The petiole length seems to have potential as a factor for early indirect selection of stem height growth (good heritability and positive correlation). But we must first test whether measurement in the nursery will give the same result.

Resistance to anthracnosis could also be selected for in collections or in nurseries, as heritability is very high.

Our measurements of susceptibility to aphid attacks did not reveal any opportunities for selection. This lack of variability is confirmed by studies on controlled aphid colony development on other clones preformed simultaneously to ours in Italy and in England (F. DUCCI and F. NICOLL, pers. comm).

In our set of sites, clone · site interaction for growth traits does not play a major role for three of the sites. The sites, though diverse (*Table 1*), do not represent all the potential sites for wild cherry. However, the selected clones have also been tested on some other sites with the same satisfactory results. These clones will be proposed as suitable for most French ecological conditions. Better estimation of adaptation with new plantations will nevertheless be necessary for final certification of clones (10 years later).

Final multisite selection did not bring any improvement of branch thinness. However, clones of interest for this trait (i.e. clone 139, 7th with Sarrazac index or clone 184, 2nd with Vaux-les-prés index) but unsuitable for certification due to crookedness or instability, will be included in future crossings.

The estimated gain on stem height increment and breast height girth is limited as we wanted to propose enough clones for certification (proportion selected: 25%), to retain enough genetic diversity. In future, additional clones will be selected while our younger tests are ageing and furnishing us with more pertinent genetic information. Final certification of clones will also limit the number of selected clones and thus allow better gains.

Acknowledgments

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RAPD Markers for the Identification of *Populus* Species

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Abstract

Twenty five poplar clones, namely, 5 of *Populus nigra*, 5 of *P. deltoides*, 5 of *P. alba*, 5 of *P. tremula*, 1 of *P. trichocarpa*, 3 of *P. x canescens* and 1 of clone *"Platero"* (*P. tremula* x *P. alba "Bolleana"*) were screened for random amplified polymorphic DNA (RAPD) markers in order to evaluate the use of RAPD analysis for distinguishing the cited species. One of the markers revealed different banding patterns between species and similar banding patterns for clones of the same species. For hybrids such as *P. x canescens* and *"Platero"*, the banding pattern was the same as either *P. alba* or *P. tremula.* On the other hand, for other hybrids analysed, such as *P. x euroamericana* I-214 and *P. deltoides x P. alba* 7/32 B, the banding pattern differed from both parents. The mentioned marker showed characteristic bands for every species, and in the particular case of *P. alba* three different groups could be distinguished.

Key words: Populus, Random amplified polymorphic DNA (RAPD), fingerprinting, species identification.

FDC: 165.3; 165.5; 176.1 *Populus.*

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Introduction

The importance of a proper identification of poplar clones was appreciated long ago, based on the observation of morphological and phenotypic traits (UPOV, 1981). Isozyme analysis has previously been used to distinguish poplar clones (RAJORA, 1989). However, its resolving power is limited by the number of enzymatic systems that can be conveniently analysed.

The development of the polymerase chain reaction (PCR) for amplification of DNA (SAIKI et al., 1988) has been important for the detection of DNA polymorphisms. One of the PCR-based applications, RAPDs, uses single DNA primers of arbitrary nucleotide sequence (WILLIAMS et al., 1990). Amplification of genomic DNA sequences occurs wherever the primers find sufficient sequence identity at a favorable distance and in a converging orientation. RAPD may be used to detect DNA variability at different levels, from single base changes to deletions and insertions (WILLIAMS et al., 1990). RAPDs have several advantages over other techniques, e.g. the use of restriction fragment length polymorphisms because it requires only a few nanograms of DNA, it is rapid, and it is relatively economical. These markers have been used for DNA fingerprinting of genetical and taxonomical relationships among *Populus* clones (CASTIGLIONE et al., 1993; SIGURDSSON et al., 1994; RANI et al., 1995).

