JOURNAL OF FOREST SCIENCE, *48*, 2002 (3): 115–120

Introgression in black poplar (*Populus nigra* L. ssp. *nigra*) and its transmission

V. BENETKA, K. VACKOVÁ, I. BARTÁKOVÁ, M. POSPÍŠKOVÁ, M. RASL

Silva Tarouca Research Institute for Landscape and Ornamental Gardening, Průhonice, Czech Republic

ABSTRACT: Introgression was studied in the offspring of *P. nigra* and *P.* × *canadensis* female trees using 3 enzymatic patterns (6-PGD, LAP and GPI). Our investigations were aimed at the offspring from open pollination and controlled pollination by the pollen mixture from 3 clones of *P. nigra* and 3 clones of *P.* × *canadensis*. The rate of introgression in *P. nigra* offspring from open pollination was 0.67%. Introgression in 3 offsprings of *P. nigra* from controlled pollination was 7.14%. In *P.* × *canadensis* the heterozygous: homozygous phenotype ratio in 2 offsprings from open pollination was 1:1; it documents prevailing fertilization by *P. nigra* pollen. After fertilization by the pollen mixture this segregation ratio was 1:1 (6-PGD; GPI) or 3:1 (LAP). The offspring of *P.* × *canadensis* maternal components are assumed to have a major share in introgression.

Keywords: introgression; open pollination; controlled crossing; *Populus nigra* L. ssp. *nigra*; *Populus* × *canadensis* Moench.

In the genus *Populus* spontaneous crossing of some species of this genus can occur. It is documented by some well-known interspecific hybrids that were introduced into cultivation in the 18th century (DATABASES FAO 2000), and by relatively easy crossability of some species in controlled conditions (CAGELLI, LEFÈVRE 1995).

Introgression is considered to mean a transmission of genes of one species into the gene pool of another species by crossing or backcrossing (ANDERSON, HUBRIGHT 1938). It is one of the factors that can cause genetic disturbance of a species in the open nature. Introgression of genes of *P.* × *canadensis* Moench. that is an interspecific hybrid of *P. deltoides* Marsh. and *P. nigra* L. is reported in the autochthonous species of *P. nigra* in the conditions of this country. Mutual pollination of *P. nigra* and *P.* × *canadensis* is a backcross. It is hardly possible to distinguish these backcrosses from *P. nigra* on the basis of morphological traits and to identify introgression in this way. However biochemical markers or DNA analyses can be used to determine the parental components of the plant in question that belong to different species (HEINZE 1997; BENETKA et al. 1999). An isozyme analysis is fully sufficient for simple identification of the particular poplar species (VACKOVÁ et al. 1998).

Considering the *Populus nigra* endangerment, it is to ask a question about the percentage of *P.* × *canadensis* introgression into the gene pool of *P. nigra*. As plants of the genus *Populus* are dioecious, it is important whether there are differences in introgression transmission between *Populus nigra* and *Populus* × *canadensis* in relation to mutual pollination or failure of such pollination and production of germinable seeds.

MATERIAL AND METHOD

Seeds from open pollination were harvested from female trees of *P. nigra* and *P.* × *canadensis* in the populetum of Silva Tarouca Research Institute for Landscape and Ornamental Gardening at Průhonice in Michovka locality. Most of twelve-year trees were fully productive at the time of seed sampling. Clones 301, 310, 311 and Ivachnová were selected from a *P. nigra* compartment, and cultivars I-214, Marylandica, Regenerata and Virginiana de Frignicourt from a *P.* × *canadensis* compartment.

For controlled pollination the same clones as for seed harvest from open pollination (except Ivachnová clone) were used as female plants while from among the male plants clones 315, 316 and 327 were selected as *P. nigra* representatives and Serotina, Blanc du Poitou and Robusta as *P.* × *canadensis* clones. The pollen of six paternal components was mixed at identical volume ratios and the mixture (MI) was used for pollination.

