Indian J. Genet. Plant Breed., (2022) 82(1): 73-80

ISSN: 0975-6906

doi: 10.31742/IJGPB.82.1.10

www.isgpg.org

RESEARCH ARTICLE

# Genetic diversity and population structure of Indian willow (*Salix tetrasperma* Roxb.) with dominant molecular markers along its distribution range in Himalayan region

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## Abstract

Indian willow (*Salix tetrasperma*) is an agriculturally useful tree which occurs over a wide geographic area across South Asia and bears importance. So far, this species has never been studied for molecular genetic diversity. The present study was, therefore, carried out to assess the genetic diversity and population structure analysis using RAPD and ISSR molecular markers in diverse genotypes from five populations covering North India. The mean number of effective alleles, Shannon information index and gene diversity i.e., 1.38 ± 0.013, 0.35 ± 0.010 and 0.23 ± 0.007, respectively were obtained with RAPD + ISSR markers. The analysis of molecular variance generated by RAPD + ISSR revealed a higher genetic variation (87%) within population as compared to that of among population (13%). Nei genetic distance was maximum (0.185) between Jammu and Kashmir and Punjab populations. Significant Mantel correlation (r=0.551,) was obtained between RAPD and ISSR markers. Bayesian clustering pattern obtained through STRUCTURE software showed four gene pools. Based on the genetic information obtained with regards to Indian willow by combining the RAPD and ISSR marker systems, it is proposed that an individual tree be selected within populations rather than among populations for the improvement of economic traits of Indian willow alongwith conservation of entire ecological populations.

**Keywords:** Differentiation, genetic, ISSR, mantel correlation, nei genetic distance

## Introduction

Willows (*Salix* species) comprised of 350-500 species of trees, shrubs and osiers, occurring worldwide (Argus 1997). The centre of origin of the genus *Salix* is China (Fang 1987). There are about 33 *Salix* species occurring in India, out of which seven species of tree willows, namely *S. tetrasperma, S. acmophylla, S. alba, S. fragilis, S. babylonica, S. daphnoids* and *S. excelsa* are found from northern temperate parts of Jammu and Kashmir to Arunachal Pradesh state of India. However, *S. tetrasperma* is occurring throughout the country (Sharma et al. 2011) and *S. acmophylla* is distributed in north-west India (Singh et al. 2012), and rest of the species are confined to sub-Himalayan track to higher reaches (Wol 1972). *S. tetrasperma* also known as Indian willow, from the section *Humboldtianae* is a paleotropical species distributed across the low elevations in India, South China, and Indo-china, reaching upto Java (Skvortsov 1968). The occurrence of *S. tetrasperma*, an agriculturally useful tree species, has been reported in South India, Indo Malaya, Egypt, Iran, Sri Lanka (Sharma et al. 2021) and Pakistan (Sher et al. 2014). Genetic diversity among populations of a species is a

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**How to cite this article:** Sharma J. P., Thakur S., Sankhyan H. P., Jha S. K., Sharma R., Kanwar P., Gautam V. and Sankhyan N. 2022. Genetic diversity and population structure of Indian willow (*Salix tetrasperma* Roxb.) with dominant molecular markers along its distribution range in Himalayan region. Indian J. Genet. Plant Breed., **82**(1): 73-80.

**Source of support:** Nil

**Conflict of interest:** None.

**Received:** June 2021 **Revised:** Dec. 2021 **Accepted:** Jan. 2022

prerequisite for genetic improvement (Porth and El-Kassaby 2014) of that species through breeding metnodologies and making it adaptable to the changing environment (Rao and Hodgkin 2002; Ingvarsson and Dahlberg 2018). The molecular markers, RAPD and ISSR have been previously used to study genetic diversity in *Salix* species (Sulima and Przyborowski 2013; Corneanu et al. 2016; Ghaidaminiharouni et al. 2017; Hao et al. 2019) and in conserved DNA-derived polymorphism marker techniques to find out the genetic variation in trees and crops (Liu et al. 2020; Srivastav et al. 2021). The genetic differentiation or fixation indices ($F_{ST}$) are the simplest parameter for assessing diversity among populations (Weir and Basten 1990) that calculate standardized variances in allele frequencies among pairs of populations (Weir and Cockerham 1984) for the partitioning of genetic diversity (Mburu et al. 2003).

Many of the native *Salix* species are not suitable for industrial use due to slow growth, inferior bole and also lacking other useful wood characters (Sharma et al. 2011). However, *Salix tetrasperma* willow has great adaptability to saline as well as drought conditions, which makes it a suitable parent for breeding with fast growing *Salix* species. *Salix tetrasperma*, though widely distributed throughout India, has not been extensively studied for genetic diversity and population structure. Considering the scanty information, the stem cuttings of natural populations of Indian willow were collected from North India and resprouted in the experimental field to study the genetic diversity and population structure with RAPD and ISSR molecular markers.

