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Allozyme Variation and Inheritance in Leaves of *Populus deltoides*, *P. nigra*, *P. maximowiczii* and *P. x canadensis* in Comparison to Those in Root Tips

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Abstract

Methods for extraction and electrophoresis of leaf tissue enzymes in *Populus deltoides* MARSH., *P. nigra* L., *P. maximowiczii* HENRY, and *P. x canadensis* MOENCH are described. Eleven enzymes were assayed in 54 individual clones of these poplars, and in progeny and parents of seven controlled crosses of *P. deltoides* with *P. deltoides*, *P. nigra*, and *P. maximowiczii*. 10 of these enzymes were analysed earlier in root tips of the same individuals. In addition, colorimetric esterase was analysed. A total of 33 genes coding for 11 enzyme systems were observed. 87% to 91% of the genes expressed in leaf tissue of the 3 *Populus* species and one interspecific hybrid were also expressed in root tips. Allozymes of 26 loci expressed identically in leaves and root tips. Four loci, *Per-1*, *Per-2*, *Per-3*, and *Sdh-2* that expressed in root tips of the *Populus* species, however were not detected in leaf tissue. In addition, 2 peroxidase loci *Per-L1*, and *Per-L2*, were detected only in leaves. Single-gene control was observed for isozyme variants of each of the 4 enzyme zones (*CE-1*, *CE-2*, *PER-L1*, *PER-L2*) investigated or detected only in leaves. Allozymes in one species were allelic to allozymes in other species at 16 loci. *Populus deltoides*, *P. nigra*, and *P. maximowiczii* could be differentiated by isozymes of *Ce-1* in leaves.

Key words: Poplars, enzyme electrophoresis, clones, clone genotypes, gene expression, hybrids.

Introduction

Populus deltoides MARSH. (section *Aigeiros* DUBY), *P. nigra* L. (section *Aigeiros*), *P. maximowiczii* HENRY (section *Tamahaca* SPACH.) and their interspecific hybrids are important for poplar breeding and intensive plantation programs (ZSUFFA, 1976; ANONYMOUS, 1979; DICKMANN and STUART, 1983). *Populus deltoides* x *P. nigra* hybrids are named as *P. x canadensis* MOENCH syn. *P. x euramericana* (DODE) GUINIER. Allozymes and their multilocus genotypes are useful for various genetic, breeding and phylogenetic studies in these *Populus* species (RAJORA, 1986, 1988, 1989a and b, 1990a; RAJORA and ZSUFFA, 1986, 1989, 1990).

Methods of enzyme electrophoresis, allozyme genotypes of clones, inheritance and linkage of allozymes, and diagnostic (species-specific) allozyme genes and alleles in root tips of *P. deltoides*, *P. nigra*, *P. maximowiczii*, and *P. x canadensis* have been described previously (RAJORA, 1988, 1989a and b, 1990a and b; RAJORA and ZSUFFA, 1989). Leaves

are the most abundant and easily available tissues for electrophoretic analysis, thus, it is highly desirable to develop methods of enzyme extraction and electrophoresis in leaf tissue. In poplars, such methods have been developed for leaf tissues of *P. tremuloides* MICHX. (CHELIAK and PITEL, 1984), and *P. balsamifera* L. (FARMER et al., 1988).

We undertook the present investigation to develop methods of enzyme extraction, electrophoresis and detection in leaf tissue of *P. deltoides*, *P. nigra*, *P. maximowiczii*, and *P. x canadensis*. We examined (i) enzyme banding patterns and allozyme coding genes, (ii) allozyme genotypes of the clones, (iii) diagnostic loci and alleles for species and their interspecific hybrids, and (iv) isozyme inheritance. The results of these investigations are compared with the results earlier obtained for root tips.

Materials and Methods

Populus Species and Clones

54 individuals of *P. deltoides*, *P. nigra*, *P. x canadensis*, and *P. maximowiczii* were studied: 16 individuals of *P. deltoides* representing var. *deltoides* and var. *occidentalis* (RAJORA, 1989a); 13 individuals of *P. nigra* representing var. *nigra*, var. *italica*, var. *plantierensis*, cv. Vereecken, and cv. Ichenheim (RAJORA, 1989b); 17 individuals of *P. x canadensis* each representing a different cultivar (RAJORA and ZSUFFA, 1989); and 8 individuals of *P. maximowiczii* (RAJORA, 1988). Except for *P. x canadensis* cv. I-214, all of these individuals were picked from among the individuals whose allozyme genotypes and phenotypes were determined earlier in root tips (RAJORA, 1988, 1989a and b; RAJORA and ZSUFFA, 1989).

