CHARACTERISATION OF *Populus alba* L. BY ISOZYMES

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SUMMARY

A characterisation of *Populus alba* L by isozymes has been carried out. Eight isoenzymatic systems were analysed: **ADH, MDH, GOT, LAP, PGM, PGI, SKDH,** and **IDH;** all of them showing polymorphic loci. A total of 18 loci, 12 of them polymorphic, and 33 alleles were identified.

Several half-sib and full-sib families from controlled crosses have been analysed in order to establish the genetic interpretation of the loci. Mendelian segregation was tested in controlled crosses families by Chi-square test (goodness fit) for five loci: Adh-2, Got-1, Got-2, Pgi-2 and Mdh-3. Segregation distortion was observed in Got-1, Got-2 and Pgi-2 in several families; in this cases pool of families was anlysed. Genetic control was determined in half-sib families for: Adh-2, Got-1, Got-2, Mdh-1, Mdh-3, Pgm-2 and Skdh; significant differences from the mendelian segregation were not found in this case. A putative asignation of loci and alleles was infered in LAP and IDH. This study identify a total of 18 loci, as an useful tool for studying diversity in *Populus alba.*

KEY WORDS: *Populus alba*
Isozymes
Segregation

INTRODUCTION

Populus alba L. (Section *Leuce* Duby, Subsection *Albidae* Dode) covers a wide natural range. It grows in valleys from the South, Centre and East of Europe to Middle Asia and the North of Africa. It is found throughout the Iberian Peninsula, except for the Cantabrian Mountains and Galicia.

Riparian ecosystems, a natural habitat for the white poplar, are threatened areas due to intensive human activities.The main goals for breeding in Spain are adaptation and growth. *P. alba* L. is able to grow in drought and in conditions of salinity, and thus offers an alternative land use under these environmental conditions.

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There are different types of breeding activities in Spain related to *Populus alba:*

Assesment of its parental role in controlled hybridisation with *Populus deltoides* to obtain new clones to be used under saline conditions (Alba, 1992).

Studies on salinity tolerance (Cuevas *et al.,* 1997).

Alternative vegetative propagation methods designed to maintain valuable clones (Bueno *et al.,* 1992).

Isozyme markers have been used in genetic variation studies of different *Populus* species (Legionet and Lefèvre, 1996), in the characterisation and differentiation of clones and species (Malvolti *et al.,* 1991 a; Rajora and Dancik 1992a, 1992b; Castillo and Padró 1987) and in genetic studies (Müller-Starck, 1992; Gallo and Geburek, 1991).

Isoenzymatic systems analysed and patterned are presented in this study to support the research on genetic variation in natural stands of *Populus alba* in Spain.

MATERIAL AND METHODS

Plant material

A stool-bed collection established by CIFOR-INIA, including 24 half-sib families and approximately 25 trees per family was analysed. The collection represents a part of the geographical range of the species in Spain, particularly from the Ebro Valley and the south of the country. Also, in order to establish genetic control in locus and alleles, nine males and four females from the river Henares were selected for controlled crosses. Sixteen full-sib families were obtained and a range from 20 to 50 trees per family was maintained in the greenhouse.

Leaves and buds from the collection (half-sib families) were collected from branches in October, and from three months old seedlings (full-sib families) to study segregation.

Extraction procedures, enzyme electrophoresis and staining procedures

The buffer conditions and procedures for electrophoresis are presented in Table 1; the extraction buffer of Fernández-López (1996), was used. Migration in the gel was considered to identify loci and alleles, so in all enzymatic systems, locus-1 describes the faster migration zone; and allele-1 describes the faster migrating band.

Genetic control

Controlled crosses were performed between parentals from Henares River, near Madrid, and genetic control in the full-sib families was tested using the chi-square test, and in the case of half-sib families the estimated maternal genotype was tested by the G-test (Gillet and Hattemer, 1989). In cases what the genetic control could not be demonstrated, the pattern was inferred from phenotypes and other descriptions from different studies on *Salicaceae.* A segregation distortion was considered when the deviation from the Mendelian expectation are significant at 0.05 level.(Strauss and Conkle, 1986).