## Materials and methods

### *Plant materials*

Fifty genotypes were randomly collected from 20 sites in the Himalayan region comprising the states of Uttarakhand, Punjab, Himachal Pradesh and Jammu and Kashmir. The state wise distinct populations of three states viz., Uttarakhand (UK), Punjab (PB) and Jammu and Kashmir (JK) were considered due to the eco-geographic similarity of the sites within each state selected for collection of genotypes. However, the state of Himachal Pradesh (HP) was divided into two populations (HP1 and HP2) due to diversity of site characters, climate and vegetation etc. (Sharma et al. 2019, Table 1, Fig 1). The distance among the genotypes within sites ranged from 200 meters (minimum) to 4 km (maximum) to avoid resampling of the same genotype. The stem cuttings were collected in the period from February to May 2016 from the trees having desirable characters such as healthy growth, clean and straight bole. The material was resprouted in the nursery/glasshouse to take the leaf samples for DNA extraction.

### *DNA isolation*

Nuclear DNA was isolated from fresh and young leaves (1.00 g) from 2 to 3 genotypes of each site with the CTAB method as per protocol developed by Doyle and Doyle (1987). The quantified samples DNA (5ng/ml) was used for the molecular analysis. A total of 21 RAPD and 26 ISSR markers were chosen out of about 80 markers from previous studies (Sulima and Przyborowski 2013) of which 18 RAPD and 21 ISSR primers showing consistent and reproducible amplification were used for molecular analysis (Table 2).

The 25 µl PCR reaction contained 5ng template DNA,10x Taq buffer, 2.5 mM dNTP with 1.5 mM $MgCl_2$, 10 mM of primers, 0.5 unit *Taq* DNA polymerase and nuclease-free $H_2O$. The amplification reactions were carried out in a 96 well 2720 thermal cycler under the following conditions: 3 min at 94 $^0$C; then 45 cycles consisting of 45 s at 92 $^0$C, 60s at 35-44 $^0$C and 120s at 72 $^0$C, followed by final extension of 72 $^0$C for 10 minutes. Amplification products were separated on 1.5% agarose gel using 1 X TAE buffer on horizontal gel electrophoresis apparatus.

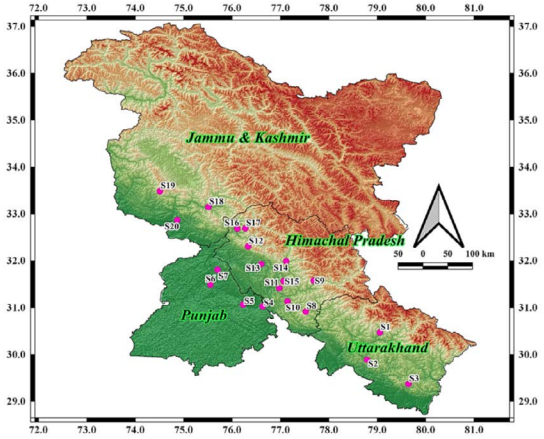


**Fig. 1.** Location of collection sites of Indian willow (*Salix tetrasperma*)

**Table 1.** Population description and coding of Indian willow genotypes

| Population | No. of sites | No of genotypes | Site name (Site serial number, site code, genotype serial number) |
|---|---|---|---|
| UK | 3 | 7 | Jakholi (S1:JA,1-3), Pauri Garhwal (S2:PG,4-5), Jyolikote (S3:JL,6-7) |
| PB | 4 | 10 | Rupnagar (S4: RN, 8-10), Tandi (S5:TD,11-12), Suhanpur (S6: SP,13-15), Dasua (S7: DS,16-17) |
| HP1 | 4 | 11 | Devamanal (S8: DM,18-20), Rampur (S9: RP, 21-23), Deothi (S10: DE, 24-25), Namhol (S11: NM, 26-28) |
| HP2 | 6 | 14 | Balh (S12:BL, 29-31), Hamirpur (S13:HM, 32-34), Kangra (S14: KG, 35-36), Bhunter (S15: BN, 37-38), Chowari (S16: CW, 39-40), Chamba (S17: CH, 41-42) |
| JK | 3 | 8 | Chinani (S18: CN, 43-45), Rajouri (S19: RJ, 46-47), Jammu (S20: JM, 48-50) |

