Taxonomical studies on some *Populus* species based on DNA polymorphism as fingerprinting

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Abstract: Four *Populus* species studied by using gel electrophoresis of DNA molecules based on five primers, and the obtained results apparently cleared that there were similarity grade among the four *Populus* species where the similarity was the highest value of 90.53 between the *P. deltoides* and *P. euroamericana,* followed by *P. nigra* and *P. deltoides* who register 81.19, followed by *P. nigra* and *P. euroamericana* record of 79.17, followed by *P. euroamericana* and *P. alba* who record 75.862, followed by *P. deltoides* and *P. alba* where recorded 73.91, after that the lowest value was between the *P. nigra* and *P. alba* which recorded 68.82. It means that *P. deltoides* and *P. euroamericana* were much more similar.

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1. Introduction

Using selective genotype linkage analysis, two rapid amplified polymorphic DNA (RAPD) markers, OPAI17-1550 and OPAI13-900, were shown to be linked to resistance locus (Zeng *et al*., 2004). Two cDNA libraries viz. L45-72 and L69-72 were constructed on the basis of anti disease cDNA fragments obtained by fluorescent differential display reverse transcription polymerase chain reaction (DDRT-PCR) using ready-to-use kits and pathogen-inoculated poplar leaves of I-69 and I-45, and two clones either extremely resistant or susceptible to black spot disease in poplar (Zhang *et al*., 2002)and two lipoxygenase genes were cloned in *P. deltoides* (Cheng *et al.,* 2006). However, the global responses of host to pathogen are not known.

In the 1970s*, P. deltoides* cv. 'Lux' (I-69/55) that has a natural distribution in North America was introduced in China as a promising genetic resource for improving resistance of poplars to *M. brunnea* f. sp. *multigermtubi*. Microarray technology has become a useful tool for analyzing the transcription abundance of thousands of genes in parallel. Recently, several successful studies of gene expression profiles responsive to environmental stresses have been reported in poplars (Smith *et al*., 2004; Christopher, 2004).

Sixty-four codons are found in the universal genetic code, which encode 20 different amino acids in the organism world. Owing to the degeneracy of the genetic code, each amino acid may be coded by two or more codons (synonymous codons). However, coding sequences in DNA do not use synonymous codons with equal frequencies within and between organisms (Grantham *et al*., 1980; Lu *et al*., 2005). Previous studies have shown that codon usage is a modulator of the gene expression because of the high correlation between codon usage, tRNA abundance, and the level of gene expression (Holm 1986; Liu *et al*., 2003). Highly expressed genes have shifted their codon usage toward a more restricted set of preferred synonymous codons than other less highly expressed genes. Apparently, the analysis of codon usage of species that express exogenous genes provides a guide for increasing the expression efficiency of exogenous genes.

Populus has tremendous economic and ecological values, which has several advantages, such as rapid growth, prolific sexual reproduction, ease of cloning, small genome, and strong correlation between the physiological traits and the biomass productivity. During the last 20 years, there have been several reports about the genetic transformation in different poplar species including the resistance to herbicide, insect, disease, and salinity (Zhang *et al.,* 2006). It is important to improve the understanding of the mechanism of codon distribution and variation in different poplar species for increasing the expression efficiency of exogenous genes in transgenic poplar plant.

Interspecific *Populus* hybrids are the fastest-growing trees in the temperate zone and have been recognized as an important source of pulp, lumber, and biofuel (Zsuffa *et al*., 1996; Tuskan, 1998). The

positional cloning of genes controlling important traits in *Populus*, and other forest trees, has been difficult (Stirling *et al*., 2001; Zhang *et al*., 2001), in large part because of their long generation time (4–10 years) and poorly known genomes. If there is substantial synteny and collinearity between the genomes of *Populus* and *Arabidopsis*, comparative genomics could provide a powerful alternative approach to gene discovery and isolation in *Populus*, given that the complete DNA sequence of the *Arabidopsis* genome is known (The Arabidopsis Genome Initiative 2000) and rapid progress is being made toward a functional understanding of all *Arabidopsis* genes (Somerville and Dangl, 2000).