TABLE 1

ENZYMES ANALYSED AND BUFFERS USED IN THE STUDY OF *P. alba*

Enzimas analizadas y sistemas de tampones empleados

Enzimes analised		E.C. Code	Buffers
Alcohol dehydrogenase	ADH	1.1.1.1	Litio pH 8.3 (Scandalios, 1969)
Glutamate oxalacetate transaminase	GOT	2.6.1.1	
Leucin aminopeptidase	LAP	3.4.11.1	
Malato dehygrogenase	MDH	1.1.1.37	Morfoline pH 6.7 (Wendel and Weeden 1989)
Isocitrate dehydrogenase	IDH	1.1.1.42	
Sikimate dehydrogenase	SKDH	1.1.1.25	
Phosphoglucomutase	PGM	2.7.5.1	Morfoline pH 7.7
Phosphoglucose isomerase	PGI	5.3.1.9	

RESULTS AND DISCUSSION

Eight enzymatic systems we have considered. All the analysed systems were polymorphic. The amount of identified alleles is 33 and, of a total of 17 loci, 12 are polymorphic. Figure 1 shows the pattern loci and alleles for each enzymatic system. Table 2 shows the observed and expected genotypes for locus.

Segregation analysis is shown in Table 3, and Table-4 to support the inferred interpretation specially for loci and alleles.

ADH (E.C. 1.1.1.1.) *Alcohol dehydrogenase.* One locus and three alleles have been considered in this dimeric enzyme, genetic control was tested in the controlled crosses, it would be as the usual Adh-2 locus in the Salicaceae. A faster zone showing irregular enzymatic activity was not scored. **ADH** has been analysed in *Populus* to characterise and distinguish species. Rajora and Dancik (1992 a) in *P. alba* identified two loci and two alleles for each one.

Some problems in the enzyme activities related with the vegetative phases of the plant are present. We recommend using leaves at the end of the growing period.

MDH (E. C. 1.1.1.37) *Malato dehygrogenase.* A dimeric enzyme is characterised by the presence of several bands having different intensity of staining. We have considered five loci; Mdh-1 and Mdh-3 are polymorphic and with tree and two alleles respectively. Mdh-2, Mdh-4, Mdh-5 are monomorphic. Different heterodimeric bands in the same position of some alleles have been identified.

Rajora and Dancik (1992 a) identified in *Populus alba, P. tremula* and *P. x canescens* a fast zone, not observed in other studies, scored as two loci, the fastest with two alleles and the slowest monomorphic, which in our analysis had variable activity and was not identified.

