validation of genetic relationships in *Mangifera indica* L. using RAPD markers. Theor. Appl. Genet. **90**: 269–274 (1995). — SHAANKER, R. U. and GANESHAIAH, K. N.: Mapping genetic diversity of *Phyllanthus emblica:* Forest gene banks as a new approach for *in situ* conservation of genetic resources. Curr. Sci. **73**(2): 163–168 (1997). — SHAH, F. H., RASHID, O., SIMONS, A. J. and DUNSDON, A.: The utility of RAPD markers for the determination of genetic variation in oil palm (*Elaeis guineensis*). Theor. Appl. Genet. **89**: 713–718 (1994). — WHITKUS, R., CRUZ, M., MOTA-BRAVO, L. and GOMEZ-POMPA, A.: Genetic diversity and relationships of cacao (*Theobroma cacao* L.) in southern Mexico. Theor. Appl. Genet. **96**: 621–627 (1998). — WILLIAMS, J. G. K., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. and TINGEY, S. V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. **18**: 6531–6535 (1990).

Genetic Characterisation of *Populus tremula* Regions of Origin in Spain Using RAPD Fingerprints

By N. SÁNCHEZ, J. M. GRAU, N. ALBA, J. A. MANZANERA[1]) and M. DE LOS ANGELES BUENO

CIFOR INIA, Ctra. de la Coruña Km 7, 28040 Madrid, Spain

(Received 5th August 1999)

Abstract

Random Amplified Polymophic DNA (RAPD) was used to identify 89 *Populus tremula* trees sampled from different Spanish origins. The combination of four primers provided a set of four banding patterns that permitted the identification of all genotypes with a high discrimination capacity. Some trees located in the same stand or in near places showed the same fingerprint, probably due to clonal propagation.

Multidimensional scaling analysis of the amplification bands showed low genetic variability between different Spanish origins, but two different groups could be distinguished. The first one formed by individuals from the Northwest (León, Lugo, Orense and Zamora) and Huesca; and the second one by Huesca, Segovia and Navarra. Huesca province (near the Pyrenees, in Northeast Spain) contained both groups, showing high variability between individuals of the same origin. Analysis of the RAPD fingerprints from different origins showed that Huesca had the greatest number of banding patterns in common with others origins, suggesting a genetic flow from the Northeast to the rest of the Iberian Peninsula.

Key words: DNA fingerprinting, PCR, RAPD markers, *Populus* Aspen.

Introduction

Interest in the culture of fast growing trees such as *Populus* species for wood, fuel and fiber plantations has been developed in the last few decades (ZSUFFA *et al.,* 1984). Furthermore, European aspen (*P. tremula*) may also be used for restoration or creation of permanent forests. This species does not form extended forests in Spain but appears in small groups dispersed in other main species forests (HUNTLEY and BIRKS, 1983). It has been suspected by foresters that these groups are not formed by seedlings but by ramets vegetatively propagated by root suckers. Thus, it is important to find a tool for identification at the genetic level, especially if large scale plant production by tissue culture is going to be used for reforestation (BUENO *et al.,* 1993).

[1]) ETSI. Montes. UPM, Madrid, Spain.
Fax no.: 34-1-3572293
E-mail: Bueno@inia.es

Progress in Molecular Biology has permitted the development of new analytical tools for taxonomic classification and identification. RFLP have been used for clone differentiation in poplars (KEIM *et al.,* 1989; D`OVIDIO *et al.,* 1991; FAIVRE-RAMPANT *et al.,* 1992). Nevertheless RFLP is time consuming and expensive (CASTIGLIONE *et al.,* 1993). Polymerase Chain Reaction (PCR) (SAIKI *et al.,* 1988) can be applied to different techniques, for instance in PCR-RFLPs (AKOPYANZ *et al.,* 1992). This technique has been applied to *Populus nigra* (HEINZ, 1998). SSRs (HEARNE *et al.,* 1992), have been isolated recently in *P. tremuloides* (DAYANANDAN *et al.,* 1998; RAHMAN *et al.* 1999). AFLPs (ZABEAU and VOS, 1992) were used to study the genetic diversity in *P. nigra* (WINFIELD *et al.,* 1998). RAPD screening (WILLIAMS *et al.,* 1990; ERLICH *et al.,* 1991) has some advantages, e.g., only DNA nanograms are needed, it is rapid, less expensive and there is no need DNA sequencing. RAPD markers have been used for DNA fingerprinting and for the study of genetic and taxonomic relationships among poplar clones (CASTIGLIONE *et al.,* 1993; LIN *et al.,* 1994; SIGURDSSON *et al.,* 1997; RANI *et al.,* 1995).

In our study, DNA fingerprints by RAPD markers have been used for identification of trees from European aspen populations of Spain. Sample size has been chosen proportionally to the abundance of European aspen in each region. Collected individuals have been micropropagated for reforestation purposes.

A statistical multidimensional analysis of the banding pattern obtained by PCR amplification of four selected primers was performed. Also, a study of the genetic relationship between populations was based on the presence of the different fingerprints found. Fingerprinting with molecular markers will be applied to conservation of genetic resources and breeding programs.

