Molecular Diversity in Willow Clones Selected for Commercial Plantation

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Twenty-five promising willow clones from six countries were selected after nursery screening. Molecular diversity among the clones was estimated using 16 RAPD and 14 SSR primers. A total of 363 markers were generated and out of which 351 were showing high (96.7%) rate of polymorphism. Out of 30, 18 primers showed 100% polymorphism. RAPD markers widely scored over SSR markers in polymorphic information content. UPGMA dendrogram based on both RAPD and SSR markers resolved into four major clusters. The similarity coefficient among 25 clones of willow ranged from 0.71 to 0.86. Maximum similarity (86%) was observed between 795 and PN-721 and SI-63-007 and V-99. Thus, these genotypes showed maximum degree of similarity in their genetic makeup. However, minimum values were observed between SE-69-002 and 84/11 (0.71) followed by SE-69-002 and 17-93-A (0.72). RAPD and SSR analysis proved helpful for estimating the magnitude of genetic diversity at molecular level. Clustering further indicated that the geographic distribution may not be the true index of genetic diversity in willow clones. On the basis of banding pattern RAPDs and SSRs were effectively used for molecular characterization of willow clones used in this study.

Key Words: Characterization, Molecular diversity, RAPD, SSR, Willow

Introduction

The introduction of DNA markers provided a good discriminatory system, independent of environmental conditions. The random amplified polymorphic DNA (RAPD) technique has been applied in several studies to successfully distinguish between tree species or clones. Nowadays, simple sequence repeat (SSR) have been proven to be very suitable markers for cultivar identification in olive as they are transferable, highly polymorphic and co-dominant markers (Carriero *et al*., 2002; Cipriani *et al*., 2002). These methods have no requirement for probes or radioactive material, and can, therefore, be applied immediately. They have proven particularly useful in identification of clones and in characterization of genetic diversity. Over the past decade, a number of molecular techniques have been developed that can provide information on genetic diversity and genetic relationships. Although, there are potentially many techniques to choose from, the choice becomes more restricted when few prior molecular studies have been carried out, as is the case for willow (Lin *et al*., 1994a; Lin *et al*., 1994b). Molecular markers play an essential role today in all aspects of plant breeding, ranging from the identification of genes responsible for marker-aided selection to quantitative trait loci studies.

Increasing concerns over global climate change have led to very practical emphasis in exploiting plants as sources of renewable energy for heat. High yielding perennial biomass crops under short rotation forestry are projected to make important contributions to the future energy mix due to low inputs and, thus, favorable energy and greenhouse gas balances associated with their production systems. Willows, grown as short rotation coppice, are among the most advanced biomass crops in temperate and sub-tropical regions because of their potential for high yields in short cultivation cycles, ease of vegetative propagation and ability to resprout after multiple harvests. A major advantage offered by willow, however, is the large genetic diversity that is present in the genus, which, together with the proven characteristics *i.e.* easy hybridization, provides a rich resource for crop improvement.

The genus *Salix* comprises about 350–500 species worldwide. Some of the tree species have been cultivated for a variety of end-uses *viz.* baskets, cricket bats, handles, furniture's etc. The genus is distributed over wide ecological and climatic zones ranging easterly from North America to China, excluding Australasia (Skvortsov, 1999). *Salix* tree improvement projects are vigorously taken up in many countries in the world. Research mission include the study of willow taxonomy,

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morphology, physiology, cultivation practices, advanced breeding, molecular fingerprinting and genome mapping (Kuzovkina *et al*., 2008). Wide range of collection, evaluation and multiplication of superior willow clones and production of interspecific hybrids are necessary for increasing biomass production (Zsuffa and Mosseler, 1986). Under such evaluation and distribution study, proper identification of species and clones is essential. Efforts are currently underway to breed willows for biomass production, wood production, soil conservation, energy plantation and myriad wood products for cricket bat (Singh and, Huse, 2004; Singh *et al*., 2004). Around 200 clones/strains covering twenty-five species from twenty countries were procured and subjected for repeated nursery screening at university campus. Field trials were undertaken in order to assess suitability of clones and juvenile mature correlation in different agro-climatic regions.