Seeds from open pollination were harvested from floriferous branches that were sampled from maternal trees just before capsule dehiscence. Branches were put into bottles with water where the inflorescences were let ma-

6-PGD

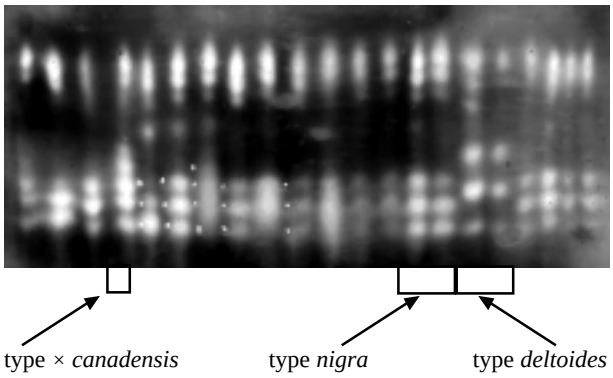

Fig. 1. Differences in the number of bands are evident in the enzymatic pattern **6-PGD** in the lower part of vertical paths. The species *P.* × *canadensis* is identified by five bands, *P. nigra* and *P. deltoides* by three bands while the bands in *P. deltoides* are more distant from each other

ture. Seeds were collected separately after capsule maturation. Controlled crossing was also carried out on floriferous branches put into bottles and in a greenhouse. Before coming into flowers the inflorescences were isolated in paper bags and rubber balloons were used to pollinate flowers through a hole in the paper bag. Pollen viability was tested by control pollination of several maternal components by the pollen of particular paternal components. A paternal genotype was identified in the offspring by an analysis of three enzymatic patterns (6-PGD, LAP and GPI). The paternal genotype could be determined with 85% probability by the combination of three isoenzymatic patterns (BENETKA et al. 1999). Homozygous phenotypes identified in zymograms corresponding to *P. nigra* genotype were designated by letter *N* while heterozygous phenotypes corresponding to *P.* × *canadensis* genotype were designated by letter C. In the enzymatic pattern 6-PGD homozygous phenotypes corresponding to *P. deltoides* genotype were designated by letter *D* (Fig. 1, Table 1 and 3). The share of paternal plants in offspring pollination was determined in *P.* × *canadensis* on the basis of segregation ratios.

ISOZYME ANALYSIS

Fresh young leaves were used for gel electrophoresis. Samples of 100 mg were homogenized with 10 mg of insoluble polyvinyl-pyrrolidone (PVP90), 50 mg of sterile sand and 400 µl of extraction buffer (100 mM Tris-HCl, pH 7.0; polyethylene glycol, 2%; sucrose, 6.8%; EDTA, 4 mM; PVP 40.4%; Triton X 100, 0.5%; isocitric acid, 0.8 mM; shikimic acid, 1 mM; glucose-1-phosphate, 0.7 mM and 2-mercaptoethanol, 128 mM). The extract was absorbed onto 3 × 9 mm filter-papers which were inserted into horizontal starch gel slabs.

Three enzyme systems were assayed by horizontal starch gel electrophoresis using two different buffer systems (Table 1).

Starch gels (11.5%) were prepared from potato starch (Sigma S 5651, St. Louis, MO, USA). After electrophoresis was completed, the gels were sliced horizontally. Gel slices were stained with specific histochemical stain assays (WENDEL, WEEDEN 1989, modified, and VALLEJOS 1983, modified).

RESULTS AND DISCUSSION

POLLEN VIABILITY

This notion is considered to mean the pollen capacity to fertilize ovules that will develop into germinable seeds after fertilization. In *P. nigra* the pollen of clones 315, 327 and 316 was tested in three female genotypes in order to exclude a potential failure caused by incompatibility. Germinable seeds were produced in all cases; it confirmed good viability of the pollen used.