Controlled Crosses

10 progeny of each of the 2 intraspecific *P. deltoides* crosses, 3 *P. deltoides* x *P. nigra* crosses, and 2 *P. deltoides* x *P. maximowiczii* crosses were studied. These crosses were made in 1983 (RAJORA, 1986, 1990b), and the sampled progenies were established in a field-progeny test in Ontario. Root-tip allozyme genotypes of these offspring were obtained previously. Inheritance and linkage of allozymes were studied earlier in root tips of much larger numbers of progeny (40 to 104 per cross, with a total of 807 for 12 crosses) of these and 5 other controlled crosses (RAJORA, 1990b).

Tissue Preparation and Enzyme Extraction

Tissues of very young expanding leaves from the sprouts of rooted cuttings were used for enzyme electrophoresis.

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Dormant shoot cuttings were collected from each individual tree in the last week of March, 1989, and were rooted in April, 1989, in a greenhouse. Leaf tissue samples for electrophoresis were collected in late May and the first week of June, 1989 on the same day electrophoretic analysis was conducted. For one sample, 1 or 2 emerging leaves were collected, cleaned, and placed in an extraction buffer (0.1 M Tris, 0.2% ascorbic acid w/v, 1% tween, 0.2% of magnesium chloride w/v, 0.2% calcium chloride w/v, 17.1% sucrose w/v, and 0.03% 2-mercaptoethanol, pH 7.5).

A crude enzyme extract was prepared by grinding the leaf tissue in the extraction buffer manually with the help of mortar and pestle. Filter paper wicks were soaked in the homogenate to absorb the enzyme extract.

Enzyme Electrophoresis and Detection

11 enzyme systems were assayed by horizontal starch gel electrophoresis using 5 buffer systems (Table 1). Except for colorimetric esterase and the lithium-borate buffer system, all 10 enzyme systems and 4 buffer systems were the same as used earlier on root tips (RAJORA, 1988, 1989a and b; RAJORA and ZSUFFA, 1989). Other enzyme electrophoretic techniques were also essentially the same as described earlier for root tips (RAJORA, 1989a and b; RAJORA and ZSUFFA, 1989), with the following two exceptions: starch gels, 12.5% w/v, were prepared from only Connaught starch (Connaught Lab., North York, Ontario, Canada), and a different type of gel apparatus (trays) was used.

Allozyme Genes, Genotypes and Inheritance

The conventions for designating gene loci and their alleles, and genotypes of the trees were the same as de-

scribed in RAJORA (1986, 1989a and b, 1990a), and RAJORA and ZSUSFA (1989). For enzymes encoded by multiple loci the numbering of loci (in numerals; PER loci expressed only in leaves were prefixed by L) and alleles (in letters) within an enzyme system progressed sequentially in the cathodal direction.

Inheritance of isozymes was tested by examining offspring genotypes and segregation of isozymes in offspring relative to putative genotypes of parents (RAJORA, 1990b).

Results

Zymograms, Allozyme Genes, Genotypes, and Inheritance

(i) Enzymes with identical zymograms in root tips and leaves

Zymograms were identical in all clones and progeny sampled for 8 of the 10 enzyme systems examined in both tissues (ACO, GOT, IDH, LAP, MDH, 6-PGD, PGM, and PGI). Thus, the same 25 loci coding for these enzymes in root tips (RAJORA, 1990b) are expressed in leaf tissue (Table 1), and genotypes of the 54 clones and 70 progeny of the controlled crosses at these 25 gene loci are identical in both tissues. SDH had only one monomorphic zone of activity, *SDH-1*, in leaves corresponding to *SDH-1* in roots of these *Populus* species, whereas this enzyme had two zones (*SDH-1*, and *SDH-2*) in root tips. Thus, 26 gene loci appear to be expressed in the same way in both leaf and root tip tissues. However, not all of these 26 enzyme loci expressed in all three *Populus* species (Table 1; RAJORA, 1990a, and b).

(ii) Enzymes with different expression

Peroxidase (PER): 3 PER zones (*PER-L1*, *PER-L2*, and *PER-4*) were observed in leaves of all 3 *Populus* species

Table 1. — Enzyme systems assayed and number of loci scored in leaves of *Populus* species and hybrids. PD = *P. deltoides*, PN = *P. nigra*, PM = *P. maximowiczii*, and PC = *P. x canadensis*.