Molecular markers specially RAPD and SSR are being applied to a greater extent in forest trees to study genetic diversity and contributes about 25 per cent and 19 per cent respectively of the total molecular markers used in forest biotech activities (FAO, 2004). Molecular markers assess variations in the nucleotide sequence of DNA of different individuals. Molecular markers are numerous and therefore a large genome can be easily assayed for existence of any variation, such genetic markers are easy to score. Molecular markers were earlier used for studying genetic relationships among 154 genotypes, including 50 species, held within the UK National Willow Collection (Trybush *et al.,* 2008). Use of molecular markers therefore provides an objective assessment of genetic diversity in a plant species and enables unequivocal identification of elite genotypes.

In *Salix* various molecular markers *viz.,* RAPD, SSR and AFLP have been used to assess genetic diversity for germplasm characterization (Barker *et al*., 1993; Barker *et al*., 2003). However, the clones selected in the present study were not investigated earlier for molecular characterization. The present investigation was undertaken with the objective of estimation of genetic diversity using RAPD and SSR markers.

Materials and Methods

Plant Material

A set of twenty five willow clones (Table 1) of genus *Salix* were procured from five countries namely, UK, Italy, New Zealand, Croatia and Turkey. These clones were selected based on their performance from nursery screening experiments done on the basis of plant height and collar diameter.

Table 1. Original identity with scientific name and source country

Treat-ment	Original Identity	Scientific name	Source country (procured from)
T1	PN-733	*Salix nigra* Marsh.	New Zealand
T2	17-93-A	*S. alba* Linn.	New Zealand
T3	SE-63-016	*S. jessonensis* Seeman.	Italy
T4	PN-722	*S.matsudana* Kiodz.	New Zealand
T5	212/03	*S.matsudana* X *S. caprea*	UK
T6	84/11	*S.alba* Linn.	Turkey
T7	MB-368	*S.alba* Linn.	Croatia
T8	PN-227	*S.matsudana* Koidz.	New Zealand
T9	SI-64-017	*S.alba* Linn.	Italy
T10	NZ-1002	S.matsudana X S.alba	New Zealand
T11	795	S.matsudana X S.alba	UK
T12	SI-63-007	*S.alba* Linn.	Italy
T13	V-99	Salix X rubens	Croatia
T14	NZ-1179	S.matsudana X S.alba	UK
T15	PN-721	S.matsudana X S.alba	New Zealand.
T16	131/25	S. alba X S. babylonica	UK
T17	799	S.matsudana X S.alba	UK
T18	PN-731	*S.nigra* Marsh.	New Zealand
T19	NZ-1040	S.matsudana X S.alba	New Zealand
T20	NZ-1140	S.matsudana X S.alba	UK
T21	SE-75-001	*S.matsudana* Koidz.	Italy
T22	NZ-1130	S.matsudanaa X S.alba	New Zealand
T23	V-311	*S.matsudana* Koidz.	Italy
T24	SE-69-002	*S.matsudana* Koidz.	Italy
T25	Kashmir Willow	*S.alba cv. Coerulea* (Smith) Dumort.	UK

Genomic DNA Extraction

Young leaf tissue was powdered in liquid nitrogen. The powder was either stored at -40^{0}C or used for DNA isolation immediately. Total genomic DNA was isolated using the CTAB (Cetyle- trimethyl ammonium bromide) method advocated by Doyle and Doyle (1987) with slight modification made in buffer concentrations. The quality of DNA was tested on 0.8 per cent agarose gel (Genei Banglore, Banglore India) and quantification was done using Perkin Elmer UV/VIS spectrophotometer and diluted to 5ng/µl for further PCR (Polymerase Chain Reaction) amplification using CR Corbett thermocycler.

PCR Amplification and Electrophoresis

RAPD amplification

Sixteen out of 20 initially screened decamer primers (Genei Banglore, Banglore India) were used for the current study (Table 2). DNA was amplified by PCR amplification reaction. The 25µl of reaction mixture contained 20ng of DNA, 0.75 units of Taq DNA polymerase (Genei Bangalore, Bangalore India), 2.5µl of 10X Taq buffer (50mM MgCl2, 10mM Tris-Cl), 1.25µl of pooled dNTP's (2.5mM each) and 10ng of primer (Genei Bangalore, Bangalore India). PCR conditions used for RAPD amplification included initial denaturation for 3minutes at 94^0C followed by 45 cycles of amplification (denaturation at 92^0C for 45 seconds, annealing of primer at 36^0C for 1 minute and primer amplification at 72^0C for 2 min) and final extension at 72^0C for 10 minutes.

