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ASSESSMENT OF GENETIC DIVERSITY AMONG HIGH YIELDING SELECTED *SALIX* CLONES, USING RAPD AND SSR MARKERS

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Willows are ecofriendly, fast growing, multipurpose, widely distributed species throughout the world and improvement in breeding programs for biomass and wood quality production is the need of the hour, which can be achieved throughout hybridization programs amongst the desired clones. In this study, molecular characterization, using RAPD and SSR markers, was carried out for the 33 clones, of which 25 clones developed through crossing were more productive with respect to biomass characteristics and the other 8 were check clones. Each RAPD generated a unique set of amplification products ranging in size from 100-2500bp, the total number of alleles ranged from 6 (Deca-7) to 10 (OPO-16). All the eight primers yielded 66 scorable bands with an average band of 8.25 per primer. Of the 10 SSRs used, only eight primers produced SSR profiles with intense banding pattern, which showed some polymorphism between 33 accessions used in the study. Each marker generated a unique set of amplification products. The Jaccard's coefficient values ranged from 0.13 to 0.76 based on RAPD and 0.39 to 0.93 based on SSR Markers. The results indicated fair range of variability in the similarity coefficient values suggesting a wide genetic base of thirty three clones taken in the experiment. Both RAPD and SSR analysis revealed high levels of genetic diversity within the reference set of willow clones.

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Based upon RAPD markers use, clone 14 [PN227 (*S.matsudana*) ×NZ1140 (*S. matsudana* x *S. alba*)] appeared to be most diverse from rest of genotype on the basis of dendrogram. To achieving better genetic gains through hybridisation programs for different desired traits in the species further research work will be helpful.

Keywords: clones, diversity, hybridization, molecular markers, *Salix*.

INTRODUCTION

Short rotation crops (SRC) grown commercially for heat and power generation belong to the plant family *Salicaceae* and include two genera: *Populus* (poplar) and *Salix* (willow). Willow can be found, through natural distribution or introduction, in most parts of the world (NEWSHOLME, 1992), with about 350 – 500 species worldwide (ARGUS, 1997) and generally higher-yielding than poplar (KUZOVKINA *et al*., 2008). In India, there are about 31 indigenous and 4 exotic species of willows (SAINI and SHARMA, 2001). Out of these most of them are categorized as shrubs except *S. alba, S. babylonica, S .daphnoids,S. fragilis, S. elegas* and *S. tetrasperma.* Regarding ploidy level in willows, the haploid chromosome number of genus *Salix* (willow) is n=19, but many species are tetraploid and higher ploidy levels are common. The genus is very heterogeneous and thus shows considerable variation in size, growth form and crown architecture. In 1920s, a willow collection was started at Long Ashton in England; a breeding programme was also initiated around the same time (KARP, 2007).

Willows are ecofriendly, fast growing, multipurpose, widely distributed species and are chiefly used for cricket bats, furniture, plywood, paper and pulp, baskets, hurdles, rope making etc. (KUZOVKINA *et al*., 2008). Others include their carbon sequestration and phytoremediation potential, reclamation of different types of soil, even compacted, swampy, acidic or alkaline under moist conditions. The annual returns from its cultivation are estimated to be around one lakh Indian rupees (1200 Euro) per acre per year. Growing willows especially high quality English willows can help the cricket bat industry to become competitive at global level and penetrate the global markets. The use of plants of the genus *Salix* for phytoremediation of metal-contaminated soils has received much attention in recent times (KUZOVKINA *et al.*, 2004). Different species of *Salix* as well as some clones vary considerably with respect to metal translocation patterns and tolerance to heavy metals (ALI *et al*., 1999). Tolerance to heavy metals such as Pb, Cd, Cu, and Zn has been documented for *Salix* spp and several studies have focused on their potential for phytoextraction of heavy metals from soils (FRICK *et al.,* 1999). Remediation by willow plantation can clear or mitigate hazardous waste stabilize and restore a site and produce wood for fuel besides the world famous willow based cricket industry (WANI *et al*.2011).Willows are also important species in agroforestry, as shelterbelts and windbreaks, soil erosion control, slope stabilization, stream bank stabilization.