This work reports on the use of RAPDs to type clones of 5 *Populus* species, including 2 American species and 3 autochthonous Spanish species. The American species (*Populus deltoides* and *Populus trichocarpa*) provided clones of commercial use. The clones from autochthonous species (*Populus nigra, Populus alba* and *Populus tremula*) have been selected from representative regions of the pure species. A primer has been identified which detects polymorphisms among these 5 species. This primer produced several informative bands for every species and in *P. alba* identified three groups.

The use of molecular markers for differentiation between species, namely of autochthonous species, is especially interesting in programmes concerned with the conservation of genetic resources and breeding.

Material and Methods

Plant material

The analysis of RAPD molecular markers has been conducted on the DNA from poplar clones shown in *table 1* and *figure 3*. Clones *P. x euroamericana* I-214, *P. deltoides x P. alba* 7/32 B and the other cultivated clones (GONZALEZ and DOMINGO, 1987) used in this work are from several origins (Turkey, Italy, USA). The rest are all of Spanish origin. DNA was extracted from leaf, branch or cutting samples. For some clones of *P. alba* and *P. tremula*, DNA was extracted from leaves of "in vitro" explants (BUENO et al., 1993; SANCHEZ et al., 1997).

DNA extraction, previous preparation and amplification

Genomic DNA was extracted by DOYLE's method (DOYLE and DOYLE, 1990). 0.02 g of tree material was macerated in an eppendorf with a sterile tip before and after adding the extraction buffer. DNA concentration was estimated by comparison

Table 1. – Clones used for the DNA amplification.

KEY	CLONE	ORIGIN (and COUNTRY)	TREE MATERIAL
1D	*P. deltoides* "N.Dakota"	Populetum M. Central. (USA)	cutting
2D	*P.deltoides* "Ohio"	" (USA)	cutting
3D	*P.deltoides* "Misouri"	" (USA)	cutting
4D	*P.deltoides* "Harvard"	" (USA)	cutting
5D	*P.deltoides* "77/55"	" (USA)	cutting
1N	*P.nigra* "NA2"	Navarra (Spain)	leaf
2N	*P.nigra* "NA8"	Navarra (Spain)	leaf
3N	*P.nigra* "(♂)"	Populetum M. Central.(Spain)	cutting
4N	*P.nigra* "Covat"	" Guadalajara (Spain)	cutting
5N	*P.nigra* "Tr.56/75"	" (Turkey)	cutting
1A	*P.alba* "5-41"	Jaén (Spain)	leaf
2A	*P.alba* "T"	Torremocha (Spain)	leaf
3A	*P.alba* "FA"	Almería (Spain)	leaf
4A	*P.alba* "Bolleana"	(The Netherlands)	branch
5A	*P.alba* "Raket"	Populetum M. Central, (Belgium)	cutting
1C	*P. x canescens* "Bet"	Cuenca (Spain)	leaf
2C	*P. x canescens* "LPG5"	Granada (Spain)	leaf
3C	*P. x canescens* "7"	Teruel-Alfambra (Spain)	leaf
4P	"Platero" *P. tremula x P. alba* "Bolleana"	Populetum M. Central (Spain)	cutting
1T	*P.tremula* "5C"	Canencia (Spain)	leaf
2T	*P.tremula* "3B"	Balsain (Spain)	leaf
3T	*P.tremula* "3"	Teruel (Spain)	leaf
4T	*P.tremula* "3L"	Riaño (Spain)	leaf
5T	*P.tremula* "4J"	La Jarosa (Spain)	leaf
1TC	*P.trichocarpa* "F.Pauley"	Populetum M. Central. (USA)	cutting
6N	*P.nigra* "Bordils"	" Gerona (Spain)	cutting

with know amounts of lambda DNA. DNA was stored at 4 °C when it was to be used in a few days and at –20 °C for longer time intervals.