SSR amplification

A set of fouteen SSR primers (Genei Bangalore, Bangalore India) were selected (Barker *et al.,* 2003) for PCR analysis based upon their performance and reproducibility, among them 10 primers had given distinct polymorphism (Table 4). PCR mixture of 25µl contained 20ng of genomic DNA template, 0.6 unit of Taq DNA polymerase (Genei Bangalore, Bangalore India), 2.5µl of 10X Taq buffer (50mM MgCl2, 10mM Tris-Cl), 1.25µl of pooled dNTP's (2.5mM), 20ng of each reverse and forward primer (Genei Bangalore, Bangalore India). PCR conditions used for SSR amplification were initial denaturation at 94^0C for 3 minutes followed by 35 cycles of amplification (denaturation at 94^0C for 45 sec, annealing of primer ranged from 55-60^0C for 1 minute and primer amplification at 72^0C for 2 min) and final extension at 72^0C for 20 minutes.

Electrophoresis

The amplified products as developed by the primers were separated on agarose 1.4 % and 2.5 % gel in 1X TAE, stained in ethidium bromide respectively for RAPD and SSR through electrophoresis and photographed in Alpha Imager Gel Documentation System. 1kb and 100bp DNA mass ladder (Genei Bangalore, Bangalore India) were used as molecular weight markers in first well of respective gel.

Data Analysis

The RAPD and SSR marker amplification profile of 25 clones was used to estimate genetic diversity/relatedness based on number of shared amplified bands. The presence or absence of a particular amplification product was used as an index of genetic diversity/relatedness. The similarity matrix value based on Jaccard's coefficient of similarity (Simqual function) was used to generate dendrogram.

Table 2. Total numbers of amplified and polymorphic fragments generated by PCR using RAPD primers along with their nucleotide sequences

S. No	Primer name	Base sequences (5'-3')	Total no. of scorable bands (y)	Total no. of polymorphic bands(x)	Total no. of monomorphic bands	Polymorphism (%) $\frac{x}{y}X100$	Size range of amplified products (bp)
1	OPJ-05	CTCCA-TGGGG	15	15	0	100	300-3500
2	OPO-03	CTGT-TGCTAC	25	25	0	100	350-2250
3	OPO-04	AAGTC-CGCTC	21	21	0	100	350-3500
4	OPO-06	CCACG-GGAAG	8	7	1	87.5	300-1500
5	OPO-07	CAGCA-CTGAC	24	24	0	100	300-1750
6	OPO-12	CAGTG-CTGTG	22	22	0	100	250-2000
7	OPO-15	TGGC-GTCCTT	19	19	0	100	400-2250
8	OPO-19	GGTGC-ACGTT	20	20	0	100	300-1750
9	OPO-16	TCGGC-GGTTC	10	10	0	100	200-2500
10	OPA-17	GACCG-CTTGT	24	24	0	100	300-2000
11	OPA-18	AGGTGACCGT	29	29	0	100	150-2500
12	OPA-19	CAAACGTCGG	24	24	0	100	250-2000
13	OPA-20	GTTGCGATCC	18	18	0	100	300-2500
14	DECA-05	CCAAGGGGGC	32	32	0	100	200-3800
15	DECA-07	CCGCCCGGAT	28	28	0	100	200-3500
16	DECA-12	CTTGCCCACG	20	20	0	100	200-2000
Total			339	338	1	99.7	150-3800

Clustering was done by UPGMA using SHAN module of NTSYSpc. Version 2.02e (Rohlf, 1998).

Results and Discussion

RAPD analysis

Out of 16 RAPD primers (Table 2) selected for study, three primers showed uniqueness by producing specific bands for three genotypes specific bands (Table 3). A total of 339 amplified products were obtained (using alpha imager software tool) with an average of 21.2 amplicons/primer, out of which 338 were polymorphic showing high degree of polymorphism (99.4%). The number of RAPD markers generated per primer varied from 8 to 32 because of the primer sequence and due to individual genotype (Table 2). This is consistent with the studies carried out in willow and poplar clones (Barker et al., 1993). Earlier Castiglione *et al.* (1993) performed RAPD analysis on 32 clones belonging to different species of genus Populus. Four primers out of eighteen tested were selected on the basis of number and frequency of the polymorphism produced. The results showed that the RAPD analysis allowed discriminating all the clones. DNA amplification pattern as detected by four RAPD primers used for in the present study has been presented in figures 1 (a) and 1 (b).

