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Assessment of Genetic Diversity among Poplar (*Populus deltoides* Marsh.) Clones from India using RAPD Markers

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A B S T R A C T

Here we determined the genetic diversity among 19 *Populus deltoides* clones collected from forest nurseries of four districts of Haryana State. All of these were raised in the farm area of the Department of Forestry, and the leaf samples from the young plants of different clones of poplar were collected for molecular analysis. The extraction method (CTAB), DNA purification and, PCR reaction conditions were standardized to obtain genetic diversity.Out of the 30 RAPD markers used in this study, only 11 showed polymorphic pattern and showed a total of 94 alleles. Out of these, 59 were polymorphic, and 35 were monomorphic. An average number of alleles amplified was 8.54. The genetic similarity value calculated varied from 0.20 to 0.73 for 19 Poplar clones.The maximum similarity value (0.73) was observed between clone FRIAM 100 and W32, indicating FRIAM 100 and W32 to be most closely related genotypes.Despite low number of alleles that detected polymorphism, RAP Danalysis indicated that there is high genetic diversity among *Populus deltoides* clones analyzed in this study. Since poplar is a crucial commercial agroforestry tree of Haryana state, this type of genetic characterization of the planting material is a pre-requisite to ensure a broader genetic base of the species.

Introduction

Poplar belongs to the family Salicaceae, order Salicales and group Amentiflorae. The genus comprises of nearly 35 species classified into five major sections (Dickman and Stuart 1983; Eckenwalder 1996). Because of the fast growth habit of its, its compatibility with agriculture crops, along with high industrial needs, the species is commonly cultivated in Indo Gangetic area of the nation (Kaushik and Saini, 2019). The wood on the tree is primarily used for plywood production in India. Plywood industries can also wear the

limbs, roots and tops of the forests as a gas, that will help reduce fossil fuel consumption. Due to its fast growth and broader adaptability, the tree has enormous potential to sequester carbon and mitigate CO_2 from the atmosphere (Dhiman 2009; Gera 2012). Though popular is widely planted in Haryana, yet only a few genetically improved clones have been identified and given to the farmers so far. For the last two decades, farmers have planted mostly two clones, i.e. G-3 and G-48. 90% of all clones planted have begun to exhibit signs of susceptibility to a variety of pathogens. Therefore, the introduction and evaluation of different clones have assumed a great significance in plantation forestry.

Poplar research is facing problems regarding the mixing of clones also. Forestry, unlike agriculture, is a long term proposition and mistakes committed once are reflected after several years or even recognized due to non-identification of the different clones. Identification of different clones of poplar based on morphological characters is nearly impossible due to lack of visible and contrasting traits among different clones. There was often error in interpretation due to variation in polygenic morphological features under different environmental conditions. Thus now a days of advanced biotechnological tools, such as DNA based characteristics have become essential in assessing genetic relationships.

Random amplified polymorphic DNA (RAPD), is a tool which has been used to discriminate and identify genetically diverse genotypes in many plant and animal systems (Williams *et al.,* 1990). As compared to the conventional methods based on morphological traits this method is useful to study genetic diversity in many plant genera such as eucalyptus (Kell and Griffin 1994; Kumar and Kaushik, 2020). Therefore, here we determined the genetic diversity among 19 *Populus deltoides* clones collected from forest nurseries of four districts of Haryana State.

Materials and Methods

Plant material

Healthy vigorous and disease-free cuttings (20-22 cm in length and 1-1.5 cm in thickness) of nineteen clones (Table1) obtained from forest nurseries of four separate locations of Northern India, i.e. Yamunanagar, Karnal, Kurukshetra, and Hisar all of the districts belong to the Haryana, India. Cuttings were planted at 60×80 cm in the second week of February 2015 inthe nursery area, Department of Forestry, CCS HAU, Hisar (29° 10' N latitude and 75° 46' E longitude at an elevation of 215.2 m above mean sea level, Mean annual minimum and maximum temperature was 16.2^{o}C and 31.5^{o}C, respectively). Leaf samples from the young plants of all the clones of poplar were collected for molecular analysis.