A willow breeding programme focuses on improving growth, optimizing relevant traits and lowering production costs to ensure long term viability of willow crop systems for producing energy, restoring degraded sites and improving water quality. The development of new willow clones can reduce the cost of willow crops by increasing biomass yields and optimizing wood chemistry for low input pre-treatment, fractionation and conversion in bio-refineries, besides providing raw material to the fast growing willow based industries. Scope of hybridization of outstanding clones with some local tree willows like *Salix tetrasperma* and *S. acmophylla* lies for producing more productive and ecologically adoptive clones (hybrids). In willow genetic improvement programmes, on one hand, fast growing clonal selection should be promoted for

intensive cultivation and industrial use and on the other hand, increasing tolerance against biotic and abiotic stress should be cultured extensively for commercial purpose (MINGIJAN *et al.*, 2004).

It is pre-requisite to assess the existing variation in any species starting any improvement programme. Genetic diversity can be assessed studying morphological features, but the variation in morphological trait is limited. Moreover, environment and growing conditions also affect the variation. Genetic markers have been extensively developed. Genetic marker is any trait representing genetic differences between individual organisms or species. Generally they do not represent the target genes themselves but act as ʽsignsʼ or ʽflagsʼ. Protein markers such as iso-enzymes have been extensively developed but their use declined due to lack of polymorphism and also being developmental stage dependent. Isozymes have served as genetic markers for fingerprinting clones, the several numbers of available alleles and loci made it impractical to identify all the clones under investigation (BOURNEVILLE and KORBAN, 1987).

With the advent of PCR technique, a revolution came into molecular studies by its multifarious applications. PCR based systems such as Random amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP) and Simple sequence repeat (SSR) are fast and allow maximum automation and are thus being used extensively for DNA based diversity studies. DNA fingerprinting will help to characterize populations, estimate genetic variability, within and among populations and monitor diversity and structure of populations, generated, maintained and manipulated in the breeding process. The screening and maintaining of identity during selection will improve overall reliability. Identification could open market for and prevents theft of proprietary clones.

RAPD markers have been the most widely used molecular marker type in forest trees to date (WHITE *et al.* 2007). They were the first of the PCR based markers for molecular characterization. With its dominant nature and low rate of reproducibility as disadvantages the RAPD marker system is easy to apply as no prior DNA sequence information is needed for designing PCR primers as required for other PCR- based genetic marker system. RAPD marker system has the potential to randomly survey a large proportion of the genome for the presence of polymorphisms. The small amount of DNA needed is a big advantage of the RAPD technique. A co-dominant SSR marker tend to be amongst the most polymorphic genetic marker types (WHITE *et al*. 2007) and are species specific and more reliable than RAPD markers. Application of RAPD and SSR markers at population level provides the opportunity to explore the extent and distribution of genetic variation at a wide range of loci within the species.

In this study, molecular characterization of 33 different clones was carried out using RAPD and SSR markers to evaluate the genetic diversity amongst the different clones, using both dominant and co-dominant marker systems. Of the 33 clones, 25 clones were developed through crossing of different clones and were more productive with respect to biomass characteristics, while as the other 8 were check clones for the study. The results from diversity analysis shall be helpful for further genetic improvement through cross breeding programs.

MATERIALS AND METHODS

Plant material

The Department of Tree Improvement and Genetic Resources, Dr. YS Parmar University of Horticulture and Forestry, Solan has obtained two hundred fifty clones over the years of different willow species and their hybrids from twenty different countries, covering five continents namely Europe, North America, South America, Asia and Australia. These clones were

subjected to repeated nursery screening. Amongst those, 18 clones have been found to be promising from the repeated nursery trials for better biomass productivity. On the basis of five years growth performance, seven clones namely; J-799, NZ-1140, 131/25, J-194, J-795, SI-63-007 and PN-731 were more productive with respect to height, basal diameter and volume index. The high performing clones were used as parents to produce F_1 as well as half sib progenies. These progenies were evaluated in the nursery and superior clones were used in the study. The experiment was conducted over the 25 clones of willows developed from crossing, with 8 selected check clones which have performed better characteristics in selection process and pooled in the Naganji nursery of the main campus of the university (Table 1).