Primers namely OPO-07, OPO - 16 [Fig 1(a)], and OPO-19, produced unique bands present only in genotypes 17-93-A, 212/03, and SI-64-017 respectively. This information could be used for characterizing particular genotypes (Table 3). Similar trends were obtained using allozyme loci, nuclear DNA Restriction Fragment Length Polymorphism (RFLP's) and Random Amplified Polymorphic DNA (RAPD's) in 130 trembling aspen (*Populus tremuloides* Michx) and 105 bigtooth aspen (*P. grandidentata* Michx) trees (Liu and Furnier, 1993). RAPD proved to be a very powerful tool for fingerprinting aspen individuals. Earlier working on the same lines Su *et al.* (1995) worked out the variation in the genomes of 16 clones in two *Salix* species (*S. viminalis* and *S. dasyclados*) using 12 random decamer primers. The amplified DNA fragments recorded 5-18 clear bands ranging from 220-2036 bp in length in case of S. dasyclados Wimm by the primer CHL1, while all the 16 clones could be identified by the primer Deca-4 which produced large number of polymorphic bands. Similarily Sanchez *et al.* (1998) used RAPD markers to distinguish 25 poplar clones, namely, five of *Populus nigra*, five of *P. deltodies*, five of *P. alba*, five of *P. tremula*, one of *P. trichocarpa*, three of *P.* x *canescens* and one clone (*P. tremula* x *P. alba* "Bolleana") *i.e. Platero*

Table 3. Informative RAPD and SSR markers specific for a particular clone

Primer	Approximate size of DNA band	Clone
OPO-4	300bp	17-93-A
OPO-19	1500bp	212/03
OPO-16	400bp	SI-64-017
OPO-16	1750bp	SI-64-017
SB-80	200bp	SE-75-001