Extraction of plant genomic DNA

Total genomic DNA was isolated with the modified cetyl-trimethylammonium bromide (CTAB) method (Saghai-Maroof *et al.,* 1984). Approximately, 5 g leaf material was ground to a fine powder using liquid nitrogen and quickly transferred into 10 ml of pre-warmed (60°C) isolation buffer in a capped polypropylene tube, after that, was kept for 1 h at 65°C in a water bath and mixed by gentle swirling after every 10 min.

To these tubes, a similar volume of chloroform: isoamyl alcoholic was added, and the contents was mixed for 10 min by hand. Tubes were centrifuged for 10 min at 10000 rpm; the aqueous layer was extracted 2 occasions with fresh CI, and also the final aqueous level was transferred to several

centrifuge tube. To these tubes, 0.6 V of ice-cold isopropanol was added and shaken several times.

By using a glass connect, DNA was spooled out there in the type of whitish fibers and flushed with seventy % alcohol and then dried. DNA was dissolved in a suitable amount of 1X Tris EDTA (TE) buffer.

PCR amplification

Thirty- 10base oligonucleotide random primers obtained commercially from Operon Technologies Alameda, California (Table 2) were used in this study. DNA amplification was carried out in 20 µl reaction mixture, each containing 50 ng of template DNA, primers (30ng/µl) – 1.6µl, 1.0 µldNTPs (10mM), TaqDNA polymerase (5U/µl) – 0.2µl and 10X buffer (100MmTrisHCl, 500mM potassium chloride, 1% triton X- 100, 16mM, $MgCl_2$-2µl. PCR amplification was carried out on a Thermal Cycler under the coming conditions: original denaturation at 95°C for five min, after which by forty cycles of denaturing at 95°C for one min, annealing at 36°C for one min, extension at 72°C for two min and a final extension at 72°C for ten min. Agarose gel (1.5%) was used for the amplification and the UV light for visualization.

Data analysis

The frequency of RAPD polymorphism was estimated based on the presence or perhaps absence of typical rings (Ghosh *et al.*, 1997). The binary information was utilized to calculate pairwise similarity coefficient (Jaccard, 1908) on NTSYS pc (version 2.2). A dendrogram according to the similarity coefficient was produced by making use of the unweighted pair group technique of arithmetic means (UPGMA).

Results and Discussion

In the present investigation, 19 clones of *Populus deltoides* were analyzed for polymorphism based on RAPD analysis using 30 random primers. This technique has already been used for study genetic diversity in *Populus deltoides* (Chaudhary *et al.*, 2012), *Morus alba* (Awasthi *et al.*, 2004), *Dalbergiasissoo* (Wang *et al.*, 2011), *Eucalyptus spp.* (Osman *et al.*, 2012), *Jatropha carcus* (Dhillon *et al.*, 2012) and many others. The PCR reaction conditions were standardized to obtain good amplification and clear bands. The influence of various concentrations of genomic DNA, primer, dNTPs, Taq DNA polymerase and PCR standard buffer (1X) and annealing temperatures.

Table.1 List of different Clones of poplar (*Populus deltoides*) used in this study

S. No.	genotype	S. No.	genotype
1	WSL 22	**11**	FRIAM 100
2	FRIAM 72	**12**	W 109
3	S_7C_8	**13**	W32
4	FRIAM 70	**14**	Udai
5	FRIAM 81	**15**	W 22
6	FRIAM 107	**16**	W108
7	Bahar	**17**	W39
8	FRIAM 37	**18**	W110
9	S_7C_1	**19**	G48
10	FRIAM 118		

Table.2 RAPD primers and their annealing and melting temperature used for the *P. deltoids* clones