The results for *P.* × *canadensis* are summarized in Table 2. Germinable seeds were produced only by the male clones Serotina and Blanc du Poitou crossed with female clones Marylandica and I-214 while the female clones Regenerata and Virginiana de Frignicourt did not produce any seeds. It can be deduced that pollen viability was good in two clones of *P.* × *canadensis* at least (Serotina and Blanc du Poitou) or there were no other barriers for pollination or fertilization and production of germinable seed. The crosses of female clone I-214 and

Table 1. Buffer systems, electrophoretic conditions and enzymes

Buffer system	Voltage	Name EC No.	Enzyme Abbr.	Quaternary structure	Loci*
A) Morfoline-citrate (CLAYTON and TRETIAK 1972)	230 V	6-phosphogluconate dehydrogenase 1.1.1.44	6-PGD	dimer	5
B) Lithium-borate (SELANDER et al. 1971)	290 V	leucine aminopeptidase 3.4.11.1	LAP	monomer	2
		glucosephosphate isomerase 5.3.1.9	GPI	dimer	2

*RAJORA (1986)

Table 2. Pollen viability expressed by the number of plants produced per fertilized inflorescence

Combinations		Fertilized inflorescences	Germinable seeds	Number of plants produced per fertilized inflorescence
Marylandica	× Serotina	15	0	0
I-214	× Serotina	11	10	0.90
Marylandica	× Blanc du Poitou	17	0	0
I-214	× Blanc du Poitou	5	4	0.80
Marylandica	× MI	71	12	0.16
I-214	× MI	70	30	0.40
Marylandica	× 315	9	1	0.11
I-214	× 315	11	12	1.10
Marylandica	× 327	10	0	0
I-214	× 327	18	3	0.20
Marylandica	× 316	12	0	0
I-214	× 316	9	0	0

Pollen mixture (MI) = *P. nigra* (clones 315, 316, 327) + *P.* × *canadensis* (Serotina, Blanc du Poitou, Robusta), identical volume proportions of pollen

partly Marylandica with *P. nigra* clones (315 and 327) yielded offspring; it demonstrates a possibility of crossing these two species.

INTROGRESSION IN THE OFFSPRING OF *POPULUS NIGRA* FEMALE PLANTS

Introgression was identified (in a zymogram) as a heterozygous phenotype of *P.* × *canadensis* after the analysis of three enzymatic patterns 6-PGD, LAP and GPI (Table 3). Among the offspring from open pollination of four female genotypes representing a total number of 150 analyzed individuals only one individual with heterozygous phenotype was identified. Fertilization by *P.* × *canadensis* pollen was not effective in the other individuals with 85% probability; it indicates introgression at a 0.67% level (with 85% probability). Out of 105 analyzed individuals there were 7 individuals with heterozygous phenotype in the offspring from fertilization by the pollen mixture and introgression was 7.14%. The rate of introgression in the offspring from open pollination is lower than in spontaneously pollinated stands in the open nature where it amounted to about 9% (BENETKA et al. 1999). It is to note that a chance of fertilization by foreign pollen was high in tested maternal components considering the populetum species composition. Not even after fertilization by the pollen mixture when *P.* × *canadensis* pollen was undoubtedly present did introgression achieve the values of stands in the open nature.

Table 3. Numbers of individuals with hybrid phenotype produced by two methods of pollination, determined by the isoenzymatic analysis in *P. nigra*

Female clon	Numbers of individuals after open pollination		after fertilization by the pollen mixture	
	N	*C*	*N*	*C*
301	29	0	35	2
310	30	0	25	2
311	53	1	38	3
Ivachnová	37	0	–	–
Σ	149	1	98	7
% of hybrids	0.67		7.14	

N – homozygous phenotypes identified in zymograms corresponding to *P. nigra*
C – heterozygous phenotypes corresponding to *P.* × *canadensis*

INTROGRESSION IN THE OFFSPRING OF *POPULUS* × *CANADENSIS* FEMALE PLANTS

P. × *canadensis* could be fertilized either by its own pollen or by *P. nigra* pollen. Fertilization by own pollen is the crossing of two heterozygotes and the segregation ratio at one locus is 1:2:1 (types *P. deltoides* : *P.* × *canadensis* : *P. nigra*) or 3:1 if it was not possible to distinguish the phenotype of *P. canadensis* from that of *P. deltoides*. In case it was pollination by *P. nigra*, it was a backcross with segregation ratio 1:1 (types *P. nigra* : *P.* × *canadensis*).

