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Research Article

CHARACTERIZATION OF GENETIC DIVERSITY OF POPLAR IN THREE STATES OF NORTH INDIA USING RAPD PRIMERS

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ABSTRACT

Poplar has been recognized as a model species in tree research due to its short life span and ease of propagation and hybridization. Genetic diversity was studied in nine poplar samples collected from three sates of Northern India. A total of 55 bands were amplified in nine poplar samples using 9 RAPD primers. Out of these 30 bands were polymorphic and 25 were monomorphic. Maximum numbers of bands were generated from the primer OPH-20 giving 10 bands out of which 4 were polymorphic. Highest level of polymorphism was obtained with the primer OPH-03 (75%) followed by primer OPH-15 showing polymorphism of 55.5%. The genetic dissimilarity index calculated varied from 0.16 to 0.64 for 9 Poplar samples. The generated dendrogram based on the dissimilarity matrix using the neighbor- joining approach showed three distinct clusters.

Keywords: Poplar, characterization, genetic diversity, molecular markers.

INTRODUCTION

Poplar belongs to genus Populus and family Salicaeceae, includes 32 species. It is a native to the northern hemisphere[1].In India the poplar is grown mainly in the Punjab, Haryana, Uttar Pradesh, Uttrakhand, Himachal Pradesh and Jammu and Kashmir. The genus *Populus* contains at least 35 species out ofwhichsix species of this genus have found to be indigenous. Poplar trees are used in pulp and paper industry. Their ecological relevance is reflected by their wide distribution and their ability to grow on marginal lands[2]. The plant is rich source of secondary metabolites especially tannins and flavonoids.

Molecular markers like Random amplified polymorphic DNA (RAPD), is a powerful tool which involves the use of primers of arbitrary sequence to discriminate and identify genetically diverse genotypes in many plant systems[3].The technique has been successfully used to study genetic diversity in many plant genera such as Pine[4], Torreya[5], and Dalbergia6 etc.

In the genus Populus, RAPD analysis has been performed for evaluation of genetic diversity and clonal identification[7,8]. The objective of the present study was to assess the genetic diversity among poplar of three states of northern India using RAPD primers with the aim of utilizing them for their parent selection and to assess the genetic variability for the management of genetic resources inbreeding programs.

MATERIALS AND METHODS

Leaf samples of nine poplars were collected from different regions of northern India Jammu and Kashmir (JK), Punjab (PB) and Himachal Pradesh (HP) (Table-1).

DNA Isolation

Leaves were collected from young trees and were surface sterilized. The genomic DNA was isolated from sample using the CTAB based method by Saghai-Maroof*et al*.[9]. The quality and quantity of DNA isolated was inferior due to the presence of secondary metabolites in popla. Addition of β mercapethanol and 1%PVP could improve the quantity and concentration of DNA. The RNA was eliminated from the isolated DNA by adding 5U of RNase and incubating the vials at 37°C for 50-60 minutes. The DNA was finally suspended in 1X TE (10mm TrisHCl and 1mM EDTA, pH 8.0) and quantified by UV spectrophotometer and also on 0.8% (w/v) agarose gel and was stored in -20°C till further use.

In vitro amplification of DNA using polymerase chain reaction (PCR)[10]was performed in 0.2ml PCR tubes (Tarsons) using 50-75ng of genomic DNA of each sample in a final volume of 25µl reaction mixture. A total of thirteen different RAPD primers procured from Operon Technologies were used for analysis (Table 2). The PCR reaction mixture contained 5.0 µl template DNA, 12.2 µL ddH_2O, 2.5 µl 10X PCR buffer, 3.5 µl of 100 mMdNTPs, 1.5 µl of 5 µM primer and 0.3 µLTaq polymerase (5 U/µl). Amplification of DNA was done with thermal cycler. Each reaction was performed using initial denaturation of Template DNA at 94°C for 4 min followed by 45 cycles of PCR amplification following: 1 min of denaturation at 94°C, 1 min of primer annealing at 37°C and 2 min of primer extension at 72°C. Final incubation was at 72°C for 7 min so as to complete primer extension. The amplified products were electrophoretically resolved on a 1.5% agarose gel in 0.5X Tris-acetate-EDTA (TAE) and visualized under UV light after staining with 0.1% ethidium bromide.

Data collection and diversity analysis

All the gels were scored manually for monomorphic and polymorphic bands The dissimilarity matrix used for clustering of genotypes was based on the unweightedneighbor-joining method and the analysis was performed using DARWIN 5.0 (http://darwin.cirad.fr)[11]. The genetic dissimilarity was calculated for all the poplar samples under study. To assess the repetitiveness of genotype clustering a bootstrapping of 500 times was performed.