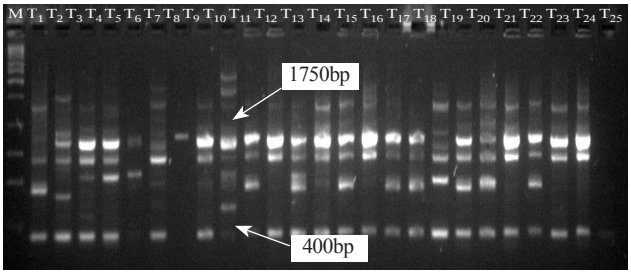

Fig. 1(a). RAPD fingerprints of willow clones using Primer OPO- 16

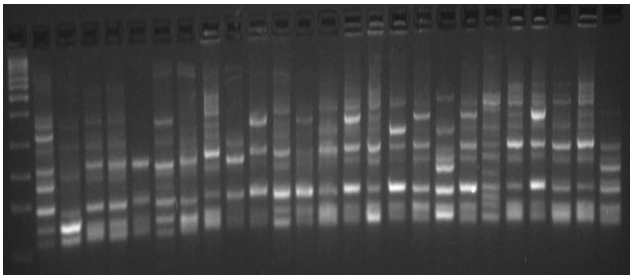

Fig. 1 (b). RAPD fingerprints of willow clones using Primer OPO- 04

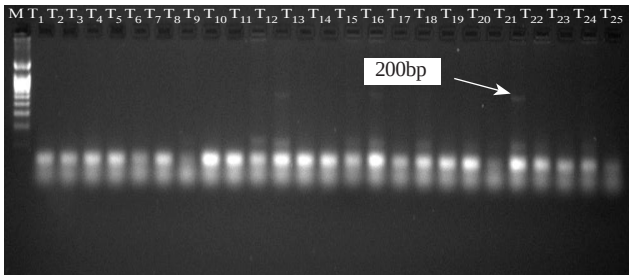

Fig. 2(a). SSR fingerprints of willow clones using Primer SB-80

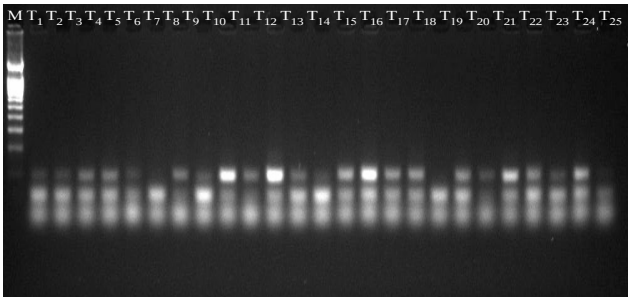

Fig. 2 (b). SSR fingerprints of willow clones using Primer SB-100

SSR Analysis

Ten out of 14 SSR primers selected for study (Table 4) showed polymorphism and selected for further analysis as given in Fig. 2 (a) and Fig.2 (b). Out of these, one primer (SB-80) exhibited unique band (Table 3) present only in clone SE-75-001 from Italy [Fig. 2(a)]. A total of 34 amplified products were obtained with an average of 3.4 amplicons/primer, out of which 27 (79.4%) bands were polymorphic. The number of SSR markers generated per primer varied from 2 to 10 because of the primer sequence and due to individual genotype (Table 4). This is consistent with the studies carried out in willow clones by Singh and Huse (2004) and Barker *et al.* (2003), on Willows by Tuskan *et al.* (2004) on *Populus trichocarpa* and for the use of poplar micro-satellites DNA markers in willow by Rajora and Rehman (2005). Similarly spatial genetic structure and diversity of alpine *Salix* species was assessed by Christoph *et al*. (2007) using microsatellite (Simple Sequence Repeat) analysis. SSR analysis, based on three loci and 16 alleles, revealed 24 different genotypes and a proportion of distinguishable genotypes of 0·18.

Cluster Analysis

UPGMA dendrogram based on both RAPD and SSR markers resolved into four major clusters (Fig. 3). The similarity coefficient among 25 clones of willow ranged from 0.71 to 0.86 (Table 5) indicating that there was more similarity among the set of clones selected. A critical perusal of dendrogram reveals that the distribution of various genotypes into clusters and within cluster was somewhat random. This is in agreement with studies carried out by Niak and Dandin (2005) on *Melia* and by Trybush *et al*. (2008) on willows. Cluster one comprised of only four genotypes *viz.* PN-733, 17-93-A, SE-63-016 and PN-722 showing 78 per cent similarity with rest of the genotypes taken for study. Cluster II comprises five genotypes viz. 212/03, 84/11, SI-64-017, Kashmir willow and MB-368 showing 79 per cent similarity among rest of the clones.

Cluster III comprises of two clones *viz.* PN 227 and PN 731 both from New Zealand showing similarity of 80 per cent with cluster IV. Cluster IV a major cluster having 14 genotypes were grouped in to four sub clusters. Sub cluster one having genotypes NZ-1002, NZ-1040 and 799 showed 85% similarity among themselves and 83% with rest of the clones of cluster IV. Second sub cluster comprises four genotypes *viz.* SI-63-007, V-99, V-311 and SE-69-002 of which SI-63-007 and V-99 showed maximum similarity between themselves (86%). Genotypes 795, PN-721, SE-75-001, 131/25, NZ-1130

Table 4. Total number of amplified and polymorphic fragments generated by PCR using SSR primers along with their nucleotide sequence

Primer name	Sequences	Total no. ofscorable bands (y)	Total no. of polymorphic bands (x)	Total no. of monomorphic bands	Polymorphism (%) $\frac{x}{y}X100$	Size range of products (bp)
SB-38	FP-CCACTTGAGGAGTGTAAGGAT RP-CTTAAATGTAAAACTGAATCT	10	9	1	90	100-400
SB-194	FP-TGTGAGATAAGATTTGTCGGT RP-CCATAAATAAAAAACGTGAAC	2	1	1	50	100-200
SB-85	FP-CTCAGCAACTTAATCCAACTA RP-GTTTGTTAGGGGAGGTAAGAA	3	3	0	100	100-150
SB-80	FP-TAATGGAGTTCACAGTCCTCC RP-ATACAGAGCCCATTTCATCAC	4	4	0	100	100-250
SB-196	FP-CTGTTTCCTGCCACTATTACC RP-TATAATCTGTCTCCTTTTGGC	2	1	1	50	100-200
SB-201	FP-CCTCTTTTTCTATTGTGGTCT RP-GGCATGTATTTTTACTCCAAC	2	1	1	50	100-300
SB-210	FP-TATAAAGACAAATACCTGGGG RP-CATCAAAGACTGCTAGAAAGG	3	2	1	66	100-200
SB-100	FP-ATGTCATTCAGGTTTGTTTTC RP-ATGGTTTAACTTGTTACTGTA	3	2	1	66	100-300
SB-88	FP-TATTGCTTTGATGGCGACTGC RP-CAGCAACGGAAATAGCAACAG	2	1	1	50	100-350
PHTR-3	FP-ATTTGCATCCAGTCTTCAGTAATT RP-CTCAAAGAAGTGCATAGAGATTTCAT	3	3	0	100	100-250
	Total	34	27	7	79.4	100-400