**Table 2.** Summary of RAPD and ISSR primers used in *Salix tetrasperma* genotypes

| Primer | Number of alleles | Polymorphic alleles (No) | Polymorphic alleles (%) | PIC | EMR | MI | RP |
|---|---|---|---|---|---|---|---|
| OPC-01 | 13 | 13 | 100.00 | 0.37 | 13.00 | 4.80 | 7.25 |
| OPC-02 | 11 | 11 | 100.00 | 0.43 | 11.00 | 4.77 | 7.51 |
| DECA-7 | 9 | 9 | 100.00 | 0.38 | 9.00 | 3.44 | 5.47 |
| OPB-12 | 7 | 6 | 85.71 | 0.31 | 5.14 | 1.61 | 3.25 |
| OPE-16 | 7 | 7 | 100.00 | 0.41 | 7.00 | 2.86 | 4.42 |
| OPE-18 | 13 | 13 | 100.00 | 0.30 | 13.00 | 3.91 | 5.74 |
| OPL-05 | 9 | 8 | 88.89 | 0.31 | 7.11 | 2.22 | 4.04 |
| OPL-07 | 6 | 6 | 100.00 | 0.41 | 6.00 | 2.43 | 3.66 |
| OPL-08 | 8 | 8 | 100.00 | 0.43 | 8.00 | 3.43 | 5.43 |
| OPL-09 | 7 | 7 | 100.00 | 0.43 | 7.00 | 3.01 | 4.94 |
| OPB-14 | 6 | 6 | 100.00 | 0.34 | 6.00 | 2.02 | 3.02 |
| OPF-08 | 7 | 6 | 85.71 | 0.30 | 7.00 | 2.07 | 2.87 |
| OPS-14 | 7 | 7 | 100.00 | 0.40 | 7.00 | 2.79 | 4.19 |
| OPT-06 | 3 | 3 | 100.00 | 0.23 | 3.00 | 0.68 | 0.98 |
| OPT-02 | 9 | 9 | 100.00 | 0.37 | 9.00 | 3.34 | 5.62 |
| OPS-07 | 9 | 9 | 100.00 | 0.38 | 9.00 | 3.38 | 4.60 |
| OPA-01 | 6 | 6 | 100.00 | 0.43 | 6.00 | 2.59 | 3.96 |
| OPA-02 | 6 | 6 | 100.00 | 0.44 | 6.00 | 2.61 | 4.00 |
| ISSR primers | | | | | | | |
| UBC835 | 8 | 8 | 100.00 | 0.38 | 12.76 | 4.85 | 10.15 |
| 840 | 7 | 7 | 100.00 | 0.31 | 8.75 | 2.71 | 7.43 |
| ISSR4 | 5 | 5 | 100.00 | 0.26 | 6.73 | 1.75 | 4.83 |
| ISSR6 | 7 | 7 | 100.00 | 0.15 | 2.30 | 0.35 | 4.79 |
| ISSR7 | 7 | 5 | 71.43 | 0.43 | 10.13 | 4.36 | 8.75 |
| ISSR12 | 5 | 4 | 80.00 | 0.34 | 10.40 | 3.54 | 7.28 |
| ISSR1 | 11 | 9 | 81.82 | 0.36 | 9.30 | 3.35 | 13.25 |
| ISSR3 | 6 | 5 | 83.33 | 0.29 | 10.01 | 2.90 | 7.74 |
| 811 | 9 | 9 | 100.00 | 0.32 | 10.35 | 3.31 | 11.13 |
| ISSR94 | 8 | 7 | 87.50 | 0.32 | 10.07 | 3.22 | 10.98 |
| ISSR98 | 7 | 6 | 85.71 | 0.31 | 10.21 | 3.17 | 8.75 |
| ISSR8 | 7 | 7 | 100.00 | 0.23 | 5.62 | 1.29 | 6.57 |
| ISSR11 | 5 | 4 | 80.00 | 0.23 | 6.28 | 1.44 | 5.21 |
| ISSR91 | 7 | 6 | 85.71 | 0.37 | 9.38 | 3.47 | 7.77 |
| ISSR97 | 6 | 6 | 100.00 | 0.29 | 10.10 | 2.93 | 7.81 |
| ISSR141 | 6 | 6 | 100.00 | 0.29 | 10.10 | 2.93 | 7.81 |
| ISSR92 | 6 | 4 | 100.00 | 0.39 | 10.93 | 4.26 | 4.23 |
| ISSR14 | 6 | 3 | 100.00 | 0.29 | 10.10 | 2.93 | 7.81 |
| ISSR93 | 6 | 6 | 100.00 | 0.29 | 10.10 | 2.93 | 7.81 |
| ISSR95 | 4 | 4 | 100.00 | 0.33 | 13.45 | 4.44 | 6.08 |
| 814 | 6 | 6 | 100.00 | 0.43 | 13.82 | 5.94 | 7.25 |

PIC = Polymorphism information contents, EMR = Effective multiplex ratio, RP = Resolving power and MI = Marker index

### *Statistical analysis*

The genetic diversity parameters, namely, per cent polymorphism, total number of bands amplified per primer and number of polymorphic band were calculated. The genotypic data obtained for different markers were used for assessing the discriminatory power of primers Polymorphism Information Content (PIC) (Cheasnokov and Artemyeva 2015), the number of observed alleles (Na),

number of effective alleles (Ne), gene diversity (He), Shannon information index, Phi-statistics, principal coordinates analysis (PCoA) and Mantel correlations were calculated with data standardization in GenAlEx 6.503 (Peakall and Smouse 2012). The analysis of molecular variance (AMOVA) generated by RAPD+ISSR was used to detect coefficient of genetic variation. POPGENE 1.32 was used to calculate the coefficient of gene differentiation (Gst) and gene flow (Nm). Population structure was analyzed by the Bayesian clustering algorithm using the admixture model in software STRUCTURE v 2.3.4 (Pritchard et al. 2000) without prior classification information. Five independent runs were performed for each value of K ranging from 1 to 10 with a burn-in of 2,00,000 iterations followed by 5,00,000 iterations. The most appropriate K value was determined by the mathematical model described by Pritchard et al. (2000) and Evanno et al. (2005). Graphical results to assess the most likely value of K from structure run were obtained by a web-based tool viz. STRUCTURE HARVESTER (Earl and Von Holdt 2012).