The primers tested in the reaction were OPN 1 to 20 Kit and OPA 1 to 5 from Operon Technologies, Inc. PCR was performed in a final reaction volume of 12.5 µl, containing 10 mM of Tris HCl pH 8.3, 1.5 mM of Cl2Mg, 200 µM each of dATP, dCTP, dGTP and dTTP (Boehringer Mannheim, Germany), 0.2 µM primer (Operon Technologies, Inc.), 0.5 ng/µl to 1 ng/µl of poplar DNA and 0.04 units/µl of Taq DNA polymerase (Boehringer Mannheim, Germany). The reaction mixture was prepared at 0 °C, overlaid with a drop of mineral oil and quickly transferred to a "personal cycler" (Biometra) thermal controller. The DNA was initially denatured at 95 °C for 5 s, followed by 1 min, 55 s at 92 °C. The amplification profile consisted of 45 cycles at 95 °C for 5 s, 92 °C for 55 s (denaturation); 34 °C for 1 min (annealing); and 72 °C for 2 min (extension). A final extension was performed at 72 °C for 7 min. The amplified DNA was stored at –20 °C. RAPD reactions were performed several times on each DNA sample to verify the reproducibility of the data.

Electrophoresis in agarose gel

A half of the amplification product was analysed by electrophoresis in 1% agarose gel with 40 mM of Tris[hydroxymethyl]aminometane-Acetic acid and 1mM of Ethylenediaminetetraacetic acid (TAE) buffer and run in the same buffer for 2 h to 3 h at 80 V. The gels were stained with 1µg/ml ethidium bromide and photographed under UV light.

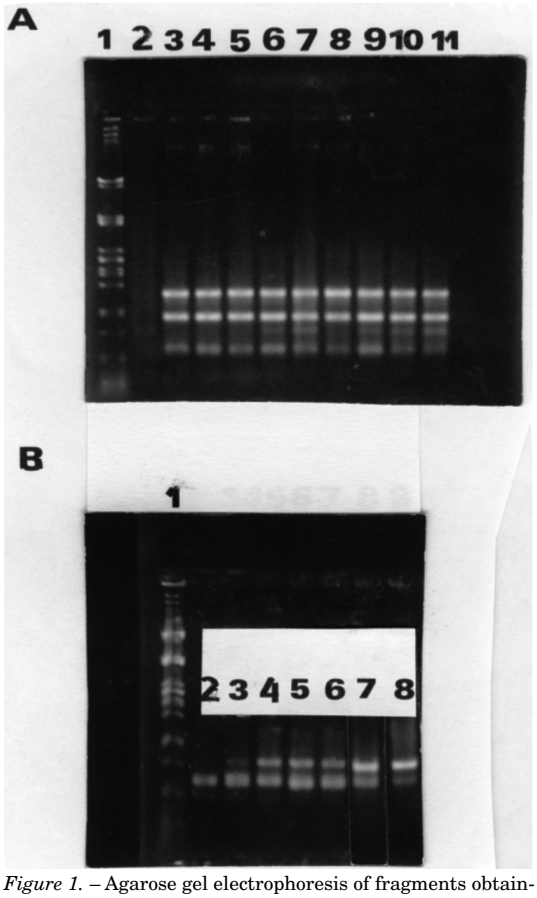

Figure 1. – Agarose gel electrophoresis of fragments obtained by PCR amplification of genomic from *P. alba* "Bolleana" (A) and *P. tremula* "5C" (B) with OPN06 primer, at different concentrations. A) Lanes: (1) lambda *Pst*I, (2) 7.5 ng/µl, (3) 3.7 ng/µl, (4) 0.75 ng/µl, (5) 0.5 ng/µl, (6) 0.375 ng/µl, (7) 0.25 ng/µl, (8) 0.18 ng/µl, (9) 0.15 ng/µl, (10) 0.125 ng/µl and (11) 0.1 ng/µl.
B) Lanes: (1) lambda *Pst*I, (2) 7.5 ng/µl, (3) 3.7 ng/µl, (4) 0.75 ng/µl, (5) 0.5 ng/µl, (6) 0.37 ng/µl, (7) 0.18 ng/µl and (8) 0.15 ng/µl.