Sr. No.	Primer	Sequence (5'-3')	Melting temperature (T_m)	Annealing temperature (T_a)
1.	M-122	GTAGACGAGC	32	34.2
2.	M-182	GTTCTCGTGT	30	32.5
3.	M-191	CGATGGCTTT	34	36.1
4.	M-198	GCAGGACTGC	34	37.2
5.	M-33	CCGGCTGGAA	34	32.6
6.	OPA-1	CAGGCCCTTC	34	32.4
7.	OPA-07	GAAACGGGTG	32	36.5
8.	OPA-12	TCGGCGATAG	32	33.5
9.	OPA-15	TTCCGAACCC	32	35.2
10.	OPA-16	AGCCAGCGAA	32	33.5
11.	OPA-20	GTTGCGATCC	32	32.6
12.	OPAF-16	TCCCGGTGAG	34	37.4
13.	OPAG-16	CCTGCGACAG	34	36.2
14.	OPB-05	GTGAGGCGTC	34	32.6
15.	OPB-10	CCCGTTGCCT	34	38.1
16.	OPB12	CCTTGACGCA	32	32.6
17.	OPB-15	GACGGATCAG	32	32.3
18.	OPB-17	AGGGAACGCA	32	33.5
19.	OPB-20	GGACCCTTAC	32	33.7
20.	OPC-02	GGAAGTCGCC	34	37.3
21.	OPC-05	GATGACCGCC	34	36.6
22.	OPC-19	GTTGCCAGCC	34	38.1
23.	OPD-01	ACCGCGAAGC	34	33.7
24.	OPD-03	GTCGCCGTCA	34	32.8
25.	OPD-20	ACCCGGTCAC	34	32.6
26.	OPE-14	TGCGCCTGAG	34	36.6
27.	OPE-20	AACGGTGACC	32	33.5
28.	OPG-09	CTGACGTCAC	34	37.3
29.	OPG-13	CTCTCCGCCA	34	36.6
30	OPI-15	AAGAGAGGGG	32	33.5

Table.3 Random primers showing polymorphism among *Populus deltoides* clones

S. No.	Primer code	The nucleotide sequence (5'-3')	Total No. of amplified fragments	No. of polymorphic fragments	Polymorphic percentage	Fragments range in (bp)
1.	M122	GTAGACGAGC	10	6	60.0	225-1148
2.	M33	CCGGCTGGAA	7	5	71.4	318-1054
3.	OPA-1	CAGGCCCTTC	5	4	80.0	332-1325
4.	OPA12	TCGGCGATAG	12	8	66.6	452-1250
5.	OPA20	GTTGCGATCC	14	10	71.4	276-1175
6.	OPB-5	GTGAGGCGTC	8	5	62.5	165-628
7.	OPB-15	GACGGATCAG	9	4	44.4	221-635
8.	OPC-02	GGAAGTCGCC	6	3	50.0	175-600
9.	OPC-19	GTTGCCAGCC	7	5	71.4	300-1155
10.	OPD03	GTCGCCGTCA	9	5	55.5	292-1146
11.	OPG09	CTGACGTCAC	8	4	50.0	200-1133
Total			94	59	----	
Mean	------		8.54	5.36	62.10	

Fig.1 Dendrogram of 19 poplar clones constructed using UPGMA, Unweighted pair group method of arithmetic means