Enzyme	Abbreviation	E.C. No.	Buffer system ¹	Number of loci scored			
				PD	PN	PM	PC
Aconitase	ACO	4.2.1.3	A	2	2	2	2
Colorimetric Esterase	CE	3.1.1.1	E	2	2	2	2
Glutamate oxaloacetate transaminase	GOT	2.6.1.1	D	4	4	3	4
Isocitrate dehydrogenase	IDH	1.1.1.42	A	3	1	1	3
Leucine amino peptidase	LAP	3.4.11.1	D	2	1	1	2
Malate dehydrogenase	MDH	1.1.1.37	A	5	6	5	6
Peroxidase	PER	1.11.1.7	B,E	3	3	3	3
Phosphoglucomutase	PGM	2.7.5.1	C	3	2	3	3
6-Phosphogluconate dehydrogenase	6-PGD	1.1.1.44	A	4	5	5	5
Phosphoglucose isomerase	PGI	5.3.1.9	B	2	2	2	2
Shikimate dehydrogenase	SDH	1.1.1.25	C	1	1	1	1

¹ Buffer systems A (citric acid-morpholine, pH 6.7), B (citric acid-morpholine, pH 7.7), C (Tris-citrate, pH 6.2), and D (lithium-borate pH 8.1 and Tris-citrate pH 8.3) are described in RAJORA (1989a, 1989b) and RAJORA and ZSUFFA (1989); E — lithium-borate, pH 8.1 (RIDGEWAY *et al.*, 1970).

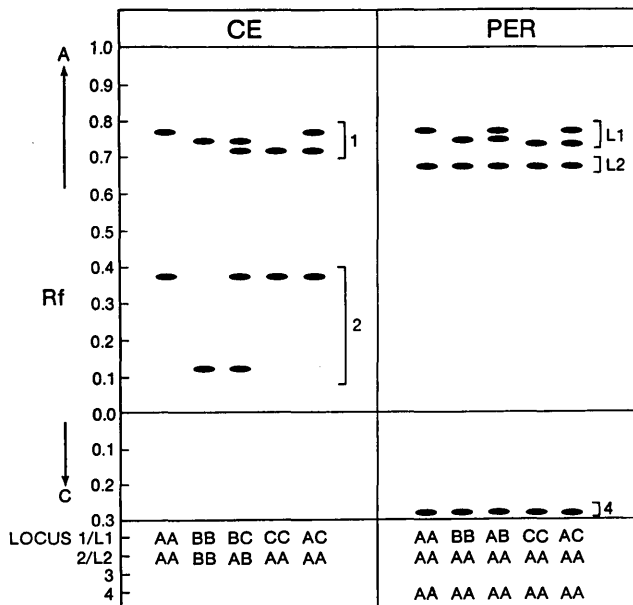


Figure 1. — Schematic representation of the allozyme phenotypes, and genotypic and allelic designations of the *Populus* individuals and progeny for loci coding for CE and PER in leaves. The loci are labelled on the right side. A indicates the anodal direction and C the cathodal direction from the origin. Rf indicates the relative migration.

and *P. x canadensis* (Fig. 1), while 4 PER zones (PER-1, PER-2, PER-3, and PER-4) were observed in root tips (RAJORA, 1988, 1989a and b; RAJORA and ZSUFFA, 1989). Only one of these zones (PER-4) was apparently the same in the 2 tissues. The 2 anodal PER zones observed in leaves migrate faster than isozymes of PER-1 and PER-2 observed in roots, and thus are designated as PER-L1 and PER-L2.

3 alleles were detected at *Per-L1*. Homozygotes showed a single-banded isozyme phenotype, and heterozygotes a double-banded phenotype at this locus. At *Per-L1*, progeny of controlled crosses between parents homozygous for the same allele were also homozygous for the same allele, progeny of crosses between parents homozygous for 2 different alleles were heterozygous for the parental alleles, and progeny of crosses between homozygous and hetero-

zygous parents segregated into 2 parental classes (Fig. 1), as expected. However, offspring sample sizes were too small to statistically test for Mendelian segregation. All individuals were monomorphic for a single-banded phenotype at *Per-L2*.

(iii) Enzymes assayed only in leaves

Colorimetric Esterase (CE): There were two zones of CE activity, CE-1 and CE-2 (Fig. 1). Each of these zones was inferred to be controlled by a single gene in the three species and figure 1 is based on our hypothesized model. CE-1 was single-banded and monomorphic among individuals within each *Populus* species but the single band had a different relative mobility in each (Fig. 1). *Populus deltoides* clones were inferred to be homozygous for allele C, *P. nigra* for allele B, and *P. maximowiczii* for allele A at this locus, consistent with genetic models tested for similar observations at other loci in root tips (RAJORA, 1990b) and leaves of these species. In agreement with the above hypothesis, all *P. x canadensis* clones were double-banded and were inferred to be heterozygotes for alleles B and C, all progeny of the 2 intraspecific *P. deltoides* crosses were single-banded homozygotes for allele C, all progeny of the 3 *P. deltoides* x *P. nigra* crosses were double-banded heterozygotes for alleles B and C, and all progenies of *P. deltoides* x *P. maximowiczii* crosses were double-banded heterozygotes for alleles A and C (Fig. 1).