Table 4 shows the frequency of homozygous and heterozygous loci of three enzymatic patterns after open pollination and controlled fertilization by the pollen mixture. Seeds were produced only in two (I-214 and Marylandica) out of the four female clones. Segregation ratios of the tested traits in the offspring from open pollination were 1:1. This segregation ratio suggests that the frequency of fertilization by black poplar pollen must have been high. Fertilization by *P.* × *canadensis* pollen is demonstrated by the phenotypes of *P. deltoides* type in the enzymatic pattern 6-PGD.

Table 4. Frequency of homozygous and heterozygous loci in three enzymatic patterns produced by two methods of pollination (in *P.* × *canadensis*)

a) after open pollination

Clon		Isoenzyme 6-PGD			LAP		GPI	
		N	*C*	*D*	*N*	*C*	*N*	*C*
I-214		11	3	2	6	11	8	9
Marylandica		4	3	0	6	3	5	4
1:1	Σ	15	8		12	14	13	13
	χ^2	2.60			0.16		0.00	

b) after fertilization by the pollen mixture

Combination		Isoenzyme 6-PGD			LAP		GPI	
		N	*C*	*D*	*N*	*C*	*N*	*C*
I-214 × MI		14	11	1	6	16	17	9
Marylandica × MI		4	6	0	4	6	1	9
1:3	Σ	18	18		10	22	18	18
	χ^2	18.00			5.24		18.00	

$\chi^2_{(0.05)} = 3.84$

MI – pollen mixture = black poplar (clones 315, 316, 327) + Canadian poplar (Serotina, Blanc du Poitou, Robusta), identical volume proportions of pollen

The appearance of a part of seedlings was not different from *P. nigra* seedlings, mainly in the offspring of the female clone Marylandica.

The segregation ratio of phenotypes in the offspring from fertilization by the pollen mixture was 1:1 in the enzymatic patterns 6-PGD and GPI. One homozygous phenotype of *P. deltoides* type was found in 6-PGD. On the contrary, the segregation ratio in the enzymatic pattern LAP was highly significantly different from 1:1, it was identical with the ratio 3:1. A sufficient amount of *P.* × *canadensis* pollen increased its share in fertilization but fertilization by *P. nigra* pollen is also documented in this case by the segregation ratios of enzymatic patterns 6-PGD and GPI.

The percentage share of *P. nigra* in fertilization of *P.* × *canadensis* female plants cannot be expressed more exactly because the number of analyzed individuals was low.

CONCLUSION

The results document that introgression of *P.* × *canadensis* into the gene pool of black poplar is not likely to exceed 10%. JANSSEN (1998) stated that pollen transmission between *P. nigra* and *P.* × *canadensis* could occur solely from *P. nigra* to *P.* × *canadensis*. MELCHIOR and SEITZ (1968) and ZSUFFA (1975) also reported that hybridization could be successful only if *P.* × *canadensis* was a maternal component. On the contrary, after fertilization by the pollen mixture in our trial the offspring of *P. nigra* female plants comprised 7.14% of hybrids; it suggests a possibility of pollination of *P. nigra* by *P.* × *canadensis*. The above results and our previously published results (BENETKA et al. 1999) confirm that *P. nigra* preferably accepts pollen of its own species. It is to ask a question which species is a more important source of seeds coming from introgression. The higher rate of introgression in seedlings in the open nature (9.7% – BENETKA et al. 1999) than introgression in the offspring from open pollination by the female plant of *P. nigra* documents that mostly female trees of *P.* × *canadensis* are a source of seeds from *P. nigra* and *P.* × *canadensis* crossing.