The cpDNA genome has been extensively studied in poplar using both the RFLP and the PCR approach (Smith and Sytsma, 1990; Mejnartowicz, 1991; Rajora and Dancik, 1992, 1995a,b,c; Sabsch, 1992; Vornam *et al*., 1994; Heinze, 1998a,b). Initial interest was directed at the development of species-specific markers and whereas (Smith and Sytsma, 1990) found no interspecific variation, several others demonstrated interspecific variation between *P. nigra* and *P. deltoides* (Vornam *et al*., 1994; Rajora and Dancik, 1995a,b,c; Heinze, 1997; Krystufek, 2001; Krystufek *et al*., 2002). Intraspecific variation was also detected in *P. nigra*, as well as in several other poplar species (Sabsch, 1992; Rajora and Dancik, 1995a; Heinze, 1998a; Krystufek, 2001). In many cases this intraspecific variation was dependent on the geographic origin of the maternal line of the tested material. The maternal inheritance of cpDNA in poplar was first demonstrated in controlled crosses by (Mejnartowicz, 1991), and this mode of inheritance was later confirmed by (Rajora and Dancik, 1992). However, in a later paper (Rajora and Dancik, 1995c) questioned the finding that cpDNA is entirely maternally inherited in poplar. The variants found in the *P. euramericana* hybrids that were studied had not been detected in any *P. deltoides*, which had previously been studied. This finding did not fit with the belief that this species had acted as the maternal parent of these hybrids. (Rajora and Dancik, 1995a) suggest that this represents evidence that cpDNA is not entirely maternally inherited in poplar and that there may be parental recombination in *P. euramericana* hybrid clones. This is an important consideration, as it would render the cpDNA molecule unsuitable for phylogenetic studies in poplar. This phenomenon has been detected in other species but not in any other studies involving poplar. Heinze (1998b) is skeptical that these results do indeed present evidence of cpDNA paternal leakage. Instead, he suggests that the results may be due to the existence of undetected cpDNA variants in *P. deltoides* or to probe contamination. In addition, Heinze (1998b) points out that as these results are confined to one particular probe which covers a region which is known to be a mutation hotspot in other species and as these results were detected in hybrids they may reflect sequence instability in hybrids rather than evidence of paternal leakage of cpDNA. Therefore, taken in its entirety, the balance of evidence indicates that cpDNA in *P. nigra* is maternally inherited and therefore variation in the cpDNA molecule has been used in this paper to study postglacial routes of colonisation.

Although no detailed data exist on the distribution of cpDNA variation in black poplar such information exists for *Alnus* glutinosa L. (black alder), which is also a wind pollinated tree species of riparian and waterlogged habitats (King and Ferris, 1998). Alder is common in Europe and the Mediterranean and extends as far as the mountains of Turkey and North Africa. The cpDNA results show that most of northern Europe was colonised from a refuge in the Carpathian region (Hungary and Romania), although two further refugia in Spain and Turkey are suggested.

2. Materials and Methods

2.1. Randomly amplified polymorphic DNA (RAPD)

In this study, RAPD was used for the identification of the four *Populus* species according to Lu *et al*., (1996).

2.1.1. DNA isolation procedure

Young and fresh leaf (Cut leaves from the top branches of developing studied species of the leaves number 3 to number 5) samples were collected separately from 4 trees for each *Populus* species.

The bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN). Isolation protocol of DNA was as follows:

1- Plant tissues were ground using liquid nitrogen to a fine powder, then, the powder was transferred to an appropriately sized tube.
2- Then, 400 µl of buffer AP1 and 4 µl of RNase a stock solution (100 mg/ml) were added to a maximum of 100 mg of ground plant tissues and vortexed vigorously.
3- Mixture was incubated for 10 min at 65oC and mixed 2-3 times during incubation by inverting

tube.

4- Then, 130 µl of buffer AP2 was added to the lysate, mixed and incubated for 5 min on ice.
5- Lysate was applied to the QIA shredder spin column sitting in a 2 ml collection tube and centrifuged for 2 min at maximum speed (10.000 rpm).
6- Supernatant from step 5 was transferred to a new tube without disturbing the cell-debris pellet. Typically, 450 µl of lysate was recovered.
7- Then, 0.5 volume of buffer AP3 and 1 volume of ethanol (96-100%) were added to the cleared lysate and mixed by pipetting.
8- Then, 650 µl of the mixture from step 7 was applied through DNeasy Mini spin column setting in a 2 ml collection tube. Then, centrifuged for 1 min at 8000 rpm and flow-through was then discarded.
9- DNeasy column was then placed in a new 2 ml collection tube. Then, 500 µl buffer AW was added onto the DNeasy column and centrifuged for 1 min at 8000 rpm.
10- Then, 500 µl buffer AW was added to DNeasy column and centrifuged for 2 min at maximum speed (10.000 rpm) to dry the column membrane.
11- DNeasy column was then transferred to a 1.5 ml microfuge tube and 100 µl of preheated (65oC) buffer AE was pipetted directly onto the DNeasy column membrane. Then, incubated for 5 min at room temperature and centrifuged for 1 min at 8000 rpm to elute.
12- Elution was repeated once as described. A new microfuge can be used for first elute. Alternatively, the microfuge tube can be reused for the second elution step to combine the elutes.

2.1.2. Randomly amplified polymorphic DNA-PCR (RAPD-PCR) procedure

PCR reactions were conducted using five arbitrary 10-mer primers. Their names and sequences are shown in table (1).

Table 1. List of the primer names and their nucleotide sequences used in the study for RAPD procedure.