Results and Discussion

The limiting factor for amplification appeared to be DNA concentration. In the case of higher concentration, the abundance of phenolic compounds from the sample interfered with

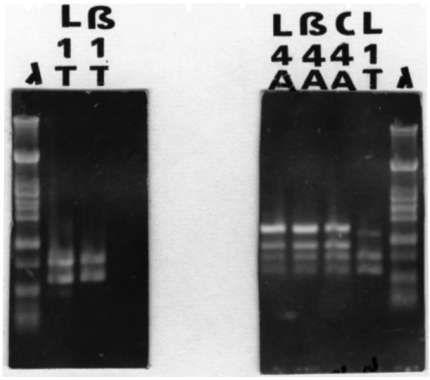

Figure 2. – Agarose-gel electrophoresis of the fragments obtained by PCR amplification from leaf (L), branch (B) and cutting (C) with OPN06 primer. (1T), *P. tremula* "5C" and (4 A) *P. alba* "Bolleana". (λ) lambda *Pst*I, size marker derived from lambda *Pst*I digest.

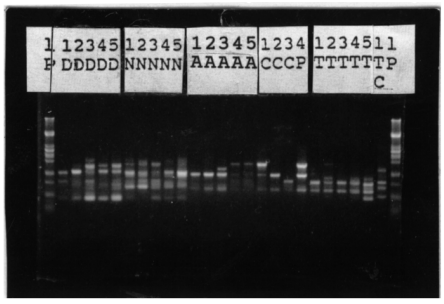

Figure 3. – Agarose-gel electrophoresis of the fragments obtained by PCR amplification of genomic DNA of different clones with OPN06 primer (see *table 1* for identification key). 1P, lambda *Pst*I, size marker derived from lambda *Pst*I digest.

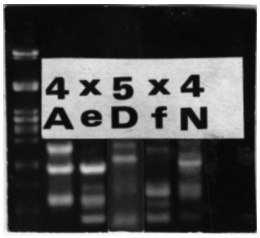

Figure 4. – Agarose-gel electrophoresis of the fragments obtained by PCR amplification of hybrid (xe and xf) and parental (4A and 4N) genomic DNAs with OPN06 primer. (4A). *P. alba,* (xe) *P. deltoides* x *P. alba* 7/32B, (5D) *P. deltoides,* (xf) *P.* x *euroamericana* (*P. deltoides* x *P. nigra*) I-214 and (4N) *P. nigra.*

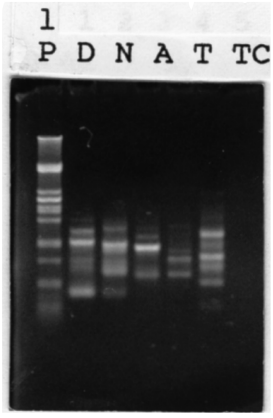

Figure 5. – Agarose-gel electrophoresis of the fragments obtained by PCR amplification with OPN06 primer. Each lane D, N, A, and T are a mix of DNA amplification products of 5 clones for each species. TC is only one clone. (1P) lambda *Pst*I, (D) *P. deltoides,* (N) *P. nigra,* (A) *P. alba,* (T) *P. tremula* and (TC) *P. trichocarpa.*

amplification. The concentration of DNA giving the most consistent results was determined by amplification of a serial dilution from 0.15 ng/µl to 7.5 ng/µl of DNA in the reaction (*Fig. 1 A, B*). The PCR-based DNA amplification from leaf, branch and cutting material from the same clone gave similar results (*Fig. 2*). Extraction from both branches and cuttings are useful for collection of plant material throughout the year.