RESULTS AND DISCUSSION

Thirteen different RAPD primers were used to evaluate the level of genetic diversity amongst the different samples of poplar. Out of thirteen RAPD primers, nine primers (OPH-03, OPH-13, OPH-15, OPH-18, OPH-20, OPBA-03, OPU-20, OPA-04, OPA-19) were polymorphic, two primers (OPY-13, OPX19) were monomorphic and two primers (OPBB10, OPBB-11) did not show any amplification.

The amplified product was scored on the basis of presence and absence of bands (Fig 1). The scoring of bands was done independently and only the distinct well separated bands were used to generate the input 1, 0 matrix that was used for all further computations. A total of 55 bands were amplified in nine poplar samples using 9 RAPD primers. Out of these 55 bands, 30 bands were polymorphic and 25 bands were monomorphic (Table 2). Maximum numbers of bands were generated from the primer OPH-20 giving ten bands out of which four were polymorphic, followed by primer OPH-18 generating nine bands with five polymorphic bands. Highest level of polymorphism was obtained with the primer OPH- 03 (75%) followed by primer OPH-15 (66.66%). Lowest polymorphism (40%) was observed with the three primers i.e. OPA-20, OPA-19 and OPU-20.Torjek*et al.*[12]established the genetic diversity of ninteenPopulus genotypes using a set of forty RAPD primers. He observed that nineteen primers gave a species or hybrid-specific pattern.

Du *et al.*[13]studied the population genetic differentiation in the *Populustomentosa* by genotyping 460 unrelated individuals using 20 species-specific microsatellite markers. The highest level of genetic variation was found in the southern followed by the northeastern and northwestern regions. No correlation was found between population genetic distance and geographic, indicating that geographical distance was not the principal factor influencing genetic differentiation in *P. tomentosa*.

Analysis of genetic dissimilarity

The clear bands were scored from the gel and were used to calculate genetic dissimilarity using Dice index of similarity where “0” and “1” were standardized as the least and maximum of dissimilarity respectively. The dissimilarity coefficients used for cluster analysis was based on the unweightedneighbor- joining method and a dendrogram was generated with the aim of analyzing the relationship among Poplar samples collected from different northern regions of India. The genetic dissimilarity index calculated varied from 0.16 to 0.83 for 9 poplar samples (Table 3). The maximum genetic diversity i.e. 0.83 was calculated for samples collected from Satwari (J-2) and Ludhiana (P-2). This shows that the two poplar samples collected from these two places are quite diverse. The lowest value obtained was 0.16 for sample collected from Shahpur (H-3) and Jallandhar (P-3) collected from. These two samples of poplar collected from two different places do not show much diversity and are very similar and may have separated due to human intervention.The genetic dissimilarity between the samples collected from same places also show diversity. This is clearly shown between sample 7 (P-1) and sample 9 (P-3) both collected from Punjab and they lie far from each other in the tree (Fig 3).

Cluster Analysis

The dendrogram revealed the presence of three distinct clusters I, II and III (Fig 2). Cluster I contains poplar sample H-1, J-1 and P-7. Sample

H-1 collected from Nurpur (H.P), sample J-1from Akhnoor (J&K) and Sample P-7 from Pathankot (Punjab) show a close relationship in the dendrogram. In Cluster II sample J-3 collected from Canal road (Jammu), sample J-2 from MBS (Jammu) shows a close relationship as both these samples are collected from same places whereas sample 5 (H-2) collected Kangra (HP) also lies in the same cluster showing that the samples collected from Kangraand the samples collected from Jammu are less diverse. Cluster - III contains samples collected from (Punjab) and Himachal Pradesh. The study shows that the samples collected show diversity. Similarly the samples collected from Kangra (H-2) and Nurpur are diverse (H-1) and distance wise also these two places are around 60kms apart. Chaudhary*et al*.[14]studied the association among the 30 genotypes based on Jaccard coefficient. The dendrogram revealed the presence of two distinct clusters, C1 and C2. The cluster C1 comprised of only three genotypes while cluster C2 comprised of 27 out of the 30 genotypes and thus was designated as a major cluster.

The study demonstrates the utility of using RAPD markers to characterize interspecific relationships and to evaluate germplasm diversity in Poplar plants collected from three different Northern states. Thus this material could be exploited in the future for breeding programs to develop new Poplar varieties.