No.	Name	Sequence
1	OP-A10	5′ CTGCTGGGAC 3′
2	OP-A12	5′ TCGGCCATAG 3`
3	OP-C09	5` CTCACCGTCC 3`
4	OP-D01	5` ACCGCGAAGC 3`
5	OP-D07	5` GGACCCAACC 3`

2.1.3. Polymerase chain reaction (PCR) condition stock solutions

A- 5X Tris-borate (TBE), pH 8.0

Tris-base	5.40 g
Boric acid	2.75 g
500 mM EDTA, 8.0	0.29 g
H_2O (d.w) up to	100.00 ml

B- Ethidium bromide

The stock solution was prepared by dissolving 1 g of ethidium bromide in 100 ml distilled water and mixed well with magnetic stirrer. Transferred to a dark bottle and stored at room temperature.

C- Sample loading dye (5x)

Na-EDTA, pH 8.0 (500 mM)	2.00 ml
Glycerol (100%)	5.00 ml
Bromophenol blue (2%)	0.75 ml
H_2O (d.w.)	1.50 ml

PCR was performed in 30-µl volume tubes according to Williams *et al*. (1990) that contained the following:

dNTPs (2.5 mM)	3.00 µl
$MgCl_2$ (25 mM)	3.00 µl
Buffer (10 x)	3.00 µl
Primer (10 pmol)	2.00 µl
Taq DNA polymerse (5U/µl)	0.20 µl
Template DNA (25 ng)	2.00 µl
H_2O (d.w.)	16.80 µl

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94ºC for 4 min followed by 45 cycles of 1 min at 94ºC, 1 min at 36ºC, and 2 min

at 72ºC. The reaction was finally stored at 72ºC for 10 min.

2.1.4. Gel preparation procedure

1- Agarose (1.40 gm) was mixed with (100ml) l x TBE buffer and boiled in microwave.
2- Ethidium bromide (5µl) was added to the melted gel after the temperature became 55°C.
3- The melted gel were poured in the tray of mini-gel apparatus and comb was inserted immediately, then comb was removed when the gel become hardened.
4- The gel was covered by the electrophoretic buffer (1 x TBE).
5- DNA amplified product (15 µl) was loaded in each well DNA ladder (100bp + 2Kbp + 3Kbp) mix was used as standard DNA with molecular weights of 3000, 2000, 1000,900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The run was performed for about 30 min at 80 V in mini submarine gel BioRad.

3. Results and Discussion

3.1. Primers descriptions

Genetic polymorphism in the four *Populus* species studied was investigated by RAPD-PCR analysis using five primers. All primers generated reproducible and informative amplified products that were used to illustrate the genetic distance among the studied species. A total of 63 DNA bands were detected among of them 29 polymorphic bands, 26 monomorphic bands and 8 unique bands. The number of amplified bands of each primer will be illustrated as follow:-

3.1.1. Primer: OP-A10

In our analysis for the four *Populus* species (Table 2), the total number of bands scored within this primer was 16; with a molecular weights ranged from 160 to 1100 bp, number of polymorphic bands was 8 which represented about 50% from the total bands scored. These bands were number and M.Wt bp (‹3,950› ‹4,880› ‹6,800› ‹7,660› ‹8,450› ‹9,370› ‹12,260› ‹14,190›).

Number of monomorphic bands scored in this primer was 5 which represented about 31.3% of the total scored bands. This bands were number and M.Wt bp (‹1,1100› ‹2,1000› ‹11,280› ‹13,220› ‹16,160›).

Three unique bands were detected in *P. alba,* these bands were used as positive specie specific marker. This bands were number and M.Wt bp(‹5,850›‹10,329›‹15,170›) also it had 4 polymorphic bands present in all species except it this bands were number and M.Wt bp (‹3,950› ‹8,450› ‹12,260› ‹14,190›).

The highest value recorded by *P. deltoides* where content 13 bands followed by *P. alba* where content 11 bands and *P. euroamericana* where content 11 bands but do not as *P. alba* in then lowest value recorded by *P. nigra* content 10 bands, so it can be used this primer as a significantly differences between *P. deltoides* and *P. nigra* but cannot used as a significantly differences between other species *P. alba* and *P. euroamericana*.

Genetic distance (GD) was measured as the difference between four studied *Populus* species. In this primer the highest genetic distance was detected between *P. alba* and *P. nigra* species which represents 0.3317. followed by genetic distance between *P. alba* and *P. euroamericana* and also between *P. alba* and P. deltoids which represents 0.2828, and the intermediate recorded between *P. nigra* and *P. deltoides* and also between *P. nigra* and *P. euroamericana* which represents 0.1732, On the other hand, the lowest distance was 0.1414 between *P. deltoides* and *P. euroamericana* species (Table 3). These results show that, there's a great variation among these four *Populus* species in genetic content.