RAPD patterns were obtained for all 25 primers of kits OPN and OPA at the appropriate dilution. Eight primers were selected and one of them (OPN06, 5'-GAGACGCACA-3') produced polymorphic bands that were informative for each of the 5 species. The amplification of DNA with the OPN06 primer showed the same banding pattern for clones of the same species but a different one for individuals of different species (*Fig. 3*). Hybrid clones *P. deltoides x P. alba* 7/32B and *P. deltoides x P. nigra* could be distinguished from their parent species (*Fig. 4 xe* and *xf*, respectively). In these two cases, the banding pattern was different from both parents although containing some bands from either one or the other parent species.

For *P. x canescens* (a *P. tremula x P. alba* natural hybrid) and *"Platero"* (*P. tremula x P. alba "Bolleana"*), the banding pattern was either the same as for *P. alba* or *P. tremula*, probably because all bands obtained in *P. tremula* samples are also found in *P. alba.* Therefore, the hybrids could not be

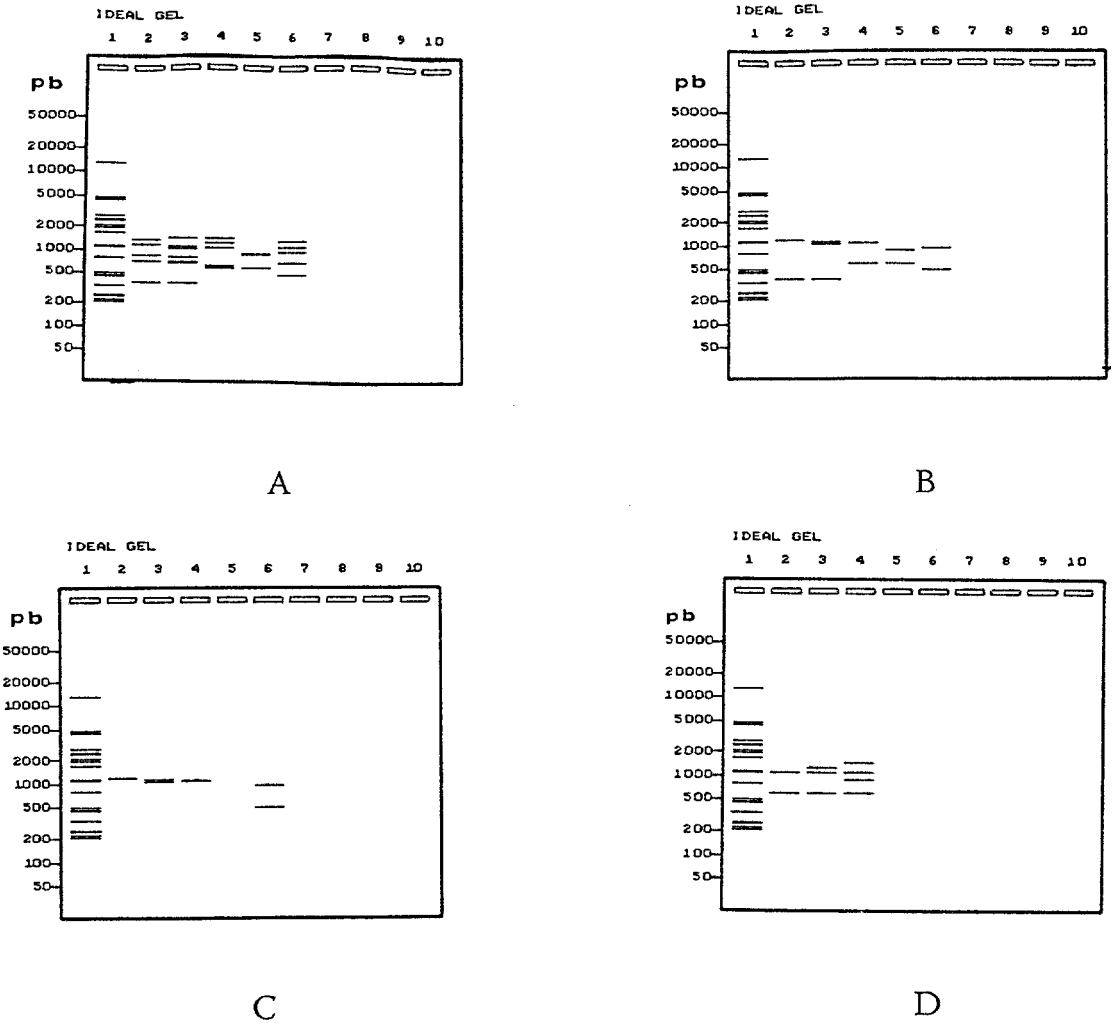

Figure 6. – A) Banding patterns obtained after mingling amplification products of 5 clones. Each species is represented in a lane. B) Banding pattern characterizing each species. C) Identificative banding patterns in each species. Lanes in A), B) and C): (1) lambda *Pst*I, (2) *P. deltoides,* (3) *P. nigra,* (4) *P. alba,* (5) *P. tremula* and (6) *P. trichocarpa.* D) Banding patterns from different groups of *P. alba.* Lanes: (I) lambda *Pst*I; (2) clones "5-4I" and "T"; (3) clone "FA"; (4) clones "Bolleana" and "Raket".

distinguished from the parents. This may be a consequence of the fact that RAPD-based markers are dominant. Alternatively, it is possible that the hybrids are genetically contaminated as a result of back-crossing to one of the parents in the wild. Finally, it is possible that some individuals may have been phenotypically mis-typed as hybrids. Nevertheless, the reliability of markers based on the OPN06 primer is demonstrated by the fact that clones of the same species from different geographic areas have a common RAPD banding pattern.

Figure 5 shows the banding pattern for each species. The products of the PCR reactions from 5 members of each species were pooled before electrophoresis. The exception is *P. trichocarpa*, since only one clone was available.