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Table 1: Poplar accessions and their codes

S. No.	LOCATION	CODE
1	Akhnoor (J&K)	J-1
2	Satwari (J&K)	J-2
3	Canal Road (J&K)	J-3
4	Nurpur (H.P)	H-1
5	Kangra (H.P)	H-2
6	Shahpur (H.P)	H-3
7	Pathankot (PB)	P-1
8	Ludhiana (PB)	P-2
9	Jallandhar (PB)	P-3

Table 2: Level of polymorphism obtained in nine Poplar samples

S. No.	Primer	Total no. of bands	Number of polymorphic bands	Polymorphic percentage %
1	OPH03	8	6	75.0
2	OPH13	5	3	60.0
3	OPH15	3	2	66.6
4	OPH18	9	5	55.5
5	OPH20	10	4	40.0
6	OPBA03	6	3	50.0
7	OPU2O	5	2	40.0
8	OPA04	4	3	75.0
9	OPA19	5	2	40.0
Total		55	30	--
Mean		6.11	3.33	55.80

Table 3: Diversity matrix between nine Poplar samples as revealed by RAPD primers

	J-1	J-2	J-3	H-1	H-2	H-3	P-1	P-2
J-2	0.49							
J-2	0.46	0.25						
H-1	0.30	0.53	0.49					
H-2	0.31	0.30	0.26	0.39				
H-3	0.52	0.62	0.59	0.56	0.49			
P-1	0.28	0.40	0.37	0.32	0.26	0.43		
P-2	0.43	0.83	0.49	0.47	0.34	0.34	0.34	
P-3	**0.54**	**0.64**	**0.60**	**0.58**	**0.50**	**0.16**	**0.46**	**0.36**

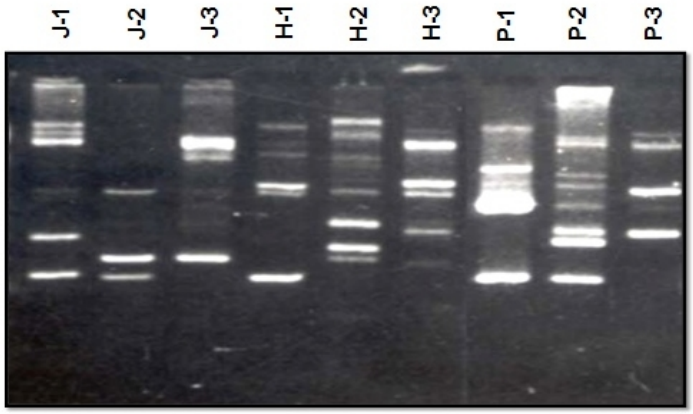

Fig. 1: PCR amplification of nine poplar sample with RAPD primer OPH- 03

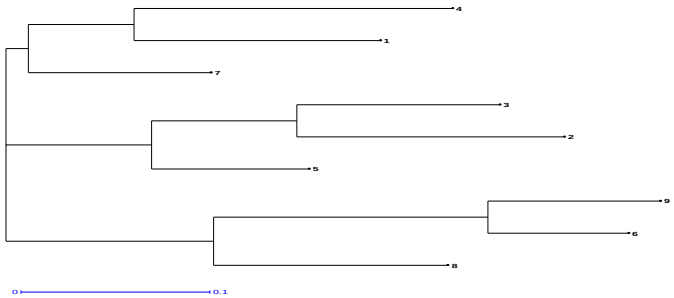

Fig. 2: UPGMA based Dendrogram on nine poplar samples (1=J-1, 2=J-2, 3=J-3, 4=H-1, 5= H-2, 6=H-3, 7= P-1, 8= P-2, 9= P-3)

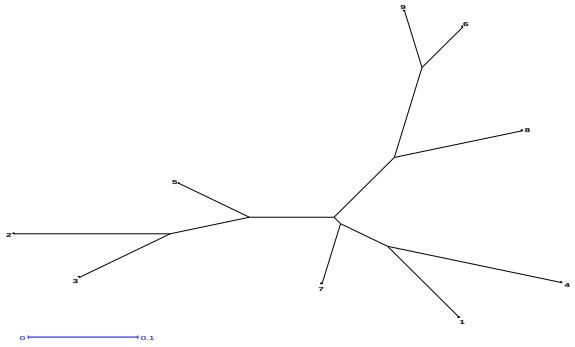

Fig. 3: Tree showing genetic dissimilarity between nine poplar samples (1=J-1, 2=J-2, 3=J-3, 4=H-1, 5= H-2, 6=H-3, 7= P-1, 8= P-2, 9= P-3)

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