Materials and Methods

Plant material

Plant material collection in the field was performed in eight Spanish provinces. Two located in the Central Mountain Range: Madrid (M) and Segovia (S); two near the Pyrenees (Northeast): Huesca (H) and Navarra (N); and four in the

Northwest: Lugo (L), Orense (O), Zamora (Z) and León (LE). Sampling location Spain is represented in *figure 1.*

Root sucker leaves from 27 adult were collected in the Central Mountain Range (Madrid and Segovia provinces). Distance between samples locations range from 13 km to 63 km. In Pyrenean group, 35 adult trees located in Huesca and Navarra provinces were analysed, with distances from 5 km to 55 km between sampling locations. In the third group (Northwestern Spain) 27 adult trees were collected in León, Lugo, Orense and Zamora (LE-L-O-Z) provinces. All four provinces are considered a single region of origin, due to the vicinity of sampling locations. Distances between samples ranged from 2 km to 30 km (*Tab. 2*).

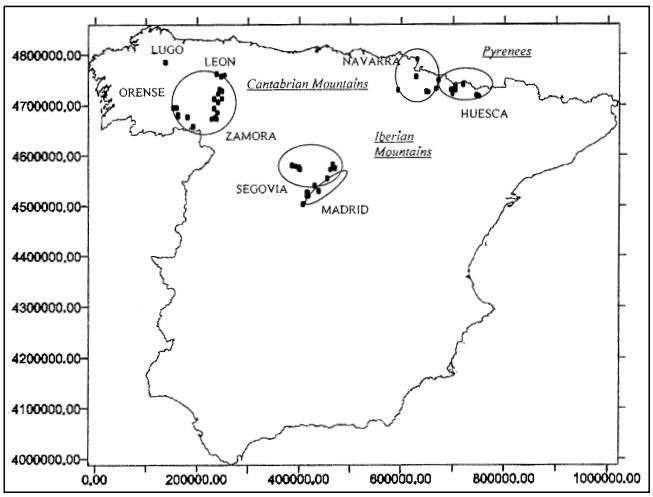

Figure 1. – Map of Spain with the sampling locations (•) of *P. tremula* trees and the different regions of origin studied (○). Approximate situation of mountain-range connections is indicated. Coordinates are indicated in the UTM system.

DNA extraction and amplification

Genomic DNA was extracted from 0.02 g leaf tissue following the method described by DOYLE and DOYLE (1990). The sample was macerated in a microcentrifuge tube with a sterile tip before and after addition of the extraction buffer. DNA was stored at 4 °C for immediate use or at –20 °C for longer preservation.

As concentration of extracted DNA is a limiting factor for amplification, serial dilutions of DNA were tested. The DNA concentration which gave a reliable band pattern for all samples was selected (SÁNCHEZ *et al.,* 1998).

Twenty six primers from Operon Technologies Inc. were used for PCR amplification. The sequences of these primers are given in *table 1.* The amplification reaction was performed in 12.5 µl, containing 10 mM of Tris-ClH (pH 8.3), 1.5 mM $MgCl_2$ 200 µM each of dATP, dCTP, dGTP and dTTP (Boehringer Mannheim or Pharmacia), 0.5 units of Taq DNA polymerase