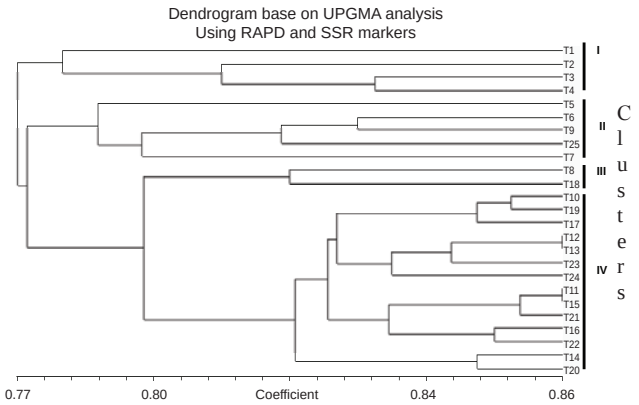

Fig. 3. Dendrogram based on UPGMA analysis of 25 clones of willow using RAPD and SSR markers

forms sub-cluster three of which 795 and PN-721 showed highest similarity in their genetic makeup. Sub-cluster four had only two genotypes *i e.* NZ-1179 and NZ-1140 showing 85 per cent similarity.

The similarity coefficient values were highest (0.86) between the genotypes 795 and PN-721, SI-63-007 and V-99. Thus these genotypes showed maximum degree of similarity in their genetic makeup. However, the minimum values of similarity coefficients were observed between SE-69-002and 84/11 (0.71) followed by SE-69-002 and 17-93-A (0.72). These genotypes are highly diverse and should be used for hybridization and improvement programmes.

Cluster I, the most diverse cluster having four species and retains four genotypes namely PN-733 (*S. nigra*), 17-93-A (*S. alba*), SE63-016 (*S. jessoensis*), PN-722 (*S. matsudana*). II and all these clones belong to the species *Salix alba* except clone 212/03 and it is the cross between *S. matsudana* and *S. caprea.* It shows clear-cut distinction from rest of the four clones of the cluster and this hybrid has all together different genetic makeup. Cluster III had two clones viz. PN-227 (*S. matsudana*) and PN- 731 (*S. nigra*) which were developed in New Zealand on the basis of plant material procured from China and U.S.A. (Kraayenoord *et al*., 1995). As regard the cluster IV, there are fourteen clones and mostly these clones were the hybrids of *S. matsudana* and *S. alba* or *S. alba* and *S. matsudana* alone. The *S. babylonica* is

Table 5. Similarity coefficient values of RAPD and SSR data using Jaccard's Similarity Correlation Coefficient