Table1 . Details of the different clones developed and used for the studies

Clone No.	Details of parents
1	PN227 (*S.matsudana*)×131/25 *(S. babylonica x S. alba)*
2	PN227 (*S.matsudana*)×131/25 *(S. babylonica x S. alba)*
3	PN227 (*S.matsudana*)×131/25 *(S. babylonica x S. alba)*
4	PN227 (*S.matsudana*)×131/25 *(S. babylonica x S. alba)*
5	PN227(*S.matsudana*)× AUSTREE *(S.alba x S. matsudana)*
6	PN227(*S.matsudana*)× AUSTREE *(S.alba x S. matsudana)*
7	PN227(*S.matsudana*)× AUSTREE *(S.alba x S. matsudana)*
8	PN227(*S.matsudana*)×JI72 *(S. babylonica x S.alba x S. matsudana)*
9	PN227(*S.matsudana*)×JI72 *(S. babylonica x S.alba x S. matsudana)*
10	PN227(*S.matsudana*)×J795 *(S. matsudana x S. alba)*
11	PN227(*S.matsudana*)×J795 *(S. matsudana x S. alba)*
12	PN227(*S.matsudana*)×J194 *(S. matsudana x S.arbutifolia x S. matsudana)*
13	PN227(*S.matsudana*)×J194 *(S. matsudana x S.arbutifolia x S. matsudana)*
14	PN227(*S.matsudana*)×NZ1140 *(S. matsudana x S. alba)*
15	PN227(*S.matsudana*)×NZ1140 *(S. matsudana x S. alba)*
16	PN227(*S.matsudana*)×NZ1179 (*S. matsudana x S. alba)*
17	PN227(*S.matsudana*)×NZ1179 (*S. matsudana x S. alba)*
18	PN227(*S.matsudana*)×NZ1179 (*S. matsudana x S. alba)*
19	PN227(*S.matsudana*)×*S.tetrasperma*
20	PN227(*S.matsudana*)×SI-63-007 *(S. alba)*
21	PN227(*S.matsudana*)×SI-63-007 *(S. alba)*
22	*S.tetrasperma*OPEN pollinated
23	SE-69-002*(S. matsudana*)OPEN pollinated
24	SE-69-002*(S. matsudana*)OPEN pollinated
25	J799 *(S. matsudana x S. alba*)OPEN pollinated
26	J799 *(S. matsudana x S. alba)*
27	NZ1002 *(S. matsudana x S. alba)*
28	J795*(S. matsudana x S. alba)*
29	SI-64-017*(S. alba)*
30	SE-63-016 (*S.jessoensis)*
31	KASHMIRI *(S. alba)*
32	NZ1040*(S. matsudana x S. alba)*
33	131/25*(S. babylonica x S. alba)*

DNA extraction

Young tender leaves were used for DNA extraction, using the protocol described by CTAB method of DOYLE and DOYLE (1987) with slight modifications. After extraction the genomic DNA (gDNA) was purified by RNase treatment and quantified by using Lambda 25 UV-vis spectrophotometer, followed by electrophoresis on 0.8% agarose gel stained with ethidium bromide. A final concentration of 20 ng/µl gDNA was used for PCR analyses.

Molecular characterization

A total of 12 RAPD primers (BARKER *et al.* 1999) and 10 SSR primers (BARKER *et al.* 2003), which had resulted polymorphic in willows, were used in the present study. Of these, eight RAPD (Table 2) and 8 SSR (Table 3) gave polymorphic amplified products.