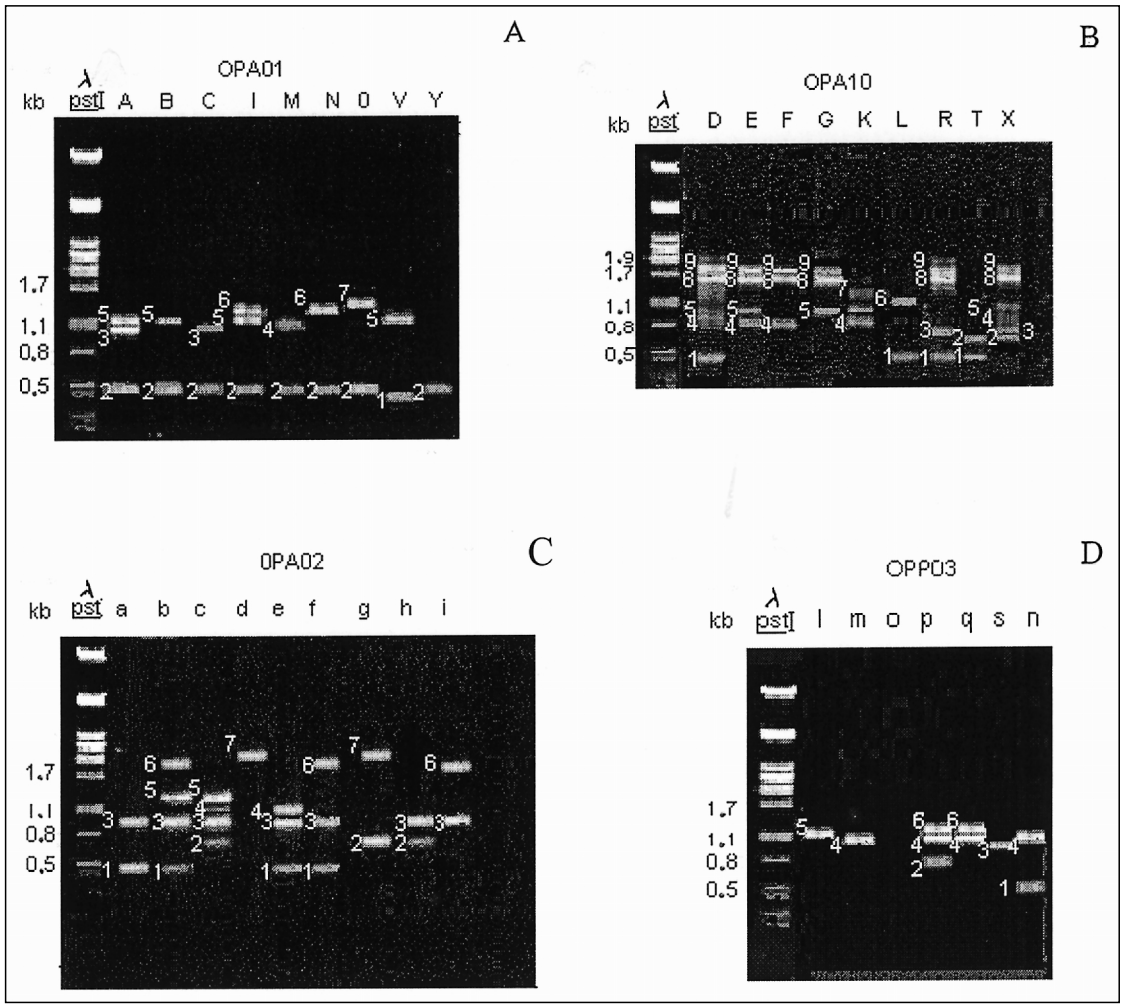

Figure 2. – RAPD fingerprints obtained by PCR amplification. *Fig. 2A:* primer OPA01, *fig. 2B:* OPA10, *fig. 2C.* OPA02 and *fig. 2D:* OPP03. Each fingerprint has been identified by a letter placed in the upper margin of the corresponding lane. Bands of the same size have been allocated a digit, which has been placed near the band for easier recognition of coincident bands between fingerprints. λ *Pst*I: λ DNA digested by *Pst*I enzyme. Left margin digits indicate band molecular weight (kb).

(Boehringer Mannheim or Pharmacia), 0.2µM primer (Operon technologies, Inc.) and 0.5 to 1 ng/µl of DNA. The mixture was performed at 0 °C and the reaction mix was overlaid with a drop of mineral oil.

DNA amplification was performed in a "Personal Cycler" (Biometra) thermal controller programmed as follows: first denaturation of 5 s at 95 °C and 1 min 55 s at 92 °C followed by 44 cycles, each of 5 s at 92 °C, 55 s at 92 °C, 1 min at 34 °C and 2 min at 72 °C, followed by one final extension cycle of 7 min at 72 °C. The amplification products were stored at –20 °C.

Amplifications were repeated in the same conditions with Taq DNA polymerase from both Pharmacia and Boehringer Mannheim brands, corroborating that banding patterns obtained with both enzymes were identical.

In this study of RAPD markers there is not information on the mode of inheritance.

Electrophoresis in agarose gel

The amplification products were size-separated by gel electrophoresis in 1% agarose with TAE buffer and run at 80 V for 2 h. Gels were stained with 1 µg/ml of ethidium bromide.

Statistical analysis

A statistical analysis of the banding pattern has been performed using the software packages, Statistical for Windows Release 4.5 A. Statsoft, inc. (1993); and Population genetic analysis (Pop gene Version 1.2, 1997). Cluster and Multidimensional scaling analysis are performed using the dissimilarities matrix of the NEI's genetic distance (NEI, 1978) product on the basis of presence or absence of bands by all four primers tested in *P. tremula* individuals.

Results and Discussion

DNA concentration for PCR amplification appeared to be critical for the perfect performance of further steps. The use of a DNA concentration between 0.5 ng/µl and 1 ng/µl gave the best results in reliability and repeatability of banding patterns in gel electrophoresis (SÁNCHEZ *et al.,* 1998). Higher concentrations of the same DNA sample carried higher amounts of phenolic compounds from the plant tissue that altered amplification.

Four primers (OPA01, OPA02, OPA10 and OPP03) were chosen, out of the 26 tested (*Table 1*), on the basis of the number and frequency of polymorphisms produced among the samples. The different banding patterns obtained by PCR amplification (*Figure 2*) with the four primers mentioned separated European aspen genotypes and could be used for their characterization (*Table 2*).

Table 1. – Primers used for PCR reaction; (+) means amplification, and (–) no amplification. Primers and the corresponding code are from Operon Technologies Inc.