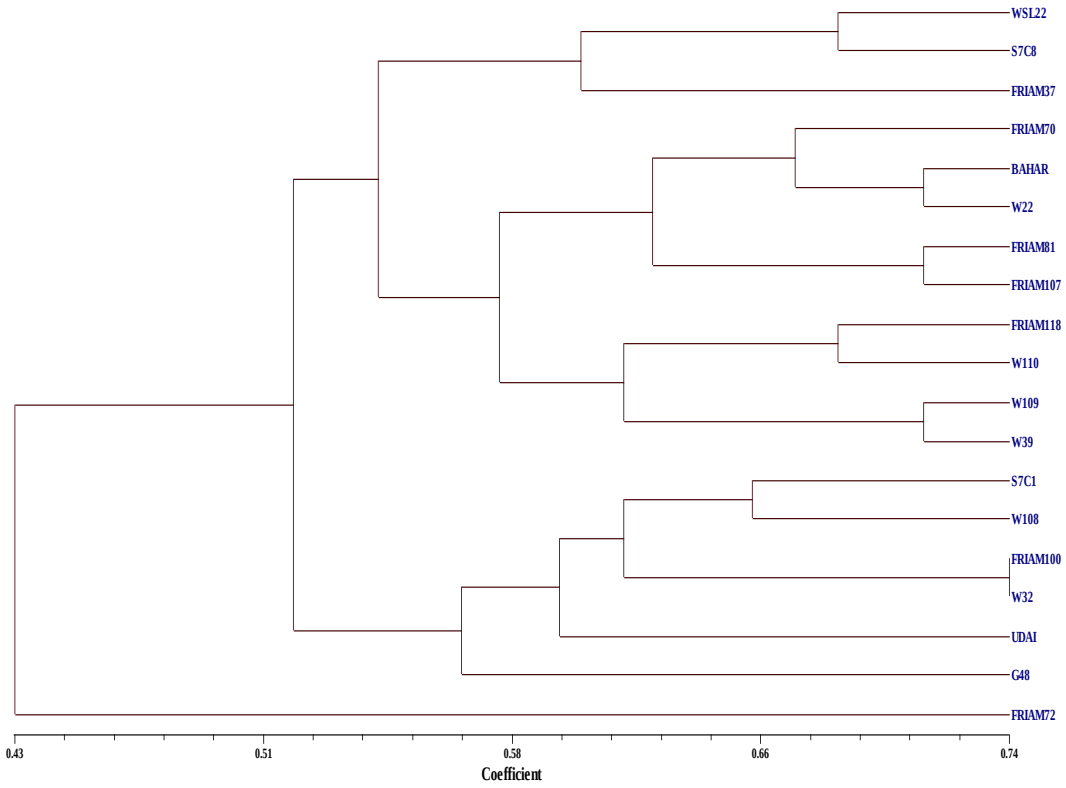
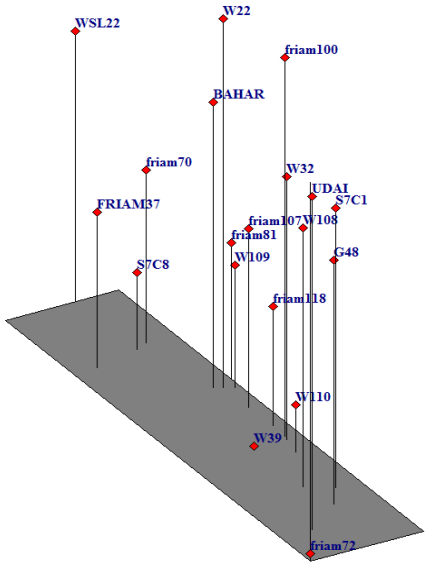


Fig.2 Three dimensional PCA scaling of 19 clones of *Populus deltoides* using RAPD primers



After analyzing amplified products, it was concluded that the concentration of template DNA, Taq DNA polymerase and annealing temperatures were important factors that influenced the banding pattern of the products and reproducibility.

In this study, a total of 30 primers screened, only 11 resulted in polymorphic banding patterns among 19 clones of *Populus deltoides* and showed a high reproducibility by using agarose gel and nucleic acid staining. Clear resolution of both major and minor bands with a consistent reproducibility of amplification patterns was obtained.