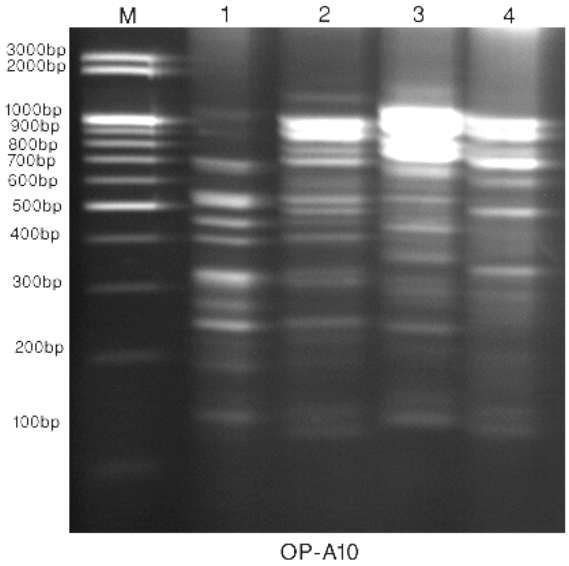

Figure 1. DNA polymorphism of four *Populus* species amplified with primer OP-A10. Lane M: DNA size marker, Lanes 1 to 4: 1- *P. nigra*, 2- *P. alba*, 3- *P. euroamericana*, and 4- *P. deltoides*.

Table 2. Scoring sheet for the polymorphism RAPD reactions resulted by primer OP-A1

Band No.	M.Wt	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*	Polymorphism
		Band score	Band score	Band score	Band score	
1	1100	1	1	1	1	Monomorphic
2	1000	1	1	1	1	Monomorphic
3	950	1	0	1	1	Polymorphic
4	880	0	1	1	1	Polymorphic
5	850	0	1	0	0	Unique
6	800	1	0	0	1	Polymorphic
7	660	0	1	1	1	Polymorphic
8	450	1	0	1	1	Polymorphic
9	370	0	1	0	1	Polymorphic
10	329	0	1	0	0	Unique
11	280	1	1	1	1	Monomorphic
12	260	1	0	1	1	Polymorphic
13	220	1	1	1	1	Monomorphic
14	190	1	0	1	1	Polymorphic
15	170	0	1	0	0	Unique
16	160	1	1	1	1	Monomorphic
Total band score		10C	11B	11B	13A	

Table 3. Genetic distance between different samples detected by primer OP-A10.

Species	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*
P. nigra	0			
P. alba	0.3317	0		
P. euroamericana	0.1732	0.2828	0	
P. deltoides	0.1732	0.2828	0.1414	0

3.1.2. Primer: OP-A12

The total number of bands scored within this primer was 8; with a molecular weight ranged from 180 to 600 bp, number of polymorphic bands was number 1with M.Wt 600bp were found in all species except *P. alba* which represented about 12.5% from the total bands scored also can be used as negative specie specific marker. Number of monomorphic bands scored in this primer was 6 which represented about 75% of the total scored bands this bands was number and M.Wt bp *euroamericana* which all both content as 7 bands all (‹3,460› ‹4,400› ‹5,360› ‹6,320› ‹7,200› ‹8,180›). One unique band number 2 with M.Wt 540 bp was detected *P. nigra* specimen representing 12.5% this can be used as positive specie specific marker (Table 4).

After this show we can resulted that result can be used as a significant differences between *P. nigra* and *P. alba* where *P. nigra* have all bands and P. alba have 6 bands all bands except number1 with M.Wt 600 bp and number 2 with M.Wt 540 bp , but cannot used as a significant differences between *P. deltoides* and *P.* bands except number 2 with M.Wt 540 bp.

In this primer the highest genetic distance was detected between *P. alba* and *P. nigra* species which represents 0.1414. followed by genetic distance between *P. nigra* and *P. deltoides* also between *P. nigra* and *P. euroamericana* also between *P. alba* and *P. euroamericana* also between *P. alba* and *P. deltoides* which represents 0.1000. On the other hand, genetic distance was absent between *P. deltoides* and *P. euroamericana* species since they were close to each other (Table 5).

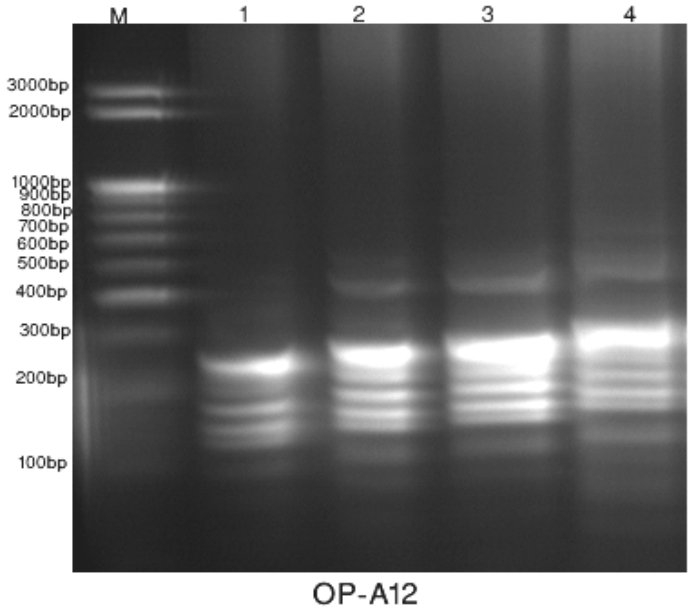

Figure 2. DNA polymorphism of four *Populus* species amplified with primer OP-A12. Lane M: DNA size marker, Lanes 1 to 4: 1- *P. nigra*, 2- *P. alba*, 3- *P. euroamericana* and 4- *P. deltoides*.