## Results and discussion

The polymorphic information content (Table 2) ranged from 0.23 (OPT-06) to 0.44 (OPA-02) in RAPD and from 0.15 (ISSR6) to 0.43 (ISSR7 and 814) in ISSR. The maximum Marker Index (MI) of 4.80 in RAPD (OPC-01) and 5.95 in ISSR was calculated by primers OPC-01 and 814, respectively. The highest resolving power (RP) of markers was exhibited in RAPD (7.51) and ISSR (13.25) by primers OPC-02 and ISSR1, respectively. The species exhibited a high level of polymorphism (Table 3), which was reflected in the number of alleles per locus (1.67 ± 0.017), number of effective alleles (1.46 ± 0.010), Shannon information index (0.40 ± 0.007) and gene diversity (0.27 ± 0.005).The maximum mean number of effective alleles was recorded for HP2 while HP1 and HP2 populations recorded same values for number of effective alleles (1.49), Shannon information index (0.43) and gene diversity (0.29).

Information on genetic diversity through molecular markers is currently limited in Indian willow. This is the first study dealing with magnitude and structuring of genetic diversity of Indian willow populations of northern India with RAPD and ISSR markers. Lin et al. (1994) used RAPD primers to differentiate clones of poplar and willow. The RAPD and ISSR primers were used previously in *Salix* (Barker et al.1999; Sulima et al. 2009; Singh et al. 2013, 2014; Pogorzelec et al. 2014, 2015; Bloju et al.2017; Sulima et al. 2018; Raja et al. 2018) and *Populus* (Liu and Furnier 1993; Torjek et al. 2001; Rajora and Rahman 2003) for genetic characterization. In the current study,the diversity parameters such as Shannon information index and gene diversity showed higher values than those in the previous study in *Salix purpurea* (Sulima et al. 2018) and *Hippophae rhamnoides* (Srihari et al. 2013) due to collection of genotypes from wider areas. The mean number of effective alleles per locus (1.46), diversity (0.27) and Shannon Index (0.40), as obtained in the current study were similar to those recorded previously by Sulima and Przyborowski (2013) in *Salix purpurea* (1.42, 0.24, 0.35, respectively) through ISSR markers. Pogorzelec et al. (2014) in *S. lapponum* got more than 90 per cent polymorphic products with ISSR markers while the expected heterozygosity in their investigation ranged from 0.143 to 0.182 which was lower as compared to the corresponding value obtained in the current study.

In a combined analysis of RAPD and ISSR marker types, AMOVA (Table 4) revealed more genetic variation (87%) within population than among population (13%) due to general characteristics of trees and shrubs (Hamrick et al. 1992). The within population variation was recorded higher

**Table 3.** A comparison of genetic variability using RAPD and ISSR primers across various populations of *S. tetrasperma*

| Population | Number of genotypes | Number of alleles per locus | Number of effective alleles | Shannon information index | Gene diversity | Unbiased diversity |
|---|---|---|---|---|---|---|
| UK | 7.00 | 1.56 ± 0.042 | 1.44 ± 0.022 | 0.38 ± 0.017 | 0.26 ± 0.012 | 0.30 ± 0.014 |
| PB | 10.00 | 1.68 ± 0.036 | 1.41 ± 0.020 | 0.38 ± 0.015 | 0.25 ± 0.011 | 0.27 ± 0.012 |
| HP1 | 11.00 | 1.74 ± 0.035 | 1.49 ± 0.021 | 0.43 ± 0.014 | 0.29 ± 0.010 | 0.32 ± 0.011 |
| HP2 | 14.00 | 1.76 ± 0.031 | 1.49 ± 0.021 | 0.43 ± 0.015 | 0.29 ± 0.011 | 0.31 ± 0.011 |
| JK | 8.00 | 1.61 ± 0.039 | 1.46 ± 0.039 | 0.39 ± 0.017 | 0.26 ± 0.012 | 0.30 ± 0.013 |
| Mean | 10.00 ± 0.065 | 1.67 ± 0.017 | 1.46 ± 0.010 | 0.40 ± 0.007 | 0.27 ± 0.005 | 0.30 ± 0.006 |

**Table 4.** Analysis of molecular variance (AMOVA) of five populations of *S. tetrasperma* with RAPD+ISSR primers

| Source | df | Sum of square | MS | Estimated variance | Per cent | Gst | Nm |
|---|---|---|---|---|---|---|---|
| Among Populations | 4 | 410.21 | 102.55 | 6.103 | 13% | 0.185 | 2.199 |
| Within Populations | 45 | 1909.65 | 42.44 | 42.44 | 87% | | |
| Total | 49 | 2319.86 | | 48.54 | 100% | | |
| Stat | Value | P(rand >= data) | | | | | |
| PhiPT | 0.126 | 0.001 | | | | | |

Gst = Coefficient of gene differentiation, Nm = Gene flow, PhiPT = Coefficient of genetic differentiation among populations

than the among population variation with SSR markers (Sharma et al. 2021). This has also been verified in case of other tree species (Sulima et al. 2018; Srihari et al. 2013; Sandeep et al. 2020) and is in line with the earlier studies which have confirmed that the high rates of out-crossing, life history and seed distribution over a wide range of area may be responsible for possession of genetic variations by woody perennial species majorly within the populations (Hamrick et al. 1992; Li et al. 2008; Khadivi-Khub et al. 2015). Gst recorded 0.185 indicating that only 18.50 % of the genetic variation existed among the populations while 81.50% of the genetic variation existed within the population. The Nm between the populations recorded was 2.199.