A total of 94 fragments were produced, out of which 35 (37.23%) were monomorphic, while 59 (62.76%) were found polymorphic, i.e. variable in at least one genotype. The number of bands (fragments) per primer ranged from 5 (OPA-01) to 14 (OPA-20), the average number of bands per primer being 8.54. The primer OPA-01 resulted in 80 percent polymorphism. The size of the amplified DNA products separated by electrophoresis in 1.5% agarose gel ranged from 165 to 1325 bp (Table 3).

Based on Jaccard's coefficients of similarity values, 19 clones of *Populus deltoides* revealed the genetic relationship among them. The similarity indices between clones ranged from 0.20 to 0.73. A maximum similarity value of 0.73 was observed between clone FRIAM 100 and W32 and minimum similarity value of 0.20 was observed between clone WSL 22 and FRIAM 72. Such a narrow range in similarity co-efficient values suggests that the *P. deltoids*clones collection represented a genetically identical population. The similar finding had already been reported by Kapoor *et al.,* (2014) in poplar.

Based on cluster tree analysis (figure 1), the dendrogram revealed the presence of two distinct clusters C1 and C2 at similarity coefficient 0.43. The former cluster C1 was found to comprise one clone, namely FRIAM 72. The latter cluster C2 was comprised of 18 of the 19 genotypes and thus designated as a major cluster. The second main cluster C2 with 18 clones separated into two major sub-clusters. The first major sub-clusters comprised of 6 clones S_7C_1, W108, FRIAM 100, W32, Udai, G48. The second major sub-cluster contained 12 clones namely WSL 22,

S_7C_8, FRIAM 37, FRIAM 70, Bahar, W22, FRIAM 81, FRIAM 107, FRIAM 118, W110, W109, W39. Other minor-sub- cluster divided into two different groups. The first group comprised of four clones namely S_7C_1, W108, W32 (from Wimco seedlings ltd.) and FRIAM 100. Clones FRIAM 100, W32 were found at the same level. The second major sub-cluster divided into two minor sub-cluster. One minor sub-cluster had 9 clones, and other minor sub-cluster was left with three clones. A detailed study of the first minor sub-cluster revealed three different groups. The first group comprised of four clones, namely FRIAM 118 and three from Wimco seedlings ltd. (W110, W39, W109). The second group had two clones FRIAM 81, FRIAM 107. The third group comprised three clones FRIAM 70, and two from Wimco seedlings ltd. namely Bahar and W22. Other minor sub-cluster had one group of 3 clones namely WSL 22, S_7C_8 and FRIAM 37. This assumption has seen further supported by Farooqui *et al.,* (1998).

Similar clustering of *Populus deltoides* clones, as shown above in dendrogram was also evident from three-dimensional principal component analysis (PCA). The PCA analysis also grouped all the clones into two major clusters. Clone FRIAM 72 was out arranged in the dendrogram, was occupying the periphery position in 3-D PCA (Figure 2). Rest of the clones were grouped into one main group. The genotypes that were closer were more similar than those that were farther. The result is coherent with the dendrogram generated employing UPGMA and is a further confirmation of the genetic similarities delineated in the present study.

Identification of genetic diversity based on genomics methods is also getting popular. It will tend to set new track as the genome sequencing cost is getting cheaper on a daily basis (Kaushik and Kumar, 2018; Kumar and Kaushik, 2019).The level of genetic variation detected within the *Populus deltoides* with RAPD analysis suggested that it is an efficient marker technology for delineating genetic relationships among clones and estimating genetic diversity, thereby enabling the formulation of strategies for management, conservation and tree improvement program.

The diverse clones create an aggressive defensive line which is relatively tough to break. Therefore, diversified plantation with the existing clones, the selection of clones from different groups formed in the dendrogram is recommended. The clones should be selected from the groups which are wide apart from each other. The several clones determined in the research will be helpful for building intraspecific hybrids to exploit hybrid vigour and for also for broadening the genetic base.

Conflict of Interest

Authors declare no conflict of interest.

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