KIT A			KIT N		
CODE	5′ ___ 3′	AMPLIF	CODE	5′ ___ 3′	AMPLIF
01	CAGGCCCTTC	+	01	CTCACGTTGG	+
02	TGCCGAGCTG	+	02	ACCAGGGGCA	+
03	AGTCAGCCAC	+	03	GGTACTCCCC	+
04	AATCGGGCTG	–	04	GACCGACCCA	+
05	AGGGGTCTTG	+	05	ACTGAACGCC	–
10	GTGATCGCAG	+	06	GAGACGCACA	+
			07	CAGCCCAGAG	+
			08	ACCTCAGCTC	–
			09	TGCCGGCTTG	–
KIT P			10	ACAACTGGGG	–
			11	TCGCCGCAAA	–
CODE	5′ ___ 3′	AMPLIF	12	CACAGACACC	–
			13	AGCGTCACTC	–
01	GTAGCACTCC	+	14	TCGTGCGGGT	–
02	TCGGCACGCA	+	15	CAGCGACTGT	–
03	CTGATACGCC	+	16	AAGCGACCTG	+
04	GTGTCTCAGG	+			

In this study, each fingerprint has been identified by a letter (*Figure 2, Table 2*). Five clones have been identified with a single primer: No. 15 by a *"V",* with primer OPA01 (*Figure 2A* and *Table 2*); No. 17 by a *"K"* and No. 32 by a *"T,"* with primer OPA10 (*Figure 2B* and *Table 2*); No. 12 by a *"n"* and No. 64 by a "q", with primer OPP03 (*Figure 2D* and *Table 2*).

In the case of OPP03, the absence of bands has been considered a different pattern, named *0*. the rationale for this is first because patterns *l, m* and *n* of this primer have only one or two characteristic bands but not several, as it is the case in other primers studied. Second, the relatively high frequency (0.21) of pattern *0* (no band) cannot be atributed exclusively to a technical failure. Therefore, it can be assumed that, at least in this case, the no-amplification pattern is due to a variation in the sequence where other individuals show amplification.

Other individuals can only be distinguished by the combination of all four banding patterns corresponding to the four primers used (*Table 2*).

KLOOSTERMAN *et al.* (1993) defined the power of discrimination (PD) as: $PD=1-\Sigma(P_i)^2$ where P_i represents the frequency of each genotype. In our study the indices i represent different and independent fingerprints of a certain primer. $\Sigma\ (Pi)^2$ for primer OPA01 is 0.28, for OPA10 is 0.2, for OPA02 is 0.14 and for OPP03 is 0.39. The probability of two individuals having the same fours primers is: p = 0.28 x 0.2 x 0.14 x 0.39 = 0.003. Therefore, the PD of this primer combination is: 1- 0.003 = 0.997. This mean that 324 individuals in a set of 325 may be identified.

LIU and FURNIER (1993) found a single primer that discriminated between 110 individuals of trembling aspen (*P. tremuloides*) but this is probably due to the fact that these trees were sampled in a bigger area (Michigan, Minnesota and Wisconsin), therefore collecting individuals at longer distances, thus showing a low consanguinity. The same authors found two adjacent trees of *P. grandidentata* that had identical RAPD genotypes for all four primers tested, suggesting that they were members of the same clone.

36% of the trees from the same stand have the same banding pattern for all the primers tested (*Table 2*). Again, this suggests that these trees from the same stand are actually ramets of a single clone propagated by root suckers that have been extended through the stand (HEYBROEK, 1984). The other 64%, showed RAPD pattern differences between individuals of the same stand, due to sexual reproduction.

In a few near stands, equal banding patterns have been obtained, e.g., Balsaín stands 5 and 6 (trees No. 5 and 7) separated at distances of 1 km. These relatively short distances suggest a high degree of consanguinity, as shown in the similar RAPD patterns. Even clonal propagation cannot be discarded, since cases have been described of a single trembling aspen clone that was extended throughout 80 ha by root suckers (HEYBROEK, 1984). In the case of both Riaza stands 31 and 32 (*Table 2:* trees No. 29 and 30), equal banding patterns are probably due to former intensive artificial plantations.

A high frequency of individuals from the same stand (36%) had the same banding pattern for all four primers tested. We considered them ramets of the same clone because the probability of two individuals with different genome having the same banding pattern is 1/325.

Cluster analysis (*Figure 3*) based in NEI's genetic distance matrix (NEI, 1978) distinguished six subgroups at 0.5 distance: Two marginal subgroups and four major subgroups, three of them (No. 3, 4, 5) composed by individuals from León-L-O-Z and Huesca; and the fourth one (No. 6) from Navarra, Segovia

Table 2. – Fingerprints obtained by RAPD amplification with primers OPA01, OPA02, OPA10 and OPP03, in European aspen (*Populus tremula*) individuals from different regions of origin. No.: number assigned to each sample; Stand/tree: the first alphanumeric character is the stand and the second is the individual; Orig: origin, where the first letter is the province: H., Huesca; L., Lugo; LE., León; M., Madrid; N., Navarra; O., Orense; S., Segovia, and Z., Zamora. The second letter is the locality: Ai, Aisa; Ast, Astorga; Ba, Balsaín; Bi, Biguezal; Bo, Borau ; Bu, Burgui; Ca, Canencia; Cab, Cabrillanes; Cf, Canfranc; Cas, Castrocontrigo; Col, Coloma de Somoza; Cu, Cuellar; Fa, Fanlo; Is, Isaba; Ja, Jaca; Jus, Justel; La, La Jarosa; Luy, Luyego; Mag, Magaz de Cepeda; Na, Navafría; Ped, Pedralba de la Pedrería; Por, Porto; Pu, Puente de la Reina de Panplona; Qui, Quintana del Castillo; Q.C., Quintana y Congosto; Ri, Riaza; Sa, Sallent de Gallego; Sl, Saldaña; So, Somosierra; Via, Viana del Bollo; Vi, Villanua; and Vil, Villagatón. A01, A10, A02 and P03, primers from Operon Technologies Inc. which show polymorphic banding patterns; Sm: symbol assigned to clones which do not show differences. The letters in columns (under primer symbols) represent the different DNA fingerprints observed for each primer. Bold letters indicate clones identified with a single primer; (–), not determined.