Table 2. Nucleotide sequences of 8 RAPD decamer random primers and allelic variation amongst different 33 clones of willow

No	Marker	Base Sequences (5'-3')	Total number of scorable bands	Polymorphism (%)	Allele size(bp)
1	DECA- 7	CCGCCCGGAT	7	85.71	200-700
2	Mosseler-31	CCGGCCTTCC	9	100	150-1000
3	Mosseler-119	ATTGGGCGA	9	100	100-1500
4	OPO - 3	CTGTTGCTAC	7	100	300-1200
5	OPO - 4	AAGTCCGCTC	9	100	150-2200
6	OPO - 12	CAGTGCTGTG	7	100	200-900
7	OPO - 19	GGTGCACGTT	8	100	200-1800
8	OPO – 16	TCGGCGGTTC	10	100	200-2500

PCR amplification reactions were performed for 25 ul mixture, containing sterile distilled water (15.0 µl), Taq buffer (10X) with $MgCl_2$ (2.50 µl), dNTPs (2.5mM) (1.25 µl), Primer (10ng) (2.00 µl; 1 µl forward primer and 1 µl reverse primer), *Taq* DNA polymerase (3U/ µl) (0.25 µl) and 4 µl of template DNA (20 ng/µl) was added to each tube for each amplification reaction in thermal cycler (Corbett Thermal Cycler). For RAPD, tests were performed for standardizing polymerase chain reaction amplification conditions mainly the annealing temperature. PCR amplification conducted at different annealing temperatures i.e. 36°C, 37°C, 38°C using standard concentrations of various components of reaction mixture. The DNA was amplified in a thermal cycler that was programmed as follows: initial denaturation for 3 min at 94°C; 45 cycles of 60 s at 92°C (denaturation), 60 s at 36°C annealing and 120 s at 72°C (extension); and a final extension at 72°C for 10 min. After this, the product was kept at 4°C till electrophoresis was conducted. For SSR markers, PCR optimization was necessary mainly in the annealing temperature. After optimization The DNA was amplified in a thermal cycler programmed at initial denaturation for 3 min at 94°C; 35 cycles of 45 s at 94°C (denaturation), 60 s at annealing temperature and 120 s at 72°C (extension); and a final extension at 72°C for 20 min.

Amplification products were separated on 2% agarose gel using 1× TBE buffer on horizontal gel electrophoresis apparatus (Genei, Bengaluru). Ethidium bromide at a concentration of (5 mg/ml) was used as intercalating agent. RAPD amplification products were mixed with 3 µl (10X orange dye) of loading dye and loaded onto the gel. Gel was run according to 5V/cm of the length of gel till the bands separate. 1 kb Ladder DNA Marker (100-10000bp) for RAPD and 100bp DNA Mass Ladder Marker (100-1000bp) for SSR markers was used as a standard in the first well of each gel.

The amplified bands after separation were visualized using Gel Documentation system and the scoring was done in the binary format for both the marker systems. Further the bands were scored for percentage polymorphism for each set of primer amplified product using NTSYS 2.2 to prepare the similarity index. The bands with same molecular weight and mobility were treated as identical fragments. Data matrices were prepared in which the presence of a band was coded as 1 whereas the absence as 0. The data matrices were analyzed by the SIMQUAL Program of NTSYS-PC (Version 2.2) and similarities between clones were estimated using Jaccard similarity coefficient, calculated as J = A /(N-D), where A is the number of positive matches (i.e. presence of band in both samples), D is the number of negative matches (i.e. absence of band in both samples) and N is the total sample size including both the number of matches and unmatches. Dendrogram was produced from the resultant similarity matrices using the UPGMA method.