	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}	T_{16}	T_{17}	T_{18}	T_{19}	T_{20}	T_{21}	T_{22}	T_{23}	T_{24}	T_{25}
T_1	1																								
T_2	.77	1																							
T_3	.76	.80	1																						
T_4	.77	.79	.82	1																					
T_5	.77	.74	.79	.78	1																				
T_6	77	.77	.77	.77	.76	1																			
T_7	.73	.75	.74	.75	.77	.79	1																		
T_8	.77	.78	.81	.82	.78	.77	.77	1																	
T_9	.75	.75	.78	.79	.78	.82	.79	.78	1																
T_{10}	.76	.77	.81	.80	.79	.76	.82	.79	.82	1															
T_{11}	.76	.78	.81	.81	.78	.77	.81	.81	.80	.84	1														
T_{12}	.77	.80	.82	.80	.79	.78	.80	.82	.82	.85	.86	1													
T_{13}	.77	.77	.81	.79	.77	.73	.77	.83	.77	.82	.85	.86	1												
T_{14}	.75	.73	.77	.76	.76	.74	.75	.79	.79	.82	.82	.81	.81	1											
T_{15}	.76	.74	.79	.77	.78	.75	.79	.79	.75	.81	.86	.80	.82	.82	1										
T_{16}	.77	.75.	.81	.78	.80	.77	.78	.78	.79	.85	.84	.85	.83	.83	.82	1									
T_{17}	.77	.78	.81	.77	.79	.78	.76	.80	.80	.85	.83	.83	.83	.80	.82	.83	1								
T_{18}	.74	.75	.78	.78	.74	.75	.77	.81	.75	.79	.81	.82	.80	.79	.78	.80	.80	1							
T_{19}	.77	.78	.80	.78	.75	.75	.78	.80	.79	.86	.84	.82	.83	.81	.82	.81	.85	.80	1						
T_{20}	.76	.77	.77	.76	.77	.75	.77	.78	.80	.81	.82	.81	.80	.85	.83	.83	.83	.80	.84	1					
T_{21}	.78	.76	.79	.79	.77	.74	.77	.82	.76	.84	.86	.81	.85	.81	.87	.82	.81	.78	.85	.82	1				
T_{22}	.75	.75	.78	.75	.78	.75	.78	.77	.77	.81	.86	.80	.83	.81	.83	.85	.80	.80	.79	.82	.84	1			
T_{23}	.78	.76	.78	.77	.76	.77	.79	.81	.77	.83	.85	.84	.85	.82	.83	.84	.84	.80	.83	.84	.84	.82	1		
T_{24}	.75	.72	.76	.75	.77	.71	.76	.80	.75	.81	.82	.82	.84	.79	.83	.81	.80	.77	.82	.80	.85	.81	.83	1	
T_{25}	.77	.78	.75	.77	.80	.81	.81	.80	.82	.82	.82	.82	.80	.78	.77	.79	.82	.79	.81	.78	.77	.77	.82	.76	1

closely allied species of *S. matsudana* on the basis of phylogenetic study (Skvortsov, 1999).

Sub-cluster one of Cluster IV had three clones and all are hybrids *i.e.* cross between *S. matsudana* and *S. alba*. Similarly, sub-cluster three had five hybrids of *S. matsudana* and *S. alba* or *S. alba* and *S. babylonica* whereas sub cluster four had only two clones and both are hybrids between *S. matsudana* X *S. alba* which were developed in New Zealand (Stott, 1984). Clone 795 and PN-721 are the hybrids of *S. matsudana* and *S. alba* developed in different countries *i.e.* China and New Zealand found most similar one.

Conclusion

The extent of polymorphism indicated was marginally higher in RAPD compared to the SSR markers and hence, the former is more informative that the later. The RAPD profile usually represent available portion of the genome while the SSR markers represent microsatellite rich regions. The result, thus, highlights the utility of two different marker systems in providing great information on the genetic structure of *Salix* clones. Markers used were also helpful in characterizing willow clones. The clustering pattern exhibited that the geographic distribution does not provide true index of genetic diversity in willow clones. This is because of the fact that willow resources have been freely exchanged all over the world and used for willow breeding for production of vigorous clones.

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References

Barker JHA, M Michaela, GM Arnold, KJ Edwards, A Inger, L Stig and A Karp (1993) Characteristics of genetic diversity in potential biomass willows *Salix* species by RAPD and AFLP analysis. *Genome* **42:** 173-183.

Barker JHA, A Pahlich, S Trybush, K Edwards and A Karp (2003) Microsatellite markers for diverse Salix species. *Mole. Ecol. Notes* **3:** 4-6.

Carriero F, G Fontanazza, F Cellini and G Giorio **(**2002**)** Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.). *Theor. Appl. Genet.* **104:** 301-307.

Castiglione E, G Wang, G Damiani, C Bandi, S Bissofi and F Sala (1993) RAPD fingerprints for identification and for taxonomic studies of elite poplar clones. *Theor. Appl. Genet.* **87:** 54-59.