No.	Stand Tree	Orig.	A01	A10	A02	P03	Sm
1	2-1	S.Ba	A	D	a	m	•
2	2-2	S.Ba	A	D	–	m	•
3	4-1	S.Ba	B	E	b	m	∪
4	4-2	S.Ba	B	E	b	m	∪
5	5-1	S.Ba	B	F	c	m	*
6	6-1	S.Ba	B	F	–	m	*
7	6-2	S.Ba	B	F	c	m	*
8	7-1	S.Ba	B	G	b	l	
9	7-2	S.Ba	B	G	a	l	
10	11-1	S.Cu	B	E	e	m	
11	12-1	S.Cu	A	E	f	m	
12	13-1	S.Cu	B	E	a	n	
13	14-1	S.Cu	B	D	a	m	
14	18-1	S.Na	B	F	f	m	
15	23-1	S.Na	V	E	e	m	
16	24-1	S.Na	B	E	c	–	
17	25-1	S.Na	B	K	c	m	
18	C-3	M.Ca	B	E	a	l	x
19	C-5	M.Ca	B	E	a	l	x
20	B-1	S.Ba	A	G	f	m	+
21	B-3	S.Ba	A	G	f	m	+
22	J-1	M.La	C	–	c	m	
23	4-1	H.Vi	B	F	e	m	
24	6-3	H.Bo	I	E	d	0	
25	7-3	H.Bo	B	F	d	0	
26	8-1	H.Ai	B	D	e	m	=
27	8-2	H.Ai	B	D	e	m	=
28	13-1	H.Sa	B	D	d	l	
29	31-1	S.Ri	A	R	c	0	♦
30	32-2	S.Ri	A	R	c	0	♦
31	42-2	S.Sl.	A	R	c	m	
32	S-1	M.So	–	T	e	0	
33	S-2	M.So	Y	–	a	0	
34	1-2	H.Cf	A	F	b	m	
35	1-3	H.Cf	B	F	b	m	
36	3-4	H.Cf	A	D	c	m	♠
37	3-5	H.Cf	A	D	c	m	♠
38	4-2	H.Vi	M	D	e	l	
39	5-5	H.Vi	A	G	f	p	
40	5-9	H.Vi	C	E	b	m	
41	6-4	H.Bo	B	G	h	m	
42	6-5	H.Bo	M	G	h	m	
43	7-4	H.Bo	B	X	c	m	
44	18-1	H.Bo	B	D	b	s	
45	18-2	H.Bo	B	D	g	m	
46	19-1	H.Ai	B	D	e	p	
47	21-1	H.Fa	O	D	e	s	
48	22-1	H.Fa	M	F	g	m	
49	27-1	H.Fa	A	F	c	m	
50	S-1	H.Vi	I	R	b	m	
51	S-2	H.Vi	O	D	g	l	
52	1-1	N.Bu	I	L	f	m	
53	3-1	N.Is	M	D	e	m	
54	3-2	N.Is	B	G	f	m	
55	4-1	N.Is	B	G	b	m	
56	5-1	N.Is	B	G	i	l	✚
57	5-2	N.Is	–	G	i	l	✚
58	6-1	N.Bi	B	D	f	m	
59	8-1	N.Bi	N	L	c	m	
60	9-1	N.Pu	A	D	e	l	
61	10-1	N.Pu	B	D	b	m	
62	10-2	N.Pu	B	G	e	m	
63	1-1	L.	M	D	g	s	
64	2-1	O.	M	D	h	q	
65	3-1	O.	M	D	h	l	
66	6-1	O.Via	Y	X	e	l	
67	7-1	O.Via	Y	L	d	m	
68	1-1	Z.Jus	N	F	e	m	
69	1-2	Z.Jus	N	X	h	m	
70	2-1	Z.Ped	Y	D	e	p	
71	4-1	Z.Por	Y	F	h	l	
72	1-1	LE.San	B	E	e	p	
73	1-2	LE.San	B	E	e	l	
74	5-2	LE.Cab	N	D	a	m	
75	8-1	LE.Cab	B	X	e	l	
76	9-1	LE.Col	B	D	c	m	
77	9-3	LE.Col	B	D	c	l	
78	16-1	LE.Vil	B	F	h	m	
79	16-2	LE.Vil	B	D	h	m	
80	19-1	LE.Vil	B	X	h	l	
81	21-1	LE.Qui	I	F	a	l	
82	21-3	LE Qui	I	F	h	m	
83	23-1	LE.Mag	I	D	h	m	
84	25-1	LE.Ast	B	X	d	l	
85	28-1	LE.Luy	I	F	h	m	
86	30-1	LE.Q.C.	I	G	a	l	
87	33-1	LE.Cas	B	X	h	p	
88	34-1	LE.Cas	I	X	h	p	
89	35-1	LE.Cas	O	E	a	l	

