1	P. maximowiczii x P. deltoides	Ontario, Canada	x	
OP45	P. maximowiczii x (P. laurifolia x P. nigra)	Ontario, Canada	x	
N166	P. nigra	Hungary	x	x
N167	P. nigra	Hungary	x	
3B23	(P. trichocarpa x P. angulata) x (P. balsamifera x P. deltoides)	K. Sax, USA	x	
1E33	(P. trichocarpa x P. angulata) x (P. balsamifera x P. deltoides)	K. Sax, USA		

TABLE II. Percentage of callus formation and organogenesis in different Populus species and hybrids in 1981.

CLONE #	SPECIES	% CALLUS FORMATION	% CALLI GREW ON ORGAN-INDUCING MEDIUM	% IN ORGANO-GENESIS
AG41	P. alba x P. grandidentata	36.1	-	-
AT12	P. alba x P. tremuloides	-	-	-
E42	P. tremula	-		-
T83	P. tremuloides	-	-	-
T84	P. tremuloides	21.3	3.0	-
TE67	P. tremuloides x P. tremula	-	-	-
TE83	P. tremuloides x P. tremula	4.2	-	-
TE86	P. tremuloides x P. tremula	9.1	-	-
ANSI	P. angulata x P. simonii	2.9	0.2	1% (leafy structure
Bacheleri	P. angulata x P. nigra	5.8	1.8	-
JACKII	P. balsamifera x P. deltoides	17.5	3.6	2% (roots)
D8	P. deltoides	15.5	7.3	2.6% (roots)
D9	P. deltoides	1.1	-	-
Carolina 1	P. x euramericana cv. eugenei	17.5	5.3	3% (roots)
V47	P. x euramericana cv. robusta	-	-	-
Verni-rubens	P. x euramericana cv. vernirubens	32.2	-	-
MD1	P. maximowiczii x P. deltoides	24.7	1.0	1% (shoots)
OP45	P. maximowiczii x (P.laurifolia x P. nigra)	-	-	-
N166	P. nigra	15.3	0.5	-
3B23	(P. trichocarpa x P. angulata) x (P. balsamifera x P. deltoides)	14.3	1.8	-
1E33	(P. trichocarpa angulata) x (P. balsamifera x P. deltoides)	18.7	1.8	1.5% (leafy structure)

TABLE III. Effect of different cytokinins and auxins on callus formation in Populus species and hybrids. 2000 anthers were incubated for each clone.

Species	MS Basal Medium and BAP+2,4D*	BAP+NAA*	K+2,4D*	K+NAA*
P. alba	-	-	-	
P. grandidentata	12	27	12	1
P. tremula	36	24	21	35
P. tremula x P. tremuloides	44	23	64	74
P. tremuloides	26	44	45	78
P. tremuloides x P. tremula	3	3	2	2
P. angulata x P. simonii	171	114	130	189
P. balsamifera x P. deltoides	286	181	340	400
P. deltoides	419	393	483	399
P. x euramericana cv. eugenei	184	215	437	458
P. nigra	129	139	131	134
(P. trichocarpa x P. angulata) x (P. balsamifera x P. deltoides)	415	301	385	344
Total Calli	1725	1464	2050	2114

*BAP = 1 mg/1
2,4-D = 2 mg/1
kinetin = 1 mg/1
NAA = 1 mg/1

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