RESULTS AND DISCUSSION

Molecular characterisation

Molecular characterizations of thirty three willow clones were investigated using RAPD and SSR markers. The different RAPD primers used in the study have shown varied polymorphism amongst the different clones (Table 2). Summary of RAPD amplified products obtained from 33 clones of Salix species examined in the study is presented in (Table 3). Each RAPD generated a unique set of amplification products ranging in size from 100-2500bp, the total number of alleles ranged from 6 (Deca-7) to 10 (OPO-16). All the eight primers yielded 66 scorable bands with an average band of 8.25 per primer (Table 3). Only eight primers produced SSR profiles with intense banding pattern, which showed polymorphisms between 33 clones used in the study (Table 4). Our observed allele size was in the range of the expected allele size for the different SSR markers used in the study. Each primer generated a unique set of amplification products. Summary of SSR amplified products obtained from 33 clones of willow (Table 3) reveals that the average number of polymorphic bands per primer was 1.625 and allele size range from 80 to 300 bp for the different SSR markers used in the study. The Jaccard's coefficient values ranged from 0.13 to 0.76 based on RAPD and 0.39 to 0.93 based on SSR Markers. The results indicated fair range of variability in the similarity coefficient values suggesting a wide genetic base of thirty three clones taken in the experiment. Both RAPD and SSR analysis revealed high levels of genetic diversity within the reference set of willow clones.

Dendrogram was obtained using the similarity coefficient and unweighted pair group mean average (UPGMA) method in order to visualize genetic differentiation among various clones of willow and to see their clustering pattern.

Table 3. Summary of RAPD and SSR amplified products obtained from 33 clones of Salix spp examined in the study

Information generated	RAPD	SSR
Total number of primers examined	12	10
Number of Polymorphic primers	8	8
Total number of bands amplified from polymorphic primers	66	21
Size range of amplified products	100-2500bp	80-300bp
Average number of bands per polymorphic primer	8.25	2.62
Total number of polymorphic bands identified	66	13
Average number of polymorphic bands per polymorphic primer	8.25	1.625
Per cent of total polymorphic bands	100%	61.90%

Table 4. Nucleotide sequences of 8 SSR primers and allelic variation amongst different clones of willow

	Primer	Sequences	Annealing temp. (°C)	Predicted product size (bp)	Observed size(bp)	Total no. of scorable bands	Polymorphism (%)
1	SB-38	FP-CCACTTGAGGAGTGTAAGGAT RP-CTTAAATGTAAAACTGAATCT	53	144	100-160	2	0.50
2	SB-194	FP-TGTGAGATAAGATTTGTCGGT RP-CCATAAATAAAAAACGTGAAC	51	122	100-300	3	0.67
3	SB-88	FP-TATTGCTTTGATGGCGACTGC RP-CAGCAACGGAAATAGCAACAG	55	87	80-100	2	0.50
4	SB-100	FP-ATGTCATTCAGGTTTGTTTTC RP-ATGGTTTAACTTGTTACTGTA	53	184	100-220	3	0.67
5	SB-196	FP-CTGTTTCCTGCCACTATTACC RP-TATAATCTGTCTCCTTTTGGC	56	212	100-230	3	0.67
6	SB-201	FP-CCTCTTTTTCTATTGTGGTCT RP-GGCATGTATTTTTACTCCAAC	57	144	100-200	4	0.75
7	SB-210	FP-TATAAAGACAAATACCTGGGG RP-CATCAAAGACTGCTAGAAAGG	54	104	100-210	3	0.67
8	SB-85	FP-CTCAGCAACTTAATCCAACTA RP-GTTTGTTAGGGGAGGTAAGAA	53	110	100-200	2	0.50

Based on the molecular characterization using RAPD markers, dendrogram was constructed which revealed clone 14 as distinct (Fig.1). All the clones where divided into two major clusters (A and B) at 31% of similarity coefficient. Genotypes 1 (*$T1_{11}$)*, 6 ($T2_{19}$), 29 (T20), 32(T24), 33(T25), 28(T19), 30(T22), 8($T3_5$), 9($T3_6$), 10($T4_6$), 23($T16_7$), 24($T15_9$), 27($T1_{13}$), 31(T23), 25($T17_7$), 26($T5_6$), 22($T16_5$), 15($T6_6$), 16($T8_{17}$), 3($T1_{14}$), 4(T26), 5($T2_{20}$), 13($T5_7$), 12(T21), 11($T4_7$), 7($T2_{25}$), 20($T9_{21}$) and 21($T9_{18}$) were found in cluster A. Genotypes 2($T1_{12}$), 17($T7_{16}$), 19($T7_6$) and 18($T7_{15}$) where found in cluster B. Cluster A was further divided into three