and Huesca. Similar results were also obtained by Multidimensional Scaling analysis of the NEI's distance matrix, with two well delimited groups (*Figure 4*). The group integrated by trees from Huesca, Segovia and Navarra origin showed small genetic distances, in contrast to the other group León-L-O-Z and Huesca. Local populations from Huesca show the highest variability. Some *P. tremula* individuals of the same locality belong to the groups and show high variability but in very different geographic origins the variability is low, as occurs in other forests and cultivated species (LANHAM *et al.,* 1998).

A schematic representation of clustered local populations having at least three fingerprints in common is shown in *figure 5.* Two main trends of genotypic relationship have been observed, deriving from Huesca, as in the statistical analysis. This suggests that the Northwestern population has maintained a genetic contact exclusively with the Pyrenean populations

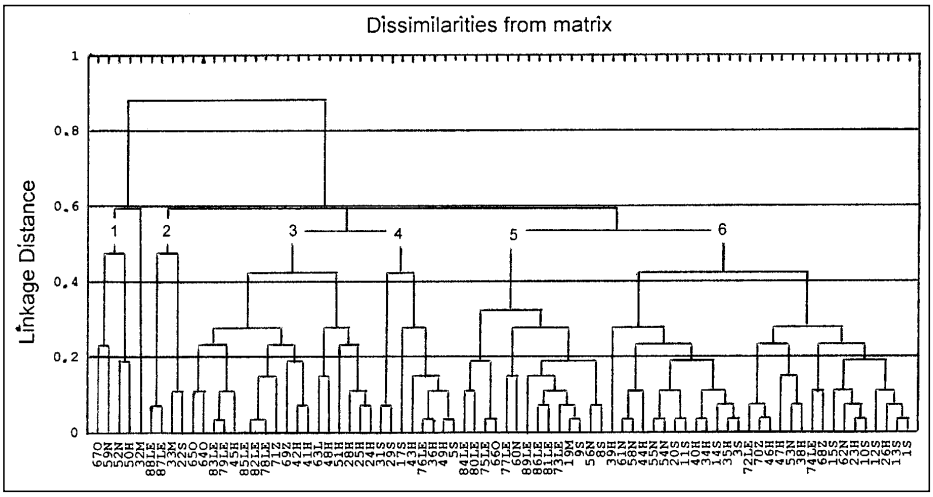

Figure 3. – Cluster analysis (dendrogram) based on the presence or absence of bands by all four primers tested in *P. tremula* individuals, using NEI's genetic distance. The geographic origin, was designated by the sample number and the Province (H., Huesca; L., Lugo; LE., León; M., Madrid; N., Navarra; O., Orense; S., Segovia; Z., Zamora). Number 1, 2, 3 ,4 , 5 and 6 are subgroups at 0.5 linkage distance.

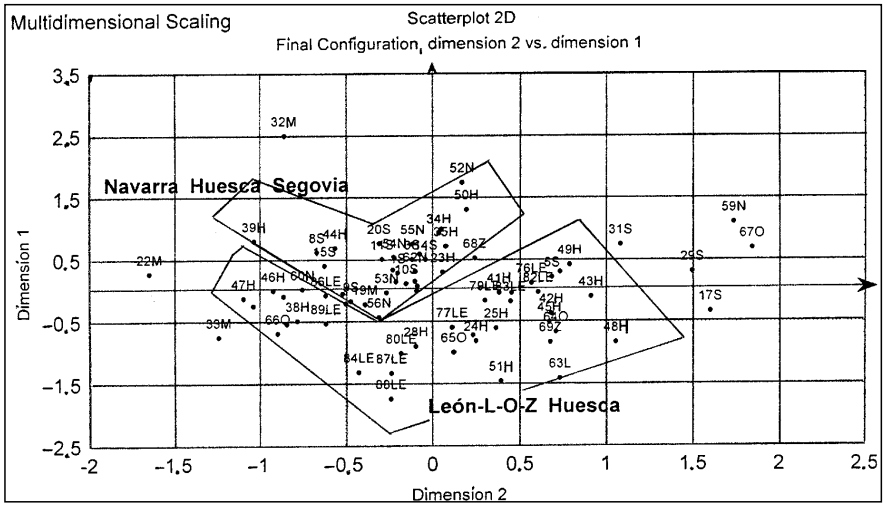

Figure 4. – Multidimensional scaling analysis based on the presence or absence of bands by all four primers tested in *P. tremula* individuals, using the dissimilarities matrix of the NEI's genetic distance. Groups represent genetic similarity. The geographic origin was designated by the sample number and Province (H., Huesca; L., Lugo; LE., León; M., Madrid; N., Navarra; O., Orense; S., Segovia; Z., Zamora).