References

ANDERSON E., HUBRIGHT L., 1938. Hybridization in *Tradescantia*. I. II. The evidence for introgressive hybridization. Amer. J. Bot., *25*: 396.

BENETKA V., MOTTL J., VACKOVÁ K., POSPÍŠKOVÁ M., DUBSKÝ M., 1999. Estimation of the introgression level in *Populus nigra* L. populations by means of isozyme gene markers. Silvae Genet., *48*: 218–223.

CAGELLI L., LEFÈVRE F., 1995. The conservation of *Populus nigra* L. and gene flow with cultivated poplars in Europe. Forest Genetics, *2*: 135–144.

CLAYTON J.W., TRETIAK D.N., 1972. Amine-citrate buffers for pH control in starch gel electrophoresis. J. Fish. Res. Board. Can., *29*: 1169–1172.

HEINZE B., 1997. A PCR marker for a *Populus deltoides* allele and its use in studying introgression with native European *Populus nigra*. Belg. J. Bot., *129* (2): 123–130.

JANSSEN A., 1998. Artbestimmung von Schwarzpappeln (*Populus nigra* L.) mit Hilfe von Isoenzymmustern und Überprüfung der Methode an Altbäumen, Absaaten von kontrollierten Kreuzungen und freien Abblüten sowie Naturverjüngungen. In: WEISGERBER H., JANSSEN A., Die Schwarzpappel, Probleme und Möglichkeiten bei der Erhaltung einer gefährdeten heimischen Baumart. Forschungsberichte Hessische Landesanstalt für Forsteinrichtung, Waldforschung und Waldökologie, *24*: 32–42.

MELCHIOR G.H., SEITZ F.W., 1968. Interspezifische Kreuzungssterilität innerhalb der Pappelsektion Aigeiros. Silvae Genet., *17*: 88–93.

RAJORA O.P., 1986. Studies on genetics and relationships of *Populus deltoides* Marsh., *P. nigra* L. and *P. maximowiczii* Henry using isozymes, pollen competition and leaf morphology. [PhD. Thesis.] Toronto, University of Toronto: 209.

SELANDER R.K., SMITH M.H., YANG S.Y., JOHNSON W.E., GENTRY J.B., 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Univ. Tex. Stud. Genet., *6*: 49–90.

VALLEJOS C.E., 1983. Enzyme activity staining. In: TANKSLEY S.D., ORTON T.J. (eds.), Isozymes in Plant Genetics and Breeding. Amsterdam, Elsevier: 469–516.

WENDEL J.F., WEEDEN N.F., 1989. Visualisation and interpretation of plant isozymes. In: SOLTIS D.E., SOLTIS P.S. (eds.), Isozymes in plant biology. Portland, Oregon, Dioscorides Press: 5–45.

VACKOVÁ K., POSPÍŠKOVÁ M., BENETKA V., 1998. Rozlišení některých druhů rodu *Populus* L. na základě isoenzymové analýzy. Acta Průhon., *66*: 43–51.

ZSUFFA L., 1975. A summary review of interspecific breeding in the genus *Populus* L. In: Proc. of the 14th Meeting of the Canadian Tree Improvement Association. Part 2. Ottawa, Ontario, Canadian Forestry Service: 107–123.

Directory of poplar and willow experts. Register of *Populus* L. cultivars. Databases FAO. Instituto di Sperimentazione per la Pioppicultura, Casale Monferrato, 2000.