Figure 6 is series of software derived schematics (ALDEA et al., 1989). *Figure 6A* is a representation of *figure 5. Figure 6B* shows a scheme of the banding pattern characterising each species, so that these bands are common to all clones of the same species. *Figure 6C* shows a scheme of identificative bands for each species, that is to say, bands that are present in a species but absent in all others. In *P. tremula* there are no identificative bands because the two characteristic bands signalled in *figure 6B,* lane 5 are present in some clones of *P. alba,* for instance in "Raket" and "Bolleana". However, *P. tremula* was identified by the absence of the *P. alba* identifiative bands (*Fig. 6B,* lane 4). *Figure 6D* shows 3 different groups of *P. alba;* all three groups have the identificative band of *P. alba,* but at the same time have other bands that identify the group.

Previous analyses of RAPD in *Populus* (CASTIGLIONE et al., 1993; SIGURDSSON et al., 1995; LIN et al., 1994; RANI et al., 1995) have identified many different clones. In this work, the discrimination has been made between species. The different species identified are *P. deltoides, P. nigra, P. alba, P. tremula* and *P. trichocarpa.* This one must be considered a previous study in the case of *P. trichocarpa,* because this hypothesis must be confirmed with more clones.

A deeper knowledge of species and clones of *Populus* fingerprints through molecular markers not only can help to clarify the genetic variability for improvement programs, but also will be useful for the recognition of patent rights.

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Bud Monoterpene Composition in *Pinus brutia* (TEN.), *Pinus halepensis* (MILL.) and their Hybrids

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Abstract

Bud terpene composition was determined by headspace gas chromatography in *Pinus halepensis* (MILL.), *Pinus brutia* (TEN.) and F1 hybrids between the two species (59 trees). The object was to explore the utility of bud monoterpenes in studying hybridization between the two species.

Sixteen components were detected in the bud resin of all the trees, twelve of which identified. No qualitative differences were found in bud terpene composition between the species and the hybrids. *Pinus halepensis* trees had much more α-pinene, myrcene and α-phellandrene whereas *Pinus brutia* had higher amounts of α-pinene and 3-δ-carene. In buds of F1 hybrids the composition was more or less intermediate for most of the

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