The Gst of the *S. tetrasperma* populations was found high according to Nei's classification standard for Gst (Low Gst<0.05, intermediate Gst = 0.05-0.15, high Gst>0.15) (Nei 1978). The Nm value of 2.199 obtained in the present study showed moderate level of gene exchange between populations indicating that despite moderate gene flow, the population differentiation is high leading to fixation of specific alleles in the population, thereby, changing its evolutionary course. The evolutionary histories and geographic isolation lead to low genetic diversity and population differentiation among populations that is depicted in Nei genetic distance among populations (Table 5). Nei Genetic distance was recorded maximum between populations from PB and JK (0.185) and minimum between HP1 and UK (0.058); showing that the geographic distance (Fig. 1) as well as land pattern genetic variation is greatly affected by the probability of gene exchange through seed or pollen in dioceous species including genus *Salix*. *Salix alba* exhibited lower gene flow upto 3 km (Van Puyvelde and Triest 2007) while a higher gene flow upto 200 km was reported in *S. caprea* (Perdereau et al. 2014). The population PB was found to be more genetically distinct from other populations due to the fact that population PB consisted of genotypes from Punjab area of India where difference in climatic and edaphic factors were different from other populations. The Punjab area falls in Agoclimatic zone VI representing Trans-gangetic Plains Region, whereas other populations fell in zone I (Western Himalayan Region) (Ahmad et al. 2017). Similar diversity parameters were obtained by Liu et al. (2020) in *S. taishanensis* studied with conserved DNA-derived polymorphism markers.

The genotypes were grouped into four major clusters based on RAPD+ISSR polymorphism using the principal coordinate analysis (Fig. 2). Mantel correlation coefficient (0.551) was significant between RAPD and ISSR markers

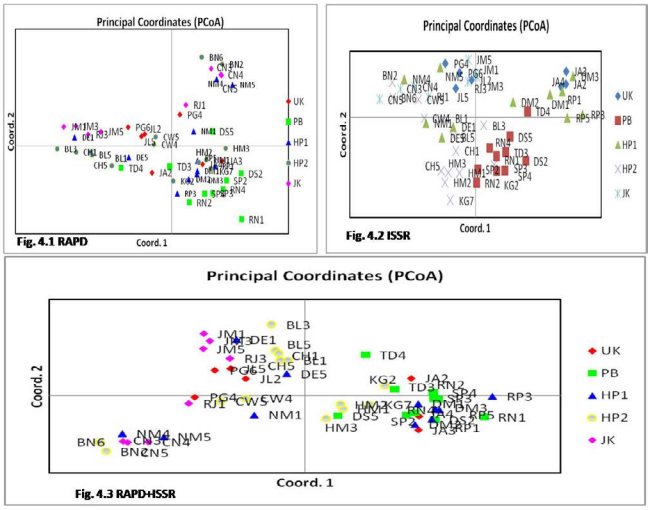


**Fig. 2. Two-dimensional plot of principal component analysis of *Salix tetrasperma* genotypes using (4.1) RAPD, (4.2) ISSR and (4.3) RAPD + ISSR analysis**

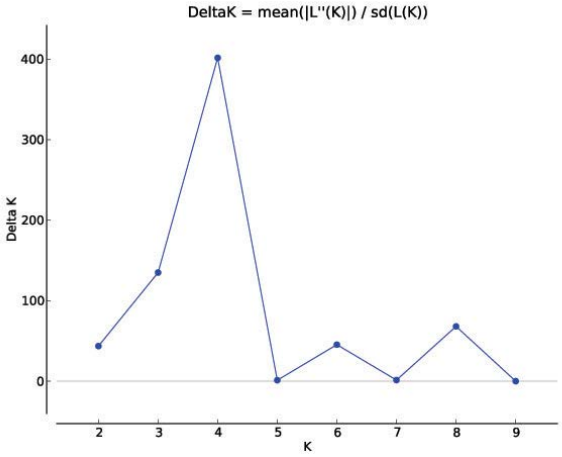


**Fig. 3. Diagram for LnP(D) and delta K of the *Salix tetrasperma* subdivided into four subpopulations based on RAPD+ISSR marker data**

**Table 5.** Pairwise population matrix of Nei Genetic Distance with RAPD + ISSR markers

| | UK | PB | HP1 | HP2 |
|---|---|---|---|---|
| PB | 0.136 | 0.000 | | |
| HP1 | 0.058 | 0.098 | 0.000 | |
| HP2 | 0.118 | 0.094 | 0.091 | 0.000 |
| JK | 0.112 | 0.185 | 0.119 | 0.098 |

**Table 6.** Mantel correlation among different marker systems and genetic/geographic distances

| Marker system | Mantel correlation coefficient | Coefficient of determination ($R^2$) | P(rxy-rand >= rxy-data) |
|---|---|---|---|
| RAPD and ISSR | 0.551 | 0.304 | 0.001 |
| RAPD and RAPD+ISSR | 0.898 | 0.806 | 0.001 |
| ISSR and RAPD+ISSR | 0.862 | 0.743 | 0.001 |
| Genetic distance and Geographic distance | 0.138 | 0.019 | 0.007 |