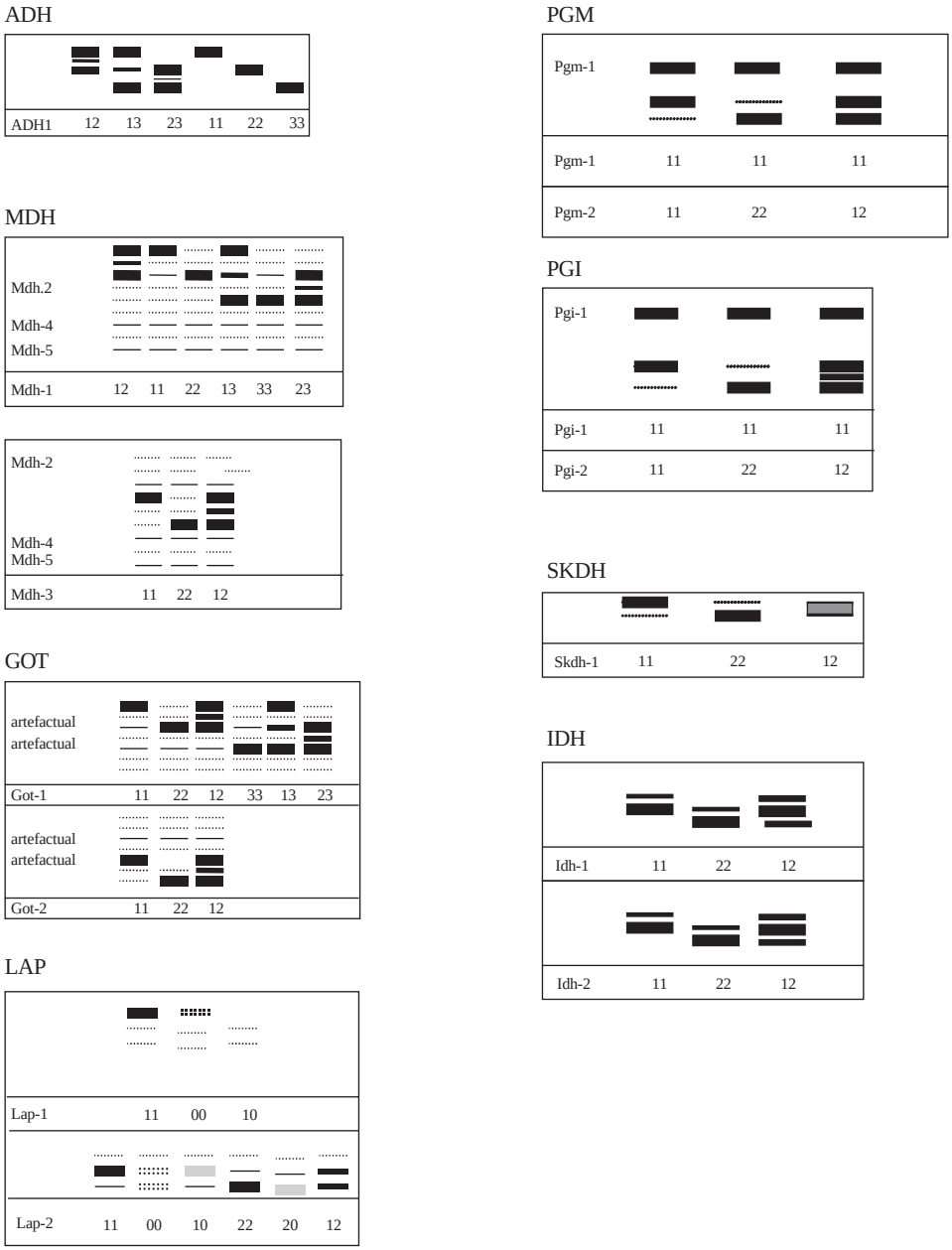

Fig. 1.–Schematic of the enzymatic systems studied

Representación esquemática de los sistemas eszimáticos estudiados

TABLE 2

OBSERVED AND EXPECTED GENOTYPES FOR LOCUS

Genotipos esperados y observados por locus

Loci	Alleles	Expected gen	Observed
Adh	3	6	6
Mdh-1	3	6	3
Mdh-3	2	3	3
Got-1	3	6	6
Got-2	2	3	3
Lap-1	3	6	–
Lap-2	2	3	–
Idh-1	2	3	–
Idh-2	2	3	–
Pgi-2	2	3	3
Pgm-2	2	3	3
Skdh	2	3	3
Total	28		

Different genetic interpretations in different species of Salicaceae are reported. In *Salix eriocephala,* Aravanopoulos *et al.* (1994) found four monomorphic loci; in *Populus nigra* and *P. deltoides,* Malvolti *et al.* (1991a, 1991b) describe four loci, two of them polymorphic and two intergenic bands. Rajora (1989, 1990a, 1990b) identified six loci in *P. nigra* and five loci in *P. deltoides.*

GOT/AAT (E.C. 2.6.1.1.) *Glutamate oxalacetite transaminase.* GOT is a dimeric enzyme. In our analysis one zone of activity was identified. This could be interpreted as the result of two polymorphic loci co-migrating in this zone. The alleles identified were: 3 in Got-1 and 2 in Got 2. Allele 1 in Got-2 is in the same position as allele 3 in Got-1.

Rajora and Dancik (1992 a) analysed *P. alba* and identified two loci, but nothing is said about mono- or polymorphic character. Zhang *et al.* (1992) studied *P. tomentosa,* a species related to *P. alba,* scoring two staining zones and identifying two alleles for each zone. Gallo and Geburek (1991) in *P. tremula* identified two loci Got-1 with tree alleles; and Got-2 with two alleles, as we have interpreted in *P. alba*.