Table 4. Scoring sheet for the polymorphism RAPD reactions resulted by primer OP-A12.

Band No.	M.Wt	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*	Polymorphism
		Band score	Band score	Band score	Band score	
1	600	1	0	1	1	Polymorphic
2	540	1	0	0	0	Unique
3	460	1	1	1	1	Monomorphic
4	400	1	1	1	1	Monomorphic
5	360	1	1	1	1	Monomorphic
6	320	1	1	1	1	Monomorphic
7	200	1	1	1	1	Monomorphic
8	180	1	1	1	1	Monomorphic
Total band score		8A	6C	7B	7B	

Table 5. Genetic distance between different samples detected by primer OP-A12.

Species	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*
P. nigra	0			
P. alba	0.1414	0		
P. euroamericana	0.1000	0.1000	0	
P. deltoides	0.1000	0.1000	0	0

3.1.3. Primer: OP-C09

Total number of bands scored within this primer was 11; with a size ranged from 100 to 1050 bp, number of polymorphic bands was 7 which represented about 63.6% from the total bands scored. This bands number with M.Wt bp was (‹1,1050› ‹2,1000› ‹3,940› ‹4,860› ‹5,760› ‹6,500› ‹11,100›). Number of monomorphic bands scored in this primer was 4 which represented about 36.4% of the total scored bands. This bands number with M.Wt bp was (‹7,420› ‹8,360› ‹9,200› ‹10,150›). Unique bands were not detected within this primer (Table 6). *P. euroamericana* content all bands Followed *P. nigra* which content 9 bands i.e. all bands except number 3 with M.Wt 940 bp and number 4 with M.Wt 860 bp. In the intimidate founded P. deltoids which content 8 bands all bands except number 2 with M.Wt 1000 bp and number 3 with M.Wt 940 bp and number 11 with M.Wt 100 bp this band do not fond in this species only so can be used it as a negative specie specific marker. And the lowest value recorded by P. alba where had 6 bands only number and M.Wt bp of this band as followed (‹3,940› ‹7,420› ‹8,360› ‹9,200› ‹10,150› ‹11,100›) and P. alba content three negative specie specific markers this markers them band number 1 with M.Wt 1050 bp and number 5 with M.Wt 760 bp and number 6 with M.Wt 500 bp which do not found in it only and founded in other species. These results cleared significant differences in value of primer OP-C09 among four *Populus* species.

In this primer the highest genetic distance was detected between *P. euroamericana* and *P. alba* species which represents 0.2449. Followed by genetic distance between *P. alba* and *P. nigra* also between *P. alba* and *P. deltoides* which represents 0.2236. The intermediate value recorded between *P. euroamericana* and *P. nigra* also between *P. euroamericana* and *P. deltoides* which recorded 0.1732. On the other hand, the lowest distance was 0.1414 between *P. deltoides* and *P. nigra* species (Table 7). The highest number of bands was scored at *P. deltoides* species and the lowest number of bands was scored at *P. alba* species (Table 6). These results show that there's a great variation between these species in genetic content.

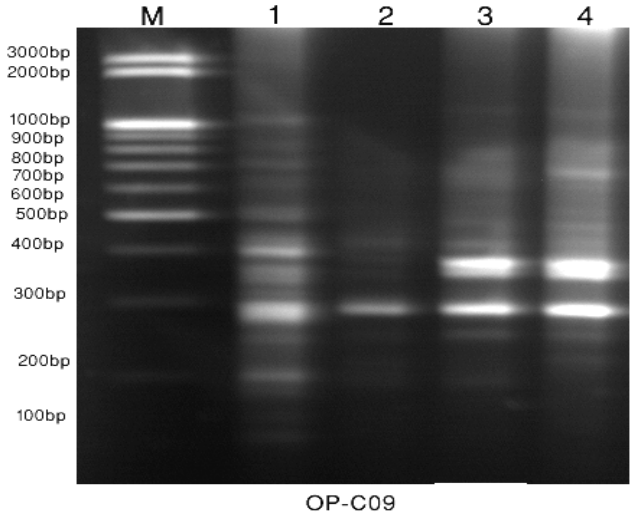

Figure 3. DNA polymorphism of four *Populus* species amplified with primer OPC-09. Lane M: DNA size marker, Lanes 1 to 4: 1- *P. nigra*, 2- *P. alba*, 3- *P. euroamericana* and 4- *P. deltoides*.

Table 6. Scoring sheet for the polymorphic RAPD reactions resulted by primer OP-C09.