Received 21 January 2002

Introgrese u topolu černého (*Populus nigra* L. ssp. *nigra*) a způsob jejího šíření

V. BENETKA, K. VACKOVÁ, I. BARTÁKOVÁ, M. POSPÍŠKOVÁ, M. RASL

Výzkumný ústav Silva Taroucy pro krajinu a okrasné zahradnictví, Průhonice, Česká republika

ABSTRAKT: U potomstev samičích stromů *P. nigra* a *P.* × *canadensis* byl sledován výskyt introgrese pomocí tří enzymových systémů (6-PGD, LAP a GPI). Byla sledována potomstva z volného opylení a kontrolovaného opylení směsí pylu od čtyř klonů *P. nigra* a dvou klonů *P.* × *canadensis*. U druhu *P. nigra* byl v potomstvech z volného opylení výskyt introgrese 0,67 %. Ve třech potomstvech *P. nigra* z kontrolovaného opylení byla introgrese 7,14 %. U druhu *P.* × *canadensis* ve dvou potomstvech z volného opylení byl poměr heterozygotních fenotypů k homozygotním 1 : 1, což svědčí o převažujícím opylení pylem *P. nigra*. Po opylení směsí pylu byl tento štěpný poměr 1 : 1 (6-PGD; GPI) až 3 : 1 (LAP). Lze předpokládat, že významný podíl na výskytu introgrese u druhu *P. nigra* mají potomstva matek *P.* × *canadensis*.

Klíčová slova: introgrese; volné opylení; řízené opylení; *Populus nigra* L. ssp. *nigra*; *Populus* × *canadensis* Moench.

Introgresí rozumíme vnesení genů jednoho druhu do genofondu druhého druhu v důsledku křížení nebo zpětného křížení. Introgrese způsobuje genetické narušení takto ovlivněného druhu. Uvádí se, že v ČR je autochtonní topol černý ohrožen introgresí hlavně ze strany topolu kanadského (*Populus* × *canadensis* Moench.).

V článku je sledován výskyt introgrese u potomstev po volném opylení od čtyř samičích klonů topolu černého a dvou samičích kultivarů topolu kanadského a u potomstev z opylení směsí pylu tří samčích klonů topolu černého a tří samčích kultivarů topolu kanadského. Výskyt introgrese byl stanoven jako podíl heterozygotů, zjištěných analýzou tří enzymových systémů (6-PGD, LAP a GPI) ve sledovaném potomstvu. Při opylení mezi topolem černým a topolem kanadským dochází ke zpětnému křížení, a proto se u potomstev z tohoto křížení poměr homozygotů a heterozygotů, zjištěný na zymogramech, blíží 1 : 1. V případě křížení dvou topolů kanadských jsou štěpné poměry 3 : 1, kdy homozygotní fenotyp topolu černého je zastoupen ve 25 %.

V potomstvech z volného opylení u topolu černého byl mezi 150 analyzovanými jedinci nalezen pouze jeden

hybridní fenotyp, tzn. že introgrese se vyskytla v 0,67 %. V potomstvech po opylení směsí pylu se hybridní fenotyp vyskytl u 7,14 % ze 105 jedinců.

Od čtyř samičích kultivarů topolu kanadského jak po volném, tak i po řízeném opylení byla sklizena semena pouze u dvou matek. V potomstvech z volného opylení u topolu kanadského se štěpné poměry sledovaných znaků shodovaly s poměrem 1 : 1, což dokazuje, že muselo docházet k rozsáhlému opylení topolem černým. Po opylení směsí pylu byl tento štěpný poměr 1 : 1 (u enzymových systémů 6-PGD a GPI) až 3 : 1 (LAP), proto i zde muselo dojít k opylení i topolem černým. Část tříletých semenáčů se morfologicky nelišila od semenáčů topolu černého.

V předchozích pokusech u přírodních populací topolu černého byla zjištěna introgrese v rozsahu až 9 %. Vzhledem k tomu, že topol černý přednostně přijímá vlastní pyl, lze předpokládat, že na jeho introgresi se významně podílejí semenáče od samičích rostlin topolu kanadského, opylené pylem topolu černého.

Corresponding author:

Ing. VOJTĚCH BENETKA, CSc., Výzkumný ústav Silva Taroucy pro krajinu a okrasné zahradnictví, 252 43 Průhonice, Česká republika
tel.: + 420 2 96 52 82 53, fax: + 420 2 67 75 04 40, e-mail: Benetka@vukoz.cz