Some differences were found in other analyses (Müller-Starck, 1992; Triest *et al.,* 1998). There are differences in the origin of the vegetal material used. This could indicate differences in the locus expression. In our analysis differences are detected when we use leaves or buds. An artefac band was present when we used leaves, in the same position as allele 1 in Got-2 (Fig. 1). Another artifact band was identified in the same position as allele 2 in Got-2.

LAP (E.C. 3.4.11.1) *Leucine aminopeptidase.* LAP, has a monomeric structure. An interpretation was difficult to obtain. We have inferred two polymorphic loci: Lap-1 with 2 alleles, one of them null, and Lap-2 with 3 alleles one of whitch was also null. The assignment of loci and alleles was based on the interpretation made by different authors. The presence of null alleles is frequent for this enzymatic system in Salicaceae. Rajora and Dancik (1992a) did no detect any variation in the two loci found for *P. alba* and *P.*

TABLE 3

SEGREGATION ANALYSIS OF CONTROLLED CROSSES

Análisis de segregación de los cruzamientos

Locus	Parental genotype	N	Family	Observed			Expected			χ^2	
Adh-2				11	13	33	11	13	33		
	13×11	6	1	2	4	–	3	3	–	0.667	ns
	13×13	22	6	8	11	3	5.5	11	5.5	2.273	ns
	13×11	24	8	14	10	–	12	12	–	0.667	ns
	13×11	37	9	20	17	–	18.5	18.5	–	0.243	ns
	13×13	39	10	8	19	12	9.75	19.5	9.75	0.846	ns
	13×11	32	11	13	19	–	16	16	–	1.125	ns
	13×11	48	13	18	30	–	24	24	–	3.000	ns
	33×11	49	16	–	49	–	–	49	–	0.000	ns
Got-1				22	23	33	22	23	33		
	23×33	36	10	25	11	–	18	18	–	5.444	ns
	23×23	47	6	7	28	12	11.75	23.5	11.75	2.787	ns
	23×23	8	8	2	3	3	2	4	2	0.750	ns
	23×23	41	9	16	13	12	10.25	20.5	10.25	6.268	ns
	23×23	46	11	12	15	19	11.5	23	11.5	7.696	**
	23×23	41	13	11	23	7	10.25	20.5	10.25	1.390	ns
	23×23	49	16	19	19	11	12.25	24.5	12.25	5.082	**
	Pool 23×23			67	101	64	58	116	58	3.95	ns
Got-2				11	12	22	11	12	22		
	12×11	8	8	2	6	–	4	4	–	2.000	ns
	12×11	37	9	16	21	–	18.5	18.5	–	0.676	ns
	12×11	32	10	2	30	–	16	16	–	24.500	***
	Pool 12×11			20	57	–	38.5	38.5		17.77	***
	12×12	46	6	3	40	3	11.5	23	11.5	25.130	***
	12×12	45	11	3	34	8	11.25	22.5	11.25	12.867	***
	12×12	39	13	8	28	3	9.75	19.5	9.75	8.692	**
	12×12	49	16	4	36	9	12.25	24.5	12.25	11.816	**
	Pool 12×12			18	138	23	44.75	89.5	44.75	52.844	***
Pgi-2				11	12	22	11	12	22		
	11×12	48	13	26	22	–	24	24	–	0.333	ns
	12×12	39	16	14	20	5	9.75	19.5	9.75	4.179	ns
	22×12	34	8		17	7		12	12	4.167	**
	22×12	39	9		33	6	–	19.5	9.75	18.692	***
	12×22	38	10		21	17	–	19	19	0.421	ns
	Pool 12×22				71	30		50.5	50.5	16.644	***
Mdh-3				11	12	22	11	12	22		
	11×11	25	8	25	–	–	25	–	–	0.00	ns
	11×12	31	11	15	16	–	15.5	15.5	–	0.032	ns

** significance level 0.05
*** significance level 0.005
ns = not significant

tremula. The pattern described by Zhang *et al.* (1991) in *P. tomentosa* referes to an one locus, three alleles systems. In *P. tremuloides* 2 loci and 2 alleles and a null allele are in the faster locus (Cheliak and Pitel, 1984). Gallo and Geburek (1991) found two loci also present in *P. tremula* each locus has a nulle allele. In *P. balsamifera* a null allele is also present in the faster locus (Farmer *et al.,* 1988).