sub clusters A_1, A_2, and A_3 at 38% similarity coefficient. Sub-cluster A_1 comprises genotype1 (*T1$_{11}$)*, 6(T2$_{19}$), 29(T20), 32(T24), 33(T25), 28(T19), 30(T22), 8(T3$_5$), 9(T3$_6$), 10(T4$_6$), 23(T16$_7$), 24(T15$_9$), 27(T1$_{13}$), 31(T23), 25(T17$_7$), 26(T5$_6$), 22(T16$_5$), 15(T6$_6$) and 16(T8$_{17}$); sub-cluster A_2 comprises genotype 3(T1$_{14}$), 4(T26), 5(T2$_{20}$), 13(T5$_7$), 12(T21) and 11(T4$_7$) whereas sub-cluster A_3 comprises genotype (T2$_{25}$), 20(T9$_{21}$) and 21(T9$_{18}$). In sub-cluster A_1 genotype 1(*T1$_{11}$)* was found distinct from rest of genotypes at 38% similarity coefficient.

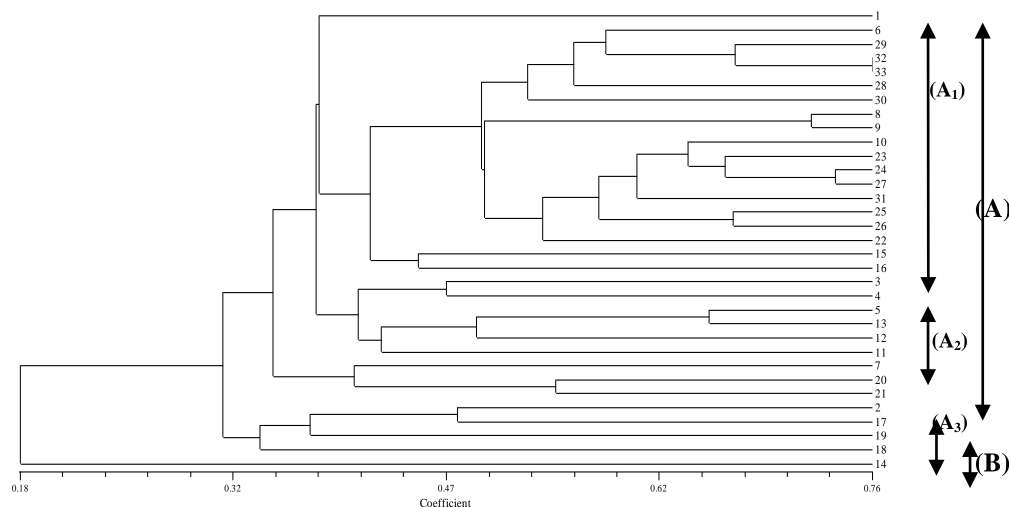

Figure 1. Dendrogram based on UPGMA analysis of 33 clones of willow using RAPD markers