Band No.	M.Wt	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*	Polymorphism
		Band score	Band score	Band score	Band score	
1	1050	1	0	1	1	Polymorphic
2	1000	1	0	0	1	Polymorphic
3	940	0	1	0	1	Polymorphic
4	860	0	0	1	1	Polymorphic
5	760	1	0	1	1	Polymorphic
6	500	1	0	1	1	Polymorphic
7	420	1	1	1	1	Monomorphic
8	360	1	1	1	1	Monomorphic
9	200	1	1	1	1	Monomorphic
10	150	1	1	1	1	Monomorphic
11	100	1	1	0	1	Polymorphic
Total band score		9B	6D	8C	11A	

Table 7. Genetic distance between different samples detected by primer OP-C09.

Species	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*
P. nigra	0			
P. alba	0.2236	0		
P. euroamericana	0.1732	0.2449	0	
P. deltoides	0.1414	0.2236	0.1732	0

3.1.4. Primer: OP-D01

Total number of bands scored within this primer was 15; with a size ranged from 608 to 2030 bp, number of polymorphic bands was 7 which represented about 46.7% from the total bands scored; This bands number and M.Wt bp was (‹1,2030› ‹4,1560› ‹8,1119› ‹11,894› ‹13,750› ‹14,691› ‹15,608›). Number of monomorphic bands scored in this primer was 8 which represented about 53.3% of the total scored bands; This bands number and M.Wt bp was (‹2,1830› ‹3,1664› ‹5,1540› ‹6,1340› ‹7,1250› ‹9,1020› ‹10.945› ‹12,820›). *P. nigra* recorded highest value which content 14 bands (all bands except number 1 with M.Wt 2030 bp); followed by *P. euroamericana* which content 13 bands also bands except number 4 with M.Wt 1560 bp and number 15 with M.Wt 608 bp, the intermediate of value recorded represents 0.2646. Followed by genetic distance by *P. deltoides* which content 12 bands all bands except number 4 with M.Wt 1560 bp and number 8 with M.Wt 1119 bp and number 11 with M.Wt 894 bp the last of two bands did not founded in *P. deltoides* only and be used as a negative specie specific markers, the lowest value recorded by *P. alba* which had 11 bands all bands except number 1with M.Wt 2030 bp and number 13 with M.Wt 750 bp and number 14 with M.Wt 691 bp and number 15 with M.Wt 608 bp. The number 13 and 14 did not founded in *P. alba* only and used as a negative specie specific markers. Unique bands were not detected within this primer (Table 8). This result cleared significant differences among four *Populus* species by primer OP-D01.

In this primer the highest genetic distance was detected between *P. deltoides* and *P. alba* species which between *P. alba* and *P. euroamericana* also between *P.*

nigra and *P. deltoides* which represents 0.2000. On the other hand, the lowest distance was 0.1732 between *P. alba* and *P. nigra*. Also, this genetic distance was detected between *P. euroamericana* and *P. nigra* as well as between *P. deltoides* and *P. euroamericana* (Table 9).

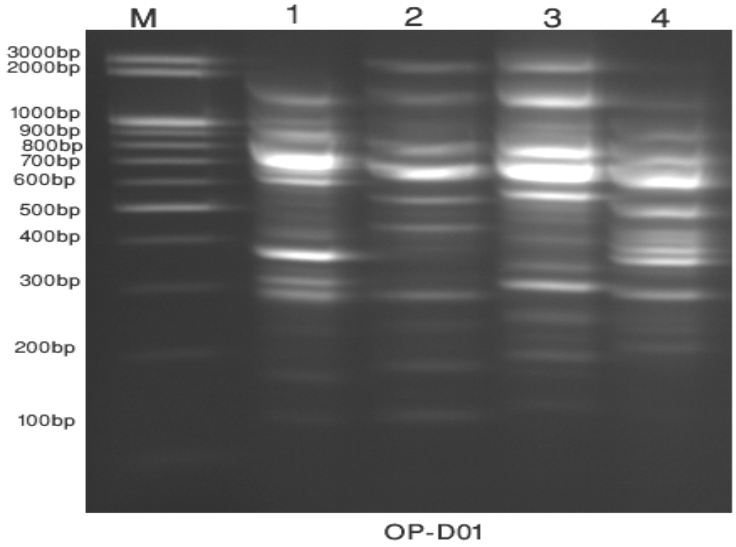

Figure 4. DNA polymorphism of four *Populus* species amplified with primer OP-D01. Lane M: DNA size marker, Lanes 1 to 4: 1- *P. nigra*, 2- *P. alba*, 3- *P. euroamericana* and 4- *P. deltoides*.

Table 8. Scoring sheet for the polymorphic RAPD reactions resulted by primer OP-D01.