CE-2 was also single-banded and monomorphic among individuals within all 3 species (Fig. 1). The single band occurred at the same position in *P. deltoides* and *P. maximowiczii*, but at a different position in *P. nigra*. Thus *P. deltoides* and *P. maximowiczii* clones were inferred to be homozygous for allele A and *P. nigra* clones for allele B, at this locus. All *P. x canadensis* clones and all progeny of the 3 *P. deltoides* x *P. nigra* crosses were double-banded and were inferred to be heterozygotes for alleles A and B. All progeny of the two intraspecific *P. deltoides* and 2 *P. deltoides* x *P. maximowiczii* crosses were single-banded homozygotes for allele A.

Allozyme Gene Identity in Leaves and Roots

87% to 91% of the allozyme loci identified in each of the 3 *Populus* species or their hybrids identically expressed in both root and leaf tissues (Table 2).

Table 2. — Overlap (identity) of allozyme genes coding for 10 enzymes in roots¹⁾ and leaves of *Populus* species and hybrids.

Species/ Hybrid	No. loci Roots	No. loci Leaves	No. loci Roots + Leaves	No. loci Identical Roots + Leaves	% Loci Identical Roots + Leaves
<i>P. deltoides</i>	30	29	32	29	90.6
<i>P. nigra</i>	29	27	31	27	87.1
<i>P. maximowiczii</i>	27	26	29	26	89.6
<i>P. x canadensis</i>	35	33	37	33	89.2

¹⁾ from RAJORA (1988, 1989a, 1989b), and RAJORA and ZSUFFA (1989).

Discussion

This study established reliable methods of enzyme extraction and allozyme analysis in very young leaves of *Populus deltoides*, *P. nigra*, *P. maximowiczii* and their hybrids.

As allozyme genotypes and phenotypes of the *P. deltoides*, *P. nigra*, *P. maximowiczii*, and *P. x canadensis* individuals at the same 25 loci encoding 8 enzymes in very young leaves and root tips were identical in both tissues, allozyme genotypes of the studied *Populus* clones at these loci reported for root tips (RAJORA, 1988, 1989a, and b; RAJORA and ZSUFFA, 1989) are good for their leaves. Also, earlier results confirming the inheritance of these enzymes in root tips, based on large samples of progeny from controlled crosses involving all 3 *Populus* species (RAJORA, 1990b), are applicable as well. Our results indicate that PER is monomeric in leaves of *P. deltoides*, *P. nigra*, and *P. maximowiczii* and that isozymes of PER-L1 are under the control of a single locus. As Mendelian segregation data was not available for CE-1 and CE-2, we can only hypothesize single-gene control of these enzymes.

Our study suggests a very high overlap in the expression of the genes encoding 10 enzyme systems, especially 9 enzymes (ACO, GOT, IDH, LAP, MDH, PGM, 6-PGD, PGI and SDH), in root tips and very young leaves of *P. deltoides*, *P. nigra*, *P. maximowiczii*, and *P. x canadensis*. Expression of the same or a high proportion of the same genes encoding one or more of these enzymes in different tissues of the same species has also been observed in other angiosperms (eg. TANKSLEY *et al.*, 1981; PEDERSON *et al.*, 1987), and in conifers (eg. LUNDKVIST and RUDIN, 1977; YAZDANI *et al.*, 1985; MUONA *et al.*, 1987; ADAMS *et al.*, 1990). These enzymes are involved in basic metabolic pathways expected to take place in any kind of tissue. Moreover, tissues of actively growing root tips and emerging young leaves may be at a similar developmental stage. Therefore, expression of the same genes in both of these actively growing tissues is not unexpected. The case of peroxidase expression in various types of plant tissues is apparently different. Highly variable and tissue-specific expression has been generally known for genes encoding peroxidase in perennial, as well as annual, angiosperms (eg. MAYBERRY and FERET, 1977; THIEBAUT *et al.*, 1982; VAN HUUSTEE and CHIBBAR, 1987). This may be explained on the basis of a different metabolic role of peroxidase in different types of plant tissue (BUTT, 1980).

In an earlier study (RAJORA and ZSUFFA, 1986), high overlap of sporophytic (root tips) and gametophytic (pollen) gene expression was observed in *P. deltoides*, *P. nigra*, *P. maximowiczii*, and *P. x canadensis*. By comparing the allozyme genes coding for 10 enzymes expressed in leaves of these *Populus* species to those expressed in pollen (RAJORA and ZSUFFA, 1986), the percentage of the common domain of the genes expressed in leaves and pollen is calculated to be 80.6 in *P. deltoides*, 83.9 in *P. nigra*, 80.0 in *P. maximowiczii* and 81.8 in *P. x canadensis*. This further indicates a high overlap in sporophytic and gametophytic gene expression in the studied *Populus* species.

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