Based on the molecular characterization using SSR markers, dendrogram was constructed (Fig 2). The dendrogram was divided into two major clusters (A and B) at 63% similarity coefficient. Major cluster A comprises genotype 1(T1$_{11}$), 15(T6$_6$), 3(T1$_{14}$), 2(T1$_{12}$), 12(T21), 29(T20), 6(T2$_{19}$), 23(T16$_7$), 25(T17$_7$), 9(T3$_6$), 13(T5$_7$), 28(T19), 4(T26), 16(T8$_{17}$), 11(T4$_7$), 10(T4$_6$), 5(T2$_{20}$), 7(T2$_{25}$), 27(T1$_{13}$), 26(T5$_6$), 14(T6$_4$), 17(T7$_{16}$), 24(T15$_9$), 19(T7$_6$) and 8(T3$_5$). Major cluster B comprised genotypes 20(T9$_{21}$), 22(T16$_5$), 31(T23), 18(T7$_{15}$), 33(T25), 21(T9$_{18}$), 30(T22) and 32(T24). Major cluster A was further divided into two sub-clusters A_1 and A_2 at 68 % similarity coefficient. Sub-cluster A_1 includes genotypes 1(T1$_{11}$), 15(T6$_6$), 3(T1$_{14}$), 2(T1$_{12}$), 12(T21), 29(T20), 6(T2$_{19}$), 23(T16$_7$), 25(T17$_7$), 9(T3$_6$), 13(T5$_7$) and 28(T19) and sub-cluster A_2 comprised genotypes 4(T26), 16(T8$_{17}$), 11(T4$_7$), 10(T4$_6$), 5(T2$_{20}$), 7(T2$_{25}$), 27(T1$_{13}$), 26(T5$_6$), 14(T6$_4$), 17(T7$_{16}$), 24(T15$_9$), 19(T7$_6$) and 8(T3$_5$).

In willow, information on genetic diversity and on genetic relationship within and among species, clones and hybrids in the gene pool is currently limited. RAPDs have been successfully used to assess the genetic diversity within and between populations of *Gliricidia sepium* and *G. maculata* (CHALMER *et al.* 1992), *Populus* species (SANCHEZ *et al.* 1998) and in willow (BARKER et al. 1999), PRZYBOROWSKI et al. (2010) in *Salix viminalis*. Our results from RAPD marker

analysis are in consistent with the studies carried out by LIN *et al.* (1994) on willow clones, BARKER *et al.* (1999) on willow and poplar and by SANCHEZ *et al.* (1998) on *Populus* species, GUPTA (2005) on *Salix alba* clones, JOSHI (2009) on Salix clones and SULIMA et al. (2018) in *Salix purpurea*. All the eight primers were found to produce distinct banding pattern for all the samples. 66 amplified products were detected, all of which 66 (100%) were found to be polymorphic; GUPTA (2005) reported 100 percent polymorphism in *Salix alba* clones. JOSHI (2009) reported 99.7 percent polymorphism. CHOUDHARY et al. (2013) while studying crossability relationship among tree willows (*Salix* spp.) and molecular genetic variation among their progenies by using RAPD primers found 100 percent and 70 percent similarity of hybrid [(*S.tetrasperma* (LG) × *S. matsudana* (TWE)] with male and female parents respectively.

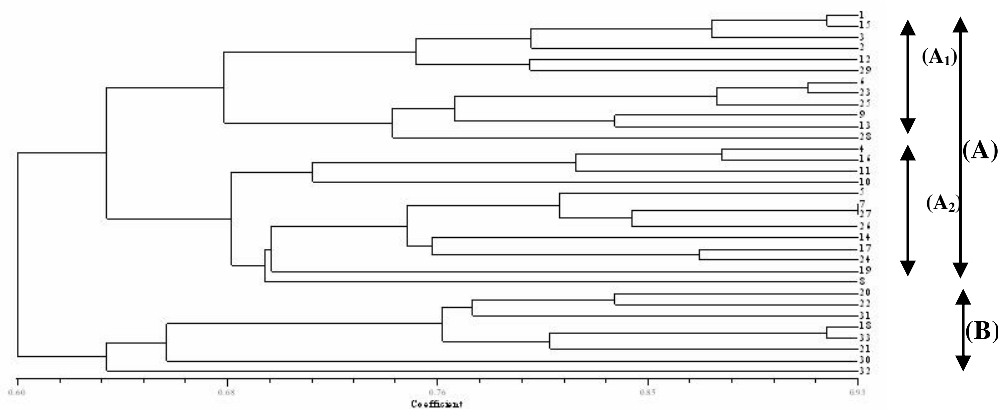

Figure 2. Dendrogram based on UPGMA analysis of 33 clones of willow based on SSR markers