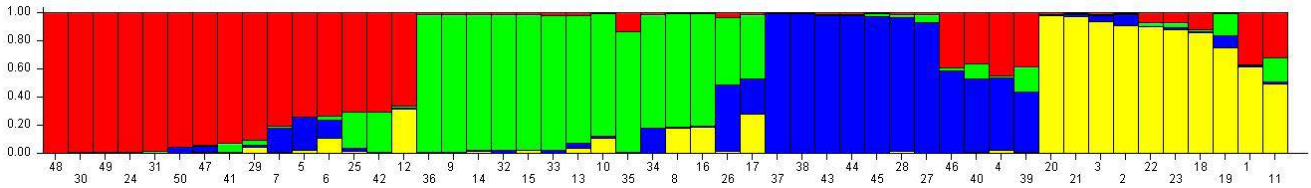


**Fig. 4. Estimated genotypic structure of *Salix tetrasperma* genotypes sorted by Q (inferred ancestry)**

whereas the combined marker system exhibited greater influence ($r^2$=0.806) with RAPD markers. A weak positive Mantel correlation (r=0.140) was observed between the genetic and geographic distance (Table 6).

The estimated likelihood of the clustering of data using STRUCTURE was found to be optimal since ΔK reached its maximum value with K=4 (Fig. 3) suggesting that the studied genotypes were subdivided into four subgroups based on RAPD+ISSR marker data and all genotypes were distributed with high probability into four subgroups (Fig.4) colored by dark orange, light green, indigo blue and yellow colours with genotypes belonging to different populations alongwith admixtures. Kayis et al. (2010) prepared dendrogram with dissimilarity index as well as PCoA in *Cannabis sativus* with RAPD, ISSR and RAPD+ISSR primers and got partially different genetic distance levels when used individually. However, when used together, RAPD-based cluster was found to be more similar to the combined cluster than ISSR-based cluster as shown by coefficient of determination values of the Mantel correlation (Table 6). Based on a set of 50 genotypes and data on 267 polymorphic markers, five populations were classified into small subgroups by STRUCTURE (Fig 4) and the pattern delineated by PCoA. Subgroups showed admixtures due to more gene flow among genotypes as a result of dioceous nature of the crop. The vegetative propagation nature of the species may also be responsible for admixture gene pools. The genetic and geographic distances of the genotypes of collection exhibited a weak correlation similar to that obtained for *Salix purpurea* (Sulima and Przyborowski 2013) based on Nei's genetic distance with ISSR markers. These results reflect the outcrossing nature and high gene flow among genotypes. The genetic diversity patterns with moderate gene flow obtained in the current study were similar to those obtained with codominant markers (Sharma et al. 2021) suggesting the use of both types of markers for studying genetic variation in this species. The present study has generated valuable information for tree breeders to exploit natural populations of *S. tetrasperma* and will prove useful for framing new conservation and breeding strategies and their effective implementation in future improvement programmes.

## Authors' contribution

Conceptualization of research (JPS, ST); Designing of the experiments (JP, ST); Contribution of experimental materials (JPS, HPS); Execution of field/lab experiments and data collection (JPS, PK); Analysis of data and interpretation (JP, RS, VG); Preparation of manuscript, language refinement and proofreading (JP, ST, NS).

## References

Ahmad L., Habib Kanth R., Parvaze S. and Sheraz Mahdi S. 2017. Agro-climatic and Agro-ecological Zones of India. *In*: Experimental Agrometeorology: A Practical Manual. Springer, Cham. https://doi.org/10.1007/978-3-319-69185-5_15.

Argus G.W. 1997. Infrageneric classification of *Salix* (Salicaceae) in the New World. Systematic botany monographs. Vol.52. The Amer. Soc. Plant Taxon., USA.

Barker J.H.A., Michaela M., Gillian M.A., Keith J.E., Inger A., Stig L. and Karp A. 1999. Characterization of genetic diversity in potential biomass willows (*Salix* spp.) by RAPD and AFLP analysis. Genome, **42**: 173-183.

Bloju O., Cornelia I.S., Rus A.B., Ciaparii R. and Popescu S. 2017. Comparative analysis of different molecular markers used for evaluation of *Salix* sp. variability. J. Hort. For. Biotech., **21**: 131-136.

Chesnokov Yu. V. and Artemyeva A.M. 2015. Evaluation of the measure of polymorphism information of genetic diversity. Agri. Biol., **50**: 571-578.

Corneanu M., Popescu S.D. and Hernea C. 2016. The molecular polymorphism evaluation in *Salix* Sp. Romanian accessions – preliminary results. Bulletin UASVM series. Agriculture, **73**: 138-139. DOI: 10.15835/buasvmcn-agr:11817.

Doyle J.J. and Doyle J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., **19**: 11-15.

Earl D.A. and von Holdt B. M. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conser. Genet. Res., **4**: 359-361. DOI: 10.1007/s12686-011-9548-7.