TABLE 4

SEGREGATION ANALYSIS IN FAMILIES OF *P. alba*, STUDIED WITH ISOENZYMES

Análisis de segregación en las familias de P. alba, estudiada con isoenzimas, a partir de los genotipos maternos estimados

Locus	Family	Maternal genotype	N.º	Observed			Expected			G-test
Adh-2				11	12/13	22/33	11	12/13	22/33	
	AL29	12	26	8	16	2	9,5	13	3.5	1,66 ns
	J4	13	22	3	14	5	4,5	11	6.5	1,70 ns
	MÑ14	13	22	16	5	1	15	3.5	3.5	3.12 ns
Got-1				11	12 13	22 23	11	12 13	22 23	
	GL2	12	28	10	4 6	3 5	8	8.5 5.5	0.5 5.5	9,27 ns
	J3	11	27	24	3 –	– –	24	3 –	– –	0,00 ns
	MÑ15	12	21	6	13 –	2 –	7.25	10.5 –	3.25 –	1,34 ns
Got-2				11	12	22	11	12	22	
	PO9	12	22	2	13	7	3	11	8	0,85 ns
	S18	12	26	6	15	5	7	13	6	0,62 ns
Mdh-1				12	22	23	12	22	23	
	J4	22	23	–	21	2	–	21	2	0,00
	MÑ14	22	23	2	20	1	2	20	1	0,00
Mdh-3				11	12	22	11	12	22	
	AL30	12	27	11	14	2	11	14	2	0,00
	GL2	12	30	12	17	1	13	15	2	0,95 ns
Pgm-2				11	12	22	11	12	22	
	GL1	22	34	–	5	29	–	5	29	0,00
	GU2	12	26	3	17	6	5	13	8	2,60 ns
Skdh				11	12	22	11	12	22	
	MÑ14	11	22	10	12	–	10	12	–	0,00
	MÑ15	12	23	3	12	8	3.25	11.5	8.25	0,05
	S18	12	26	12	12	2	11.5	13	1.5	0,25 ns

IDH (E.C. 1.1.1.42) *Isocitrate dehydrogenase.* This enzymatic system has a dimeric structure. Two activity zones have been observed, the faster one having no stable activity, while for the slower zone two loci with two alleles per locus were described. Since Mendelian segregation was not tested, the **IDH** pattern considered is an extrapolation based on the results of different authors.

Rajora and Zsuffa (1989) performed controlled crosses for *P.x euramericana* and tree loci were described: Idh-1, Idh-2 and Idh-3 with 2, 6 and 5 alleles respectivelly. Position is shared between Idh-2 and Idh-3. In *Salix eriocephala* (Aravanopoulos *et al.,* 1994) genetic control was tested, in three loci: Idh-1, Idh-3 showed monomorphic pattern and Idh-2 polymorphic with two alleles. Between Idh-2 and Idh-3 intergenic bands were present, one of theses heterodimers overlapping with the slow Idh-2 allele.

PGI (E.C:5.3.1.9.) *Phosphoglucose isomerase.* This is a dimeric enzymatic system. Two loci were identified. The fastest was monomorphic, and the slowest had two alleles present. The assignement of loci and alleles is based on the segregation obtained from controlled crosses.

In *Populus* sp (Rajora, 1990; Rajora and Zsuffa, 1989; Rajora and Dancik, 1992) and *Salix* sp. (Aravanopoulos *et al.,* 1993, 1994) a common pattern was observed. Thus two loci were described, the faster being polymorphic only in *P. tremuloides* and the slower showing two or three alleles.

PGM (E.C: 2.7.5.1.) *Phosphoglucomutase.* This is a monomeric enzymatic system. **PGM** shows two stained zones, Pgm-1 is monomorphic and Pgm-2 has 2 alleles. All the expected genotypes were found in the families obtained from controlled crosses.