A summary of amplified fragments using SSR (Table 3) revealed that all the eight primers were found to be polymorphic and amplified the genomic DNA of *willow clones* successfully. The number of SSR markers generated per primer varied from 2 to 4 (Table 4). This is consistent with the studies carried out by BARKER *et al.* (2003) on willow clones, LIN and Lawrence (2003) in *Populus* and *Salix* species and by TUSKAN *et al.* (2004) on *Populus trichocarpa and* SINGH *et al.* (2013) in willows. All the eight primers were found to produce distinct banding pattern for all the samples. 21 amplified products were detected, out of which 13 were found to be polymorphic and 8 bands were found to be monomorphic. JOSHI (2009) while working on Salix clones observed 34 amplified bands in which 27 were polymorphic and 7 monomorphic, which was almost in conformity with the present study. JOSHI (2009) while constructing dendrogram based on 10 SSR primers of *Salix* clones found all the clones into four major groups which were further divided into different sub groups. This can provide a lead for obtaining higher genetic gains through hybridization programmes for the species, as suggested by OKCU et al. (2015).

CONCLUSIONS

Although much work has been carried on the genetic improvement of *Salix*, but there is still a lot of scope in the improvement of the genera. The present study has certainly revealed the diversity pattern using RAPD and SSR markers amongst the different clones of willow. The results indicated fair range of variability in the similarity coefficient values suggesting a wide genetic base of thirty three clones taken in the experiment. Both RAPD and SSR analysis revealed high levels of genetic diversity within the reference set of willow clones. Based upon RAPD markers, clone 14 [PN227 (*S.matsudana*) ×NZ1140 (*S. matsudana x S. alba*)] appeared to be most diverse from the rest of genotype. The study shall be helpful in for genetic conservation of willows, selective breeding programs by achieving better genetic gains through hybridization and generation of productive hybrids for mass afforestation programs.

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OCENA GENETIČKE RAZNOVRSNOSTI VISOKOPRINOSNIH KLONOVA *SALIX* POMOĆU RAPD I SSR MARKERA

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Izvod

Vrbe su ekološke, brzo rastuće, višenamenske, široko rasprostranjene vrste širom sveta, a poboljšanje u programima oplemenjivanja proizvodnje biomase i drveta se može postići kroz programe hibridizacije među željenim klonovima. U radu je urađena molekularna karakterizacija, pomoću RAPD I SSR markera, 33 klona, od kojih je 25 dobijeno ukrštanjima imalo veću produkciju biomase, a osam klonova su korišćeni kao kontrola. Svaki RAPD je proizveo jedinstven set amplikovanih proizvoda veličine od 100-2500bp, dok je ukupan broj alela bio od 6 (Deca-7) do 10 (OPO-16). Svih osam prajmera dalo je ukupno 66 traka sa prosečnim brojem od 8,25 po prajmeru. Od 10 SSR-ova, samo je osam dalo profile sa izraženim trakama, i pokazalo polimorfizam između 33 ispitana uzorka. Svaki marker je dao jedinstven set amplifikovanih proizvoda. Jaccard-ov koeficijent je varirao od 0.13-0.76 za RAPD i od 0.39-0.93 za SSR markere. Rezultati ukazuju na nisku varijabilnost koeficijenata sličnosti, ukazujući na široku genetičku osnovu ispitivanih klonova. Obe vrste markera otkrile su visok stepen genetičkog diverziteta u setu ispitivanih klonova. Na osnovu primenjenih RAPD markera, klon 14[PN227 (*S.matsudana*) ×NZ1140 *(S. matsudana x S. alba)*] je najudaljeniji na dendrogramu u odnosu na ostale. Da bi se ostvarila veća genetčka dobit kroz programe hibridizacije za različita poželjna svojstva, potrebna su dalja istraživanja.

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