Evanno G., Regnaut S. and Goudet J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol., **14**: 2611-2620. DOI:10.1111/j.1365-294X.2005.02553.x

Fang Z.F. 1987. On the distribution and origin of *Salix* in the world. J. Syst. Evol., **25**: 307-313.

Ghaidaminiharouni M., Rahmani F. and Khodakarimi A. 2017. The analysis of genetic diversity in willow (*Salix* spp.) by ISSR markers. Indian J. Genet. Plant Breed., **77**: 321-323. DOI:10.5958/0975-906.2017.00044.X.

Hamrick J.L., Godt M.J.W. and Sherman-Broyles S.L.1992. Factors influencing levels of genetic diversity in woody plant species. New For., **6**: 95-124.

Hao L., Zhang G., Lu D., Hu J. and Jia H. 2019. Analysis of the genetic diversity and population structure of *Salix*

*psammophila* based on phenotypic traits and simple sequence repeat markers. Peer J., **7**: e6419. https://doi.org/10.7717/peerj.6419.

Khadivi-Khub A., Shabanian N., Alikhani L. and Rahmani M.S. 2015. Genotypic analysis and population structure of Lebanon oak (*Quercus libani* G. Olivier) with molecular markers. Tree Genet. Genomes, **11**: 102, doi: 10.1007/s11295-015-0935-1.

Li F., Gan S., Weng Q., Zhao X., Huang S., Li M., Chen S., Wang Q. and Shi F. 2008. RAPD and morphological diversity among four populations of the tropical tree species *Paramichelia baillonii* (Pierre) Hu in China. For. Ecol. Manage., **255**: 1793-1801.https://doi.org/10.1016/j.foreco.2007.12.002.

Lin D., Hubbes M. and Zsuffa L.1994. Differentiation of poplar and willow clones using RAPD fingerprints. Tree Physiol., **14**: 1097-1105. doi:10.1093/treephys/14.10.1097.

Liu Z. and Furnier G.R.1993. Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. Theor. Appl. Genet., **87**: 97-105. https://doi.org/10.1007/BF00223752.

Liu H., Zang F., Wu Q., Ma Y., Zheng Y. and Zang D. 2020.Genetic diversity and population structure of the endangered plant *Salix taishanensis* based on CDDP markers. Glob. Ecol. Conserv., 24: e01242.

Mburu D.N., Ochieng J.W., Kuria S.G., Jianlin H. Kaufmann B., 2003. Genetic diversity and relationships of indigenous Kenyan camel (*Camelus dromedarius*) populations: implications for their classification. Anim. Genet., **34**: 26-32.

Nei M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**(3): 583e591. https://doi.org/10. 0000/PMID17248844.

Peakall R. and Smouse P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. Bioinformatics, **28**: 2537-2539. DOI: 10.1093/bioinformatics/bts460.

Perdereau A.C., Kelleher C.T., Douglas G.C. and Hodkinson T.R.2014. High levels of gene flow and genetic diversity in Irish populations of *Salix caprea* L. inferred from chloroplast and nuclear SSR markers. BMC Plant Biol., **14**: 202. doi: 10.1186/s12870-014-0202-x.

Pogorzelec M., Glebocka K., Hawrylak-Nowak B. and Bronowicka-Mielniczuk U. 2015. Assessment of chosen reproductive cycle processes and genetic diversity of *Salix myrtilloides* L. in wetlands of Polesie Lubelskie: the prospects of its survival in the region. Pol. J. Ecol., **63**: 352-364. http://dx.doi.org/10.3161/15052249PJE2015.63.3.006.

Pogorzelec M., Glebocka K., Hawrylak-Nowak B. and Parzymies M., 2014. Reproduction and diversity of the endangered *Salix lapponum* L. populations in Eastern Poland. Turk. J. Bot., **38**: 1239-1247. DOI:10.3906/bot-1405-113.

Porth I. and EI-Kassaby Y.A. 2014. Assessment of the genetic diversity in forest tree populations using molecular markers. Diversity, **6**: 283-295. https://doi.org/10.3390/d6020283.

Pritchard J.K., Stephens M. and Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics, **155**:945–959.

Przyborowski J.A., Sulima P., Kuszewska A., Załuski D. and Kilian A. 2013. Phylogenetic relationships between four *Salix* L. species based on DArT markers. Int. J. Mol. Sci., **14**: 24113-24125. doi:10.3390/ijms141224113.

Rajora O.P. and Rahman M.H., 2003. Microsatellite DNA and RAPD finger printing, identification and genetic relationships of hybrid poplar (*Populus* × *canadensis*) cultivars. Theor. Appl. Genet., **106**: 470-477.

Raja R., Singh N.B. and Bhat S.S. 2018. Assessment of genetic diversity among high yielding selected *Salix* clones, using RAPD and SSR markers. Genetika, **50**: 983-99. https://doi.10.2298/gensr1803983r.

Sandeep C., Kumar A., Rodrigues V., Viswanath S., Shukla A.K. and Sundaresan V. 2020. Morpho-genetic divergence and population structure in Indian *Santalum album* L. Trees, **34**(5): 1113-1129. https://doi.org/10.1007/s00468-020-01963-2.