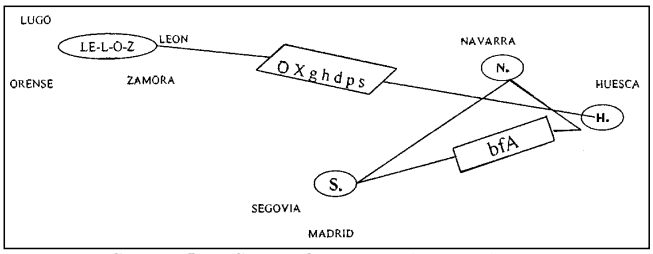

Figure 5. – Genetic flow. Geographic origins (province) containing more than two fingerprints in common are united by a line. Huesca has the greatest number of fingerprints in common with other origins. The genetic flow follows the geographic position of mountain-ranges in Northern Spain (*Fig. 1*). Province: H., Huesca; L., Lugo; LE., León; M., Madrid; N., Navarra; O., Orense; S., Segovia; Z., Zamora.

through the Cantabrian mountain-range, and has been isolated from the Central population by the central plateau during the wamer post-glacial period. The other trend of genetic flow has been conserved between the Central population (Segovia and Madrid) and that of the Pyrenees (Huesca and Navarra) trough the mountain ranges (*Figure 1*). Furthermore, the higher genetic diversity in the Huesca province is probably due to genetic interchange with populations from Central Europe. After the mid Holocene, the Northern margin of aspen distribution area in Europe was displaced South, as a result of climatic deterioration, and the range in the Alps increased (HUNTLEY and BIRKS, 1983), probably as well as in the Pyrenees and the rest of the mountains in the Iberian Peninsula.

A close relationship between the Northwestern population and that from the Pyrenees has been found, and also between the population from the Central mountain range and that from the Pyrenees but not between Northwestern and Central populations. The pyrenean population showed the highest variability.

Nevertheless, a deeper knowledge of European aspen genetic structure through molecular markers may be helpful not only for variability and improvement studies but also for identification of clones and legal implications.

Acknowledgements

This study was funded by the National Institute of Agronomical Research (INIA) project SC 94-138. SÁNCHEZ was a recipient of a INIA postdoctoral grant during the tenure of this study.