Analyses made by Aravanopoulos *et al.* (1993, 1994) and Malvolti *et al.* (1991) tested the Mendelian segregation and its structure as a monomeric enzyme. These authors also found two activity zones, Pgm-2 with three 3 alleles. For *P. tomentosa* Zang *et al.* (1992) described one locus and two alleles, while in *P. tremuloides* (Hyun, 1987) two zones were identified, Pgm-1 with tree alleles and Pgm-2 with four. Analyses made both in *P. alba* (Rajora and Dancik, 1992) and *P. x euramericana* (Rajora and Zsuffa, 1989) described three loci and 2 alleles for each one. This interpretation is different from the report of other authors.

SKDH (E.C. 1.1.1.25) *Sikimate dehydrogenase:* **SKDH** has a monomeric structure. We have identified one locus and two alleles; all possible genotypes were in our analysis.

P. tremula, P. tremuloides (Gallo and Geburek, 1991), and *P. tomentosa* (Zhang *et al.,* 1992) were described as having a monomeric structure and one locus with three alleles. In the Section Aigeiros Rajora (1990a, 1990b) identified two monomorphic loci.

Different activity related to the origin of the extraction was found, so in leaves there is a duplication in the products of the genes. For others species, like *Salix* species variation also was detected (Aravanopoulos *et al.,* 1993, 1994; Triest *et al.,* 1998).

Genetic control

Mendelian segregation was tested in controlled crosses families by Chi-square test (goodness fit) for five loci: Adh-2, Got-1, Got-2, Pgi-2 and Mdh-3. Analysis was significantly distorted for Got-1, Got-2 and Pgi-2. The analysis of pooled data grouped by parental' genotypes for Got-2 and Pgi-2, remained significant because of heterozygotes excess. In *P. tremula* Gallo and Geburek (1991) found Mendelian segregation in Got-2. The loci Lap-1, Lap-2, Pgm-2 and Skdh, remain monomorphic in all the progenies.

The distortion observed in Got-1, Got, 2 and Pgi-2, have dificult explanation. In other segregation studies on Salicaceae (Gallo and Geburek, 1991; Müller-Stark, 1992; Malvolti *et al.,* 1991a) no distortion was observed. Studies on other forest species (Stauss and Conkle, 1986) found distortion for these systems Some distortion in Got-2 and Pgi-2 for *Salix exigua* (Aravanopoulos *et al.,* 1993) were estimated and explained on the basis of error sampling, to linkage to other loci, and deleterious effect that are caused by crossing individuals from unrelated population. These explanation are not enough in our case because we had analysed a great number of samples, and the parental were from one population (Río Henares).

Genetic control was determined in half-sib families for: Adh-2, Got-1, Got-2, Mdh-1, Mdh-3, Pgm-2 and Skdh. Significant differences from the mendelian segregation were not found. Lap-1 and Lap-2 was inferred

The interpretation for LAP and IDH was inferred from half-sib families, and no genetic control was determined because of the complicate assignments of genotypes. **IDH** was polymorphic in both families and controlled crosses, although the pattern is not consistent because of some activity problems.

CONCLUSIONS

The systems available for a *Populus alba* variation study were: **ADH, GOT, MDH, PGM,** and **SKDH.** Genetic control in Adh-2, Got-1, Got-2, Pgi-2 and Mdh-3, was studied in the progenies of controlled crosses. It is necessary to study segregation distortion on **GOT** and **PGI** to clarify their causes and consequences in genetic structure populations studies on P. alba.

Some difficulties in establishing the pattern of loci and alleles led us to omit some systems of for studying variation in *Populus alba.* These systems were: **LAP,** because of the presence of null alleles that complicate the assignment of genotypes; **IDH** because of the overlapping of different alleles and intergenic bands; and **PGI** because of poor activity in the half-sib families.

The use of different material (leaves and buds) had no consequences in the staining of almost all enzymes. This was observed in **GOT** and **SKDH,** but identification of the differences is straighforward.

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RESUMEN

Caracterización de *Populus alba* L. por medio de isoenzimas

Se ha realizado la caracterización isoenzimática de *Populus alba* para ocho sistemas ADH, MDH, GOT, LAP, PGM, PGI, SKDH e IDH. Se han identificado un total de 18 loci, 12 de ellos polimórficos y 33 alelos.