Band No.	M.Wt	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*	Polymorphism
		Band score	Band score	Band score	Band score	
1	2030	0	0	1	1	Polymorphic
2	1830	1	1	1	1	Monomorphic
3	1664	1	1	1	1	Monomorphic
4	1560	1	1	0	0	Polymorphic
5	1540	1	1	1	1	Monomorphic
6	1340	1	1	1	1	Monomorphic
7	1250	1	1	1	1	Monomorphic
8	1119	1	1	1	0	Polymorphic
9	1020	1	1	1	1	Monomorphic
10	945	1	1	1	1	Monomorphic
11	894	1	1	1	0	Polymorphic
12	820	1	1	1	1	Monomorphic
13	750	1	0	1	1	Polymorphic
14	691	1	0	1	1	Polymorphic
15	608	1	0	0	1	Polymorphic
Total band score		14A	11D	13B	12C	

Table 9: Genetic distance between different samples detected by primer OPD-01.

Species	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*
P. nigra	0			
P. alba	0.1732	0		
P. euroamericana	0.1732	0.2000	0	
P. deltoides	0.2000	0.2646	0.1732	0

3.1.4. Primer: OP-D07

Total number of bands scored within this primer was 13; with a size ranged from 90 to 1600 bp, number of polymorphic bands was 6 which represented about 46.2% from the total bands scored. (‹1,1600› ‹2,1500› ‹4,700› ‹5,660› ‹7,400› ‹13,90›). Number of monomorphic bands scored in this primer was 3 which represented about 23.1% of the total scored bands. This bands was number and M.Wt bp (‹9,300› ‹11,240› ‹12,270›). Four unique bands were detected *P. nigra* specimen representing 30.7%. These bands were number and M.Wt bp (‹3,960› ‹6,480› ‹8,350› ‹10,280›) (Table 10). The resulted recorded *P. nigra* content 10 bands with the highest value four bands from them unique with it only this bands can be used as a positive specie specific markers specie specific markers and three monomorphic with other species and three polymorphic them number 5 with M.Wt 660 bp and number 7 with M.Wt 400 bp and number 13 with M.Wt 90 bp and the other polymorphic did not founded in it only also can be this as a negative specie specific markers. *P. alba* content 8 bands three with other species monomorphic and five polymorphic number and M.Wt bp of them (‹1,1600› ‹2,1500› ‹4,700› ‹7,400› ‹13,90›)followed by *P. deltoides* which recorded 7 bands three with other as monomorphic and four with polymorphic number and M.Wt bp of them (‹1,1600› ‹2,1500› ‹4,700› ‹5,660›) the lowest value recorded by *P. euroamericana* which had 6 bands three with other as monomorphic and three with polymorphic number and M.Wt bp of them (‹1,1600› ‹2,1500› ‹4,700›) this resulted can be used as significant differences between four *Populus* species by primer OP-D07.

In this primer the highest genetic distance was detected between *P. euroamericana* and *P. nigra* samples which represents 0.3162. Followed by genetic distance was detected between *P. deltoides* and *P. nigra* samples which represent 0.3000 and followed by genetic distance was detected between *P. alba* and *P. nigra* samples which represents 0.2828 and intermediated by genetic distance between *P. alba* and *P. deltoides* which recorded 0.1732 also between *P. alba* and *P. euroamericana* which represents 0.1414. On the other hand, the lowest distance was 0.1000 between *P. deltoides* and *P. euroamericana*. Also, this genetic distance was detected between *P. euroamericana* and *P. nigra* as well as between *P. deltoides* and *P. euroamericana* (Table 11).

From the previous results, genetic diversity among the four species of *Populus* was evaluated, using five primers. All primers generated polymorphism among different species. In total, 63 bands were produced, 29 of which were polymorphic. Primer OPA-10 produced the highest polymorphic bands. The percentage of polymorphic bands ranged from 12.5% to 63.6% with an average of 46%. The average number of polymorphic bands produced was 5.8 per primer. Only the amplified DNA fragments ranging in size between 90 to 2030 bp were used for statistical analyses. Number of monomorphic bands range from 3 to 8 with average 5.2 per primer. Number of unique bands ranged from 1 to 4 with an average of 1.6 per primer, it was observed that most of unique bands scored at *P. nigra* sample. All primers generated unique bands except primers OPC-09 and OPD-01. Size range of bands, No. of polymorphic bands, No. of monomorphic bands and number of unique bands scored within this study by each primer are illustrated in (table 12).

Genetic distance between samples ranged from 0.000 to 0.3317. Cluster analysis based on the presence or absence of bands was performed by dice similarity coefficient, based on Unweighted Pair Group Method with Arithmetic Averages (UPGMA). Genetic similarity ranged between 68.82 and 90.53. Similarity matrix showed that there's a great variation between species specially, between *P. alba* and *P. nigra* samples (Table 13). Dendrogram shows a high similarity between *P. deltoides* and *P. euroamericana* samples (Fig. 6).