Sharma J.P., Sankhyan H.P., Thakur S. and Gupta R.K. 2019. Estimates of genetic parameters for growth, leaf and biomass traits of Indian willow (*Salix tetrasperma* Roxb.). J. Tree Sci., **38**: 1-5. DOI: 10.5958/2455-7129.2019.00001.3.

Sharma J.P., Sankhyan H.P., Thakur S., Jha S.K., Sharma R., Poonam and Gautam V. 2021. Genetic diversity and population structure of Indian willow (*Salix tetrasperma* Roxb.) along its distributional range in the Himalayan region. Ecol. Genet. Genom., **21**: 100096. https://doi.org/10.1016/j.egg.2021.100096.

Sharma J.P., Singh N.B., Sankhyan H.P., Chaudhary P. and Huse S.K. 2011. Estimation of genetic parameters of newly introduced tree willow clones in Himachal Pradesh, India. Genetika, **43**: 487-501.DOI:10.2298/gensr1103487s.

Sher Z., Hussain F. and Ibrar M. 2014. Traditional knowledge on plant resources of Ashezai and Salarzai valleys, District Buner, Pakistan. Afr. J. Plant Sci., **8**: 42-53.

Singh N.B., Chaudhary Punit., Joshi S., Srivastva D.K., Sharma J.P., 2014. Molecular characterization of promising willow clones using RAPD markers. Indian For., **140**: 456-461.

Singh N.B., Singh M.K., Naik P. K., Thakur S. and Sharma J.P. 2013. Analysis of genetic diversity in female, male and half sibs willow genotypes through RAPD and SSR markers. Afr. J. Biotechnol., **12**: 4578-4587.

Singh N.B., Sharma J.P. and Thakur S. 2012. Willow Improvement in India with a special reference to work done at Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni. Forestry Bull., **12**(2): 43-55.

Skvortsov A.K. 1968. Willows of the USSR Willows of the USSR and adjacent lands. Multi-lingual Service Division, Bureau No. 125754, Ottawa, Canada.

Srihari J.M., Verma B., Kumar N., Chahota R.K., Singh V., Rathour R., Sunil K., Singh S.K., Sharma S.K. and Sharma T.R. 2013. Analysis of molecular genetic diversity and population structure in sea buckthorn (*Hippophae* spp L.) from north-western Himalayan region of India. J. Med. Plants Res., **7**: 3183-3196. DOI: 10.5897/JMPR12.1112.

Srivastav M., Singh S. K., Prakash J., Singh R., Sharma N., Ramchandra S., Devi R., Gupta A., Mahto A K. , Jayaswal P.K. , Singh S. and Singh N.K. 2021. New hyper-variable SSRs for diversity analysis in mango (*Mangifera indica* L.). Indian J. Genet. Plant Breed., **81**(1): 119-126. DOI: 10.31742/IJGPB.81.1.13.

Sulima P. and Przyborowski J.A. 2013. Genetic diversity of *Salix purpurea* L. genotypes and interspecific hybrids. Acta Biol. Crac. Ser. Bot., **55**: 29-36.DOI:10.2478/abcsb-2013-0020.

Sulima P., Prinz K. and Przyborowski J.A. 2018. Genetic diversity and genetic relationships of purple willow (*Salix purpurea* L.) from natural locations. Int. J. Mol. Sci., **19**: 105, DOI:10.3390/ijms19010105.

Sulima P., Przyborowski J.A. and Załuski D. 2009. RAPD markers reveal genetic diversity in *Salix purpurea* L. Crop Sci., **49**: 857-863. doi: 10.2135/cropsci2008.07.0397.

Törjék O., Kiss E., Kiss J., Kondrak M., Gyulai G., Gergacz J. and Heszky L. 2001. Evaluation of genetic diversity of poplar genotypes by RAPD and AP-PCR analysis. Biologia Futura, **52**: 345-354. doi:10.1556/ABiol.52.2001.2-3.19.

Van Puyvelde K. and Triest L. 2007. ISSRs indicate isolation by distance and spatial structuring in *Salix alba* populations along Alpine upstream rivers (alto Adige and upper Rhine). Belg. J. Bot., **140**: 100-108.

Weir B.S. and Basten C.J. 1990. Sampling strategies for distances between DNA sequences. Biometrics, **46**: 551-582.

Weir B.S. and Cockerham C.C. 1984. Estimating F statistics for the analysis of population structure. Evolution, **38**: 1358-1370.

WoI. 1972. Wealth of India: A dictionary of Indian raw material and industrial products. Vol. 9. New Delhi, CSIR. 471p.

## CORRIGENDUM

Heterotic grouping of sweet corn (Zea mays var. sachharata) genotypes based on their combining ability and molecular diversity by Anima Mahato, Jai Prakash Shahi , Pawan Kumar Singh , Monu Kumar and Ashok Singamsetti , Indian J. Genet., **81**(3): 410-421 (2021) DOI: 10.31742/IJGPB.81.3.8

Authors inform that the published version of this research article contained a typographical error in the formula of breeding efficiency in which the denominator 2 is missing. A corrected formula is provided below. Authors regret the inadvertent error.

Breeding efficiency = [{(HYintergroup/TNintergroup) x 100} + {(LYintragroup/TNintragroup) x 100}]/2