La comprobación del control genético se realizó a partir de las segregaciones obtenidas en familias procedentes de cruzamientos controlados. La comprobación de la segregación Mendeliana se ha realizado en los cruzamientos controlados mediante el test Chi-Cuadrado para cinco loci: Adh-2, Got-1, Got-2, Pgi-2 y Mdh-3; se ha observado distorsión en la segregación en los loci: Got-1, Got-2 y Pgi-2 en varias familias, en las que se ha analizado el pool de familias. La comprobación de la segregación mendeliana en las familias de polinización abierta se ha realizado mediante G-test, para siete loci: Adh-2, Got-1, Got-2, Mdh-1, Mdh-3, Pgm-2 y Skdh, en

este caso no se ha observado distorsión. La interpretación de los loci LAP y IDH, ha sido inferida. Este estudio ha identificado a 18 loci como una herramienta útil para abordar trabajos de variación genética en *P. alba.*

PALABRAS CLAVE: *Populus alba* L
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REFERENCES

ALBA N., 1992. Mejora genética de *Populus alba* L. En: El cultivo de los álamos y los sauces. Actas de la 19 sesión de la Comisión Internacional del Álamo (IPC). Zaragoza, 22-25 Septiembre. Vol II: 157-168.

ALBA N., AGÚNDEZ D., ALÍA R., 1997. Estudio de la diversidad genética de *Populus alba* L mediante el uso de soenzimas. Actas del I Congreso Forestal Hispano-Luso/ II Cogreso Forestal Español. Comunicaciones. Tomo III: 27-32.

ARAVANOPOULOS F. A., ZSUFFA L., CHONG K.X., 1993a. The genetic basis of enzymatic variation in *Salix exigua.* Hereditas 119 (1): 77-88.

ARAVANOPOULOS F. A., ZSUFFA L., 1993b. Molecular markers as probes in screening genetic diversity and gene pool sustainability in *Salix.* intensive forestry. Journal of Sustainable Forestry 1(3): 1-7.

ARAVANOPOULOS F.A., ZSUFFA L., CHONG K.X., 1994. Inheritance of isozymes in *Salix eriocephala* Michx.The Journal of Heredity, 85(5) 381-388.

BUENO M.A., ASTORGA R., MANZANERA J.A., 1992. Micropropagación de *Populus alba* «Siberia Extremeña» a partir de amentos. Invest. Agr.: Sist. Recur. For. Vol. 1(2):163-171.

CHELIAK W.M., PITEL J.A., 1984. Electrophoretic identification of clones in trembling aspen. Can. J. For. Res. 14: 740-743.

CASTILLO T., PADRÓ A., 1987. Short note: Electrophorestic characterization of the euramerican poplar clones «I-214» and «Campeador». Silvae Genetica 36 (5-6):250-251.

CUEVAS S., ALBA N., PADRÓ A., 1997 Tolerancia a la salinidad de *Populus alba* L. Estudio preliminar. Actas del I Congreso Forestal Hispano-Luso/ II Cogreso Forestal Español. Comunicaciones. Tomo III: 165-170

FARMER R.E., CHELIAK W.M., PERRY D.J., KNOWLES P., BARRETT J., PITEL J.A., 1988. Isozyme variation in balsam poplar along latitudinal transect in northwestern Ontario. Canadian Journal Forest Research 18(8): 1078-1081.

FERNÁNDEZ-LÓPEZ J. 1996. Variabilidad isoenzimática, morfológica y selección clonal en *Castanea sativa* Miller, *C. crenata* Sieb. et Zucc., *C. mollissima* Blume e híbridos interespecíficos. Tesis doctoral. Universidad Politécnica de Madrid. 212 pp.

GALLO L.A., GEBUREK TH., 1991. Genetics of isozyme variants in *Populus tremula, P. tremuloides* and their hybrids. Euphytica 53: 225-233.

GILLET, E., HATTEMER, 1989. Genetic analysis of isozyme phenotypes using single trees progenies. Heredity 63: 135-141

HYUN J.O., RAJORA O.P., ZSUFFA L., 1987a. Genetic variation in trembling aspen in Ontario based on isozyme studies. Can. J. For. Res. 17: 1134-1138.