Christoph R, S Sophia and P Peter (2007) Spatial genetic structure and clonal diversity in an alpine population of *Salix herbacea* (*Salicaceae*). *Ann. Bot.* **99:** 647-651.

Cipriani G, MT Marrazzo, R Marconi, A Cimato and R Testolin (2002) Microsatellite markers isolated in olive are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars (*Olea europaea* L.). *Theor. Appl. Genet*. **104:** 223-228.

Doyle JJ and JJ Doyle (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissue. *Phytochem. Bull.* **19:** 11-15.

FAO (2004) Preliminary Review of Biotechnology in Forestry including *Genetic Modification. Genetic Resource Working Paper FGR/59F*. Forest Resources Development Service. Forest Resource Division, Rome, Italy.

Kraayenoord CWSV, B Slui and FB Knowles (1995) Introduced forest trees in New Zealand, Recognition, Role and Seed source: The Willows *Salix* spp. *New Zealand FRI Bull.* 124.

Kuzovkina YA, M Weih, MA Romero, J Charles, S Hust, I Mcivor, A Karp, S Trybush, M Labrecque, TT Teodorescu, NB Singh, LB Smart and TA Volk (2008) Salix: botany and global horticulture. *Hort. Rev.* **34:** 447-481.

Lin D, M Hubbes and L Zsuffa (1994a) Differentiation of poplar and willow clones using RAPD fingerprints. *Tree Physiol*. **14:** 1097-1105.

Lin D, M Hubbes and L Zsuffa (1994b) Differentiation of poplar and willow clones using RAPD fingerprints. *Norwegian J. Agric. Sci*. **18:** 67-81.

Liu Z and GR Furnier (1993) Comparison of allozyme, RFLP and RAPD markers for revealing genetic variation within and between trembling aspen and big tooth aspen. *Theor. Appl. Genet.* **87:** 97-105.

Naik GV and SV Dandin (2005) Molecular characterization of some improved and promising mulberry varieties (*Morus* spp.) of India by RAPD and ISSR markers. *Indian J. Seric*. **44:** 59-68.

Rajora OP and MH Rahman (2005) Poplar microsatellite DNA markers. *Plant and Animal Genomes* XIII Conference, San Diego, USA.

Rohlf FJ (1998) *NTSYS-PC Numerical Taxonomy and Multivariate System Version 2.02. Applied Biostatis* Inc., New York.

Sanchez N, JM Grau, JA Manyanera and M A Bueno (1998) RAPD markers for the identification of *Populus* species. *Silvae Genetica* **47:** 67-71.

Singh NB and SA Huse (2004) Improvement of tree willows in India: variation on quantatic genetic parameters on growth traits. *In. International Poplar Commission,* 22nd session Santiago, Chile.

Singh NB, S Huse and RK Gupta (2004) Improvement of tree willows in India: principle component analysis of tree willow clones for genetic improvement of quantative traits. *In. International Poplar Commission* 22nd session Santiago, Chile.

Skvortsov AK (1999) Willows of Russia and adjacent countries: Taxonomical and Geographical Revision. Joensuu University, Joensuu, 307p.

Stott KG (1984) Improving the biomass potential of willows by selction and breeding. *In:* Perttu, K (ed.). Ecology and Management of Fforest Biomass Production Systems. Dept Ecol & Env. Res., Swed. Univ. *I* **15:** 233-260.

Su Xiaoheea, L Zsuffa, D Lin and Su X H (1995) Estimation of variation in *Salix* and its clones by RAPD. *Scientia-Silvae Sciencia* **31:** 211-214.

Trybush, S, S Jahodova, W Macalpine and A Karp (2008) A genetic study of a *Salix* germplasm resource reveals new insights into relationships among subgenera, sections and species. *Bioenergy Res.* **1:** 67-79.

Tuskan GA, IE Guntur, ZK Yang, TM Yin, MM Swell and S P Difazio (2004) Characterization of microsatellites revealed by genomic sequencing of *Populus trichocarpa*. *Canadian J. Forest Res.* **34:** 85-93.

Zsuffa L and A Mosseler (1986) The collection and distribution of clones of *Salix* species and hybrids, which are of importance for biomass production in energy plantation. In: *Proceedings Joint Management IEA Forestry Programme and FAO*. Network for Rural Energy October 28-30 (1995) Swedish University of Agricultural Science Publication **1:** 54-73.