References

AKOPYANZ, N. N., BUKANOV, T. u., WESTBLOM and BERG, D. E.: PCR based RFLP analysis of DNA squence diversity in the gastric pathogen *Helicobacter pylori.* Nucleic Acids Res. **20**: 6221-6225 (1992). — BUENO, M. A., GRAU, J. M. and GARCIA-DE-LOS-RIOS, M. D.: Micropropagación de árboles de *Populus tremula* e identificación de clones en rodales mediante electroforésis. Congreso Forestal Español. Tomo II., 177–182 (1993). — CASTIGLIONE, S., WANG, G., DAMIANI, G., BANDI, C., BISSOFI, S. and SALA, F.: RAPD Fingerprintints for identification and for taxonomic studies of elite poplar (*Populus* spp.) clones. Theor. Appl. Genet. **87**: 54–59 (1993). — DAYANANDAN, S., RAJORA, O. P. and BAWA.: Isolation and Characterization of microsatellites Tremblin aspen (*Populus tremuloides*). Theor. Appl. Genet. **96**: 950–956 (1998). — D'OVIDIO, R., MUGNOZZA, G. S. and TANZARELLA, O. A.: rDNA clonig and rapid hybrid identification in *Populus* spp. (Salicaceae). Pl. Syst. Evol. **177**: 165–174 (1991). — DOYLE, J. J. and DOYLE, J. L.: Isolation of DNA from fresh tissue. Focus. **12**: 13–15 (1991). — ERLICH, H. A., GELFAND, D. and SNINSKY, J. J.: Recent advances in the polimerase chain reaction. Science **252**: 1643–1650 (1991). — FAIVRE-RAMPANT, P., JEANDROZ, S., LEFEVRE, F., LEMOINE, M., VILLAR, M. and BERVILLÉ, A.: Ribosomal DNA studies in poplar *P. deltoides, P. nigra, P. trichocarpa, P. maximowiczii,* and *P. alba.* Genome **35**: 733–740 (1992). — HEARNE, C., CHOSHS and TODD, J.: Microsatellites for linkage analysis of genetic traits. Trends. Genet. **8**: 288–294 (1992). — HEINZE, B.: PCR-based chloroplast assays for the identification of native *Populus nigra* and introduced poplar hybrids in Europe. Forest Genetics (Slovak Republic), International Journal of Forest Genetics. ISSN 1335–048X. **5**: 31–38 (1998). — HEYBROEK, H. M.: Clones in Forestry and in Nature. Arboricultural Journal **8**: 275–286 (1984). — HUNTLEY, B. and BIRKS, H. J. B.: An atlas of past and present pollen maps for Europe, 0–13,000 Years ago. Cambridge University Press, Cambridge, U.K. (1983). — KEIM, P., PAIG, K. N., WHITHAM, T. G. and LAR, K. G.: Genetic analysis of an interspecific hybrid swarm of *Populus:* accurrence of unidirectional introgression. Genetics **123**: 557–565 (1989). — KLOOSTERMAN, A. D., BUDOWLE, B. and DASELAAR, P.: PCR-amplification and detection of the human D1S8 VNTR locus. Int. J. leg. Med. **105**: 257–264 (1993). — LANHAM, P. G. and BRENNAN, R. M.: Characterization of the genetic resources of redcurrant (*Riber rubrum;* subg. Ribesia) using anchored microsatellite markers. Theor. Appl. Genet. **96**: 917–921 (1998). — LIN,V., HUBBES, M. and ZSUFFA, L.: Differentiation of poplar and Wilow clones using RAPD fingerprinting. In: KLOFENSTEIN, N. B., CHUN, Y. W., KIM, M. S. and AHUJA, M. R. eds. Micropopagation, Genetic Engineering and Molecular Biology of *Populus.* Gen. Tech. Rep. RM-GTR-297. Fort Collins, Department of Agriculture, Forest Service, Rocky Mountain Research Station. 326 p. (1997). — LIU. Z. and FURNIER, G. R.: Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within an between trembling aspen and bigtooth aspen. Theor. Appl. Genet. **87**: 97–105 (1993). — NEI, M.: Estimation of avarage heterozigosity and genetic distance from a small number of individuals. Genetics **89**: 583–590 (1978). — RAHMAN, M. H., DAYANANDAN, S. and RAJORA, O. P.: Microsatellite DNA Markers in *Populus tremuloides.* Genome (in Press) (1999). — RANI, V., PARIDA, A. and RAINA, S. N.: Random amplified polymorphic DNA (RAPD) markers for genetic analysis in micropropagated plants of *Populus deltoides* MARSH. Plant. Cell. Reports. **14**: 459–462 (1995). — SAIKI, R. K., GELFAND, S., STOFFEL, S., SCHARF, S. T., HIGUCHI, R., HORN, G. T., MILLIS, K. B and ERLICH, H. A.: Primer-detected enzimatic amplification of DNA with a thermostable DNA polymerase. Science **239**: 487–491 (1988). — SÁNCHEZ, N., GRAU, J. M., MANZANERA, J. A. and BUENO, M.A.: RAPD markers for the identification of *Populus* species. Silvae Genetica **47**: 67–71 (1998). — SIGURDSSON, V., ANAMTHAWAT-JONSSON, K. and SIGURGEIRSSON, A.: DNA fingerprinting of *Populus trichocarpa* clones using RAPD markers. New Forests **10**: 197–206 (1995). — WILLIAMS, J. G., KUBELIK, A. R., LIVAK, K. J., RAFALSKI, J. A. and TINGEY, S. V.: DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic. Acids. Res. **18**: 6531–6535 (1990). — WINFIELD, M. O., ARNOLD, G. M., COOPER, F., LERAY, M., WHITE, J., KARP, A. and EDWARDS, K. J.: A study of genetic diversity in *Populus nigra* subsp. *betulifolia* in the Upper Severn area of U.K. using AFLP markers. Molecular Ecology **7**: 3–10 (1998). — ZABEAU and VOS.: Selective restriction fragment amplification a general method for DNA fingerprinting, European patents. Application. No: 0534858 A1 (1992). — ZSUFFA, L., MOSSELER, A. and RAJ, Y.: Prospects for interspecific hybridization in willows for biomass production. In: PERTTU, K. (ed.). Ecology and Management of Forest Biomass Production Systems. Dept. Ecol. and Environ. Res., Swed. Univ. Agric. Rep. **15**: 261–281 (1984).

Genetic Variation in Blister-Rust Resistance and Growth Traits in *Pinus strobus* x *P.peuce* Hybrid at Age 17: (Experiment 2)

By I. BLADA

Forest Research Institute, Sos. Stefanesti 128, Bucharest 11, Romania

(Received 31st August 1999)

Summary

The major objective of this paper was to provide basically information about genetic variation within a 17 years old *P. strobus* x *P. peuce* F_1 hybrid population. The progeny trial involved factorial mating among 5 female x 5 male parents. The 25 families and the 2 parent species controls were artificially inoculated at age 2, 3 and 4, by using leaves of *Ribes nigrum* L. heavily infected by the *Cronartium ribicola*. At age 6, the families were planted out at 3 m x 3 m spacing using a randomized complete block design with 10 trees row-plot in each of the three blocks. Eleven traits (blister-rust resistance and growth traits among them) were assessed at age 17 and the major results are presented here. (1). Highly significant ($p<0.01$; $p<0.001$) differences among hybrid families for all but one trait were found; (2) The male, female and male x female effects were highly significant ($p<0.01$; $p<0.001$) for both blister-rust resistance and growth traits; (3) The high parent heterosis was negative whereas the mid-parent heterosis was positive for all but one trait; mid-parent heterosis accounted for 27.3% for blister-rust resistance and 27.7% for volume growth; (4) Tree survivors accounted for 86% for hybrid, 93% for Balkan pine and only 21% for eastern white pine; (5) Good general combiners, not only for growth, but for blister-rust resistance, too, were found among eastern white pine parents as four of 10 parents had positive significant *gca* effects for blister-rust resistance and six for volume growth; (6) Highly significant positive correlations were found among growth traits, but no significant correlations between any growth trait and blister-rust resistance; (7) A genetic gain of 11.5% in