HYUN J.O., RAJORA O.P., ZSUFFA L., 1987b. Inheritance and linkage of isozymes in *Populus tremuloides* (Michx.). Genome 29: 384-388.

LEGIONNET A., LEFÈVRE F., 1996. Genetic variation of the riparian pioneer tree species *Populus nigra* L. I. Study of population structure based on isozymes. Heredity 77: 629-637.

MALVOLTI E., BOCCONE A., FINESCHI S., PACIUCCI M., 1991 a. Marcatori biochimici nella tassonomia del pioppo. Monti e Boschi 42(1): 49-56.

MALVOLTI M.E, TEISSIER DU CROSS E., FINESCHI S., PACIUCCI M., 1991 b. Biochemical markers in eastern cottonwood *(Populus deltoides* BARTR.). Enzymatic variation in a factorial mating design. In S. Fineshi, M.E. Malvolti, F. Cannata, HH. Hattemer (ed.), 1991. SPB Academic Publishing bv, The Hague, The Netherlands. Biochemical markers in the populations genetics of forest trees, pp. 31-40.

MÜLLER-STARCK G., 1992. Genetic control and inheritance in isoenzymes in Poplars of the Tacamahaca section and hybrids. Silvae Genetica 41(2): 87-95.

RAJORA O.P., 1989. Characterization of 43 *Populus nigra* L. clones representing selections, cultivars and botanical varieties based on their multilocus allozyme genotypes. Euphytica 43: 197-206.

RAJORA O. P., 1990 a. Marker allozyme genes and alleles for differentiation of *Populus deltoides, P. nigra, P. maximowiczii,* and their interspecific hybrids. *Silvae Genetica* 41(4-5): 289-292.

RAJORA O.P., 1990 b. Genetics allozymes in *Populus deltoides* Marsh., *P. nigra* L. and *P. maximowiczii* Henry. Journal of Heredity 81: 301-308.

RAJORA O.P., DANCIK B.P., 1992a. Genetic characterization and relationship of *Populus alba, P. tremula,* and *P. canescens,* and their clones. Theor. Appl. Genet. 84: 291-298.

RAJORA O. P., DANCIK B.P., 1992b. Allozyme variation and inheritance in leaves of *Populus deltoides,* P. *nigra, P. maximowiczii,* and *Px canadensis* in comparision to those in root tips. Silvae Genetica 41(4-5): 289-292.

RAJORA O.P., ZSUFFA L., 1989. Multilocus genetic structure, characterization and relationships of *Populus x canadensis* cultivars. Can. J. Bot. 68: 990-998.

RAJORA O.P., ZSUFFA L., 1991. Screening *Populus deltoid*es Marsh. Selections by allozymes to assure species identity. Scandinavian Journal of Forest Research 6: 471-478.

SCANDALIOS J.G.,1969. Genetic control of multiple forms of enzymes in plants: A review. Biochemical Genetics 3: 37-79

STRAUSS S.H., CONKLE M.T., 1986. Segregation, linkage and diversity of allozymes in knobcone pine. Theor. Appl. Genet. 72:483-493

TRIEST L., DE GREEF B., D'HAESELEER M., ECHCHGADDA G., VAN SLYCKEN J., COART E., 1998. Variation and inheritance of isozyme loci in controlled crosses of *Salix alba* and *Salix fragilis.* Silvae Genetica 47(2-3): 88-94.

WENDEL J.F., WEEDEN N.F., 1989. Visualization and intrepretation of plant isozymes. In: *Isozymes in Plant Biology (eds: D.E. Soltis and P. S. Soltis)* Dioscurides Press. Portland Oregon, pp. 5-44.

ZHANG Z., ZHU Z., MÜLLER-STARCK G., HATTEMER H.H., 1992. A study on the inheritance and variation of isozymes of *Populus tomentosa.*Carr. 1. Enzyme gene markers of the clones FAO/IPC, 19th Session of the International Poplar Commission. Zaragoza, Spain, 22nd-25th September 1992, pp. 1-10.