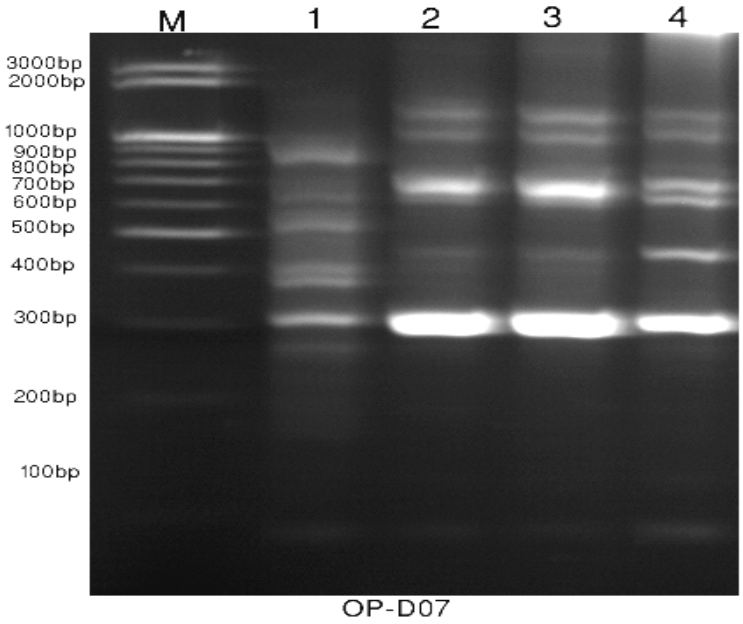

Figure.5. DNA polymorphism of four *Populus* species amplified with primer OP-D07. Lane M: DNA size marker, Lanes 1 to 4: 1- *P. nigra*, 2- *P. alba*, 3- *P. euroamericana* and 4- *P. deltoides*.

Table 10. Scoring sheet for the polymorphic RAPD reactions resulted by primer OP-D07.

Band No.	M.Wt	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*	Polymorphism
		Band score	Band score	Band score	Band score	
1	1600	0	1	1	1	Polymorphic
2	1500	0	1	1	1	Polymorphic
3	960	1	0	0	0	Unique
4	700	0	1	1	1	Polymorphic
5	660	1	0	0	1	Polymorphic
6	480	1	0	0	0	Unique
7	400	1	1	0	0	Polymorphic
8	350	1	0	0	0	Unique
9	300	1	1	1	1	Monomorphic
10	280	1	0	0	0	Unique
11	240	1	1	1	1	Monomorphic
12	270	1	1	1	1	Monomorphic
13	90	1	1	0	0	Polymorphic
Total band score		10A	8B	6D	7C	

Table 11. Genetic distance between different samples detected by primer OP-D07.

Species	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*
P. nigra	0			
P. alba	0.2828	0		
P. euroamericana	0.3162	0.1414	0	
P. deltoides	0.3000	0.1732	0.1000	0

Table 12. List of primers, number of bands, size range of products and polymorphism within each primer.

Primer	Size range of Products (bp)	No. of polymorphic bands	No. of monomorphic bands	No. of unique bands	Total No. of bands	Polymorphism (%)	Monomorphism (%)	Uniqueness (%)
OPA-10	160-1100	8	5	3	16	50	31.3	18.7
OPA-12	180-600	1	6	1	8	12.5	75	12.5
OPC-09	100-1050	7	4	0	11	63.6	36.4	0
OPD-01	608-2030	7	8	0	15	46.7	53.3	0
OPD-07	90-1600	6	3	4	13	46.2	23.1	30.7
Total bands scored		29	26	8	63	46	41.3	12.7

Table 13. Similarity among different species using Dice Coefficient method.

Species	*P. nigra*	*P. alba*	*P. euroamericana*	*P. deltoides*
P. nigra	100			
P. alba	68.82	100		
P. euroamericana	79.17	75.862	100	
P. deltoides	81.19	73.91	90.53	100

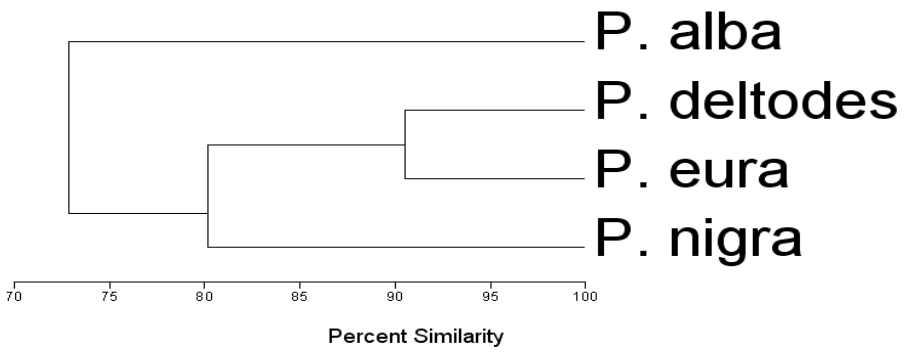

Figure 6. Dendrogram obtained by cluster analysis based on presence/absence matrix for DNA.

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