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Short Communication

The analysis of genetic diversity in willow (*Salix* spp.) by ISSR markers

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Abstract

In the present study, ISSR (Inter Simple Sequence Repeat) markers were employed to determine genetic diversity among 30 clones of *Salix* (*S. acmophylla, S. excelsa, S. alba* and *S. triandra*). The average percentage of polymorphic loci was 59.86. The pair-wise genetic similarity varied from 0.18 to 0.65. Nei's gene diversity (H_E) ranged from 0.12 to 0.18 and the average expected heterozygosity (H_E) estimated 0.15. The values obtained for genetic diversity (H_T = 0.20) and genetic differentiation (G_{ST} = 0.20) indicated a low level of genetic differentiation among examined populations of *Salix* in North West of Iran.

Key words: Genetic diversity, ISSR, polymorphism, *Salix*

The genus *Salix* belongs to the Salicaceae family and comprised of 350 to 500 species worldwide in form of trees or shrubs (Argus 1997). Thirty-one species of the *Salix* genus were reported in Iran (Maassoumi 2009). Willows are categorized as advanced biomass crops (Keoleian and Volk 2005) with many desirable traits that make them suitable for production. ISSR markers reveal higher level of polymorphism and show a higher reproducibility than RAPD and RFLP approaches (Tsumura et al.1996). This study is the first attempt to assess genetic diversity among willow clones in Iran.

Plant material consisted of *S. acmophylla* (6 clones), *S. excels* (12 clones), *S. alba* (9 clones) and *S. triandra* (3 clones). All of the studied clones were collected from different regions of the North West of Iran. Genomic DNA was extracted from fresh leaves tissue based on modified CTAB procedure (Doyle and Doyle 1987). The PCR – ISSR reaction was performed in a total volume of 25 µl using the following touchdown program: 3 min at 95°C for 1 cycle; 30 s at 95°C, 30 s at 65°C and 60 s at 72°C for 10 cycles; annealing temperature at 65°C being subsequently reduced by 1°C for the next 10 cycles and remained at 55°C for the remaining 30 cycles; 10 min at 72°C for 1 cycle. Shannon diversity index (*I*) was calculated in POPGENE 1.31 (Yeh et al. 1997). POPGENE 32 was employed to calculate number of observed alleles (*Na*), mean number of effective alleles (*Ne*), number of polymorphic loci, percentage of polymorphic loci, total heterozygosity (H_T), mean heterozygosity within the clones (H_S) and the coefficient of clone differentiation ($G_{ST} = H_T - H_S / H_T$).

Figure 1 is the representative of the extent of polymorphism observed in 20 willow clones by primer 825. The average number of scorable fragments per primer was 15.2, ranging from 9 to 23 (Table 1). Percentage of polymorphic loci was from 36.84% (*S. triandra*) to 80.26% (*S. excelsa*), with an average polymorphism of 59.86% across all four species (Table 1). The number of observed alleles per locus reached to 1.58 with the effective number of alleles equal to 1.24 (Table 2). Therefore, effective alleles accounted for 78.48% of total number of alleles. Number of polymorphic loci and Nei's gene diversity (H_E) ranged from 56 to 122 and 0.12 to 0.18, respectively. The

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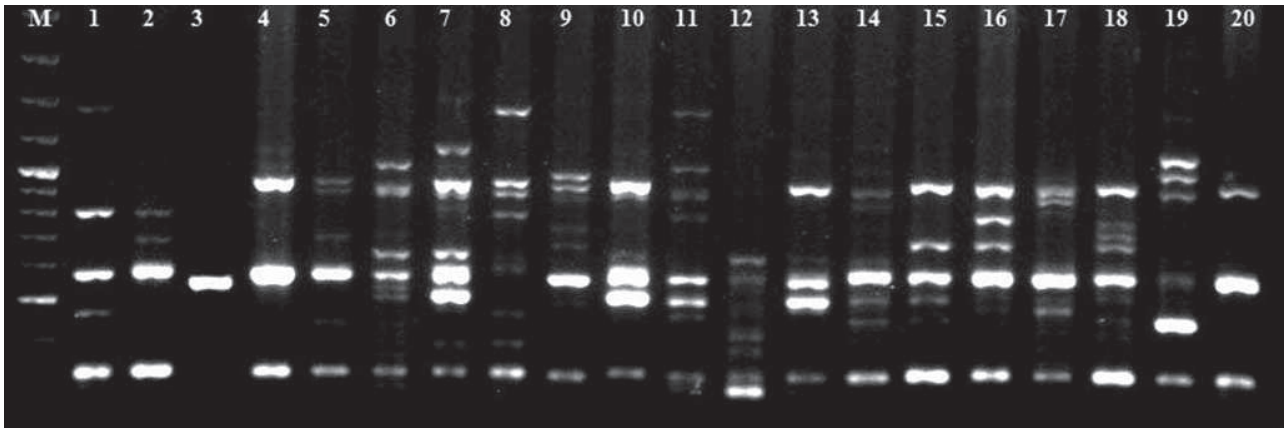

Fig. 1. Agarose gel of ISSR products representative of 20 willow clones using UBC 825. M=100 bp ladder

Table 1. List of ISSR primers used to detect polymorphism

Primer	Sequence (5' - 3')	Total of bands	Poly-morphic bands	Poly-morphism (%)
UBC 811	5'(GA)8C-3'	13	13	100
UBC 818	5'(CA)8G-3'	13	13	100
UBC 825	5'(AC)8T-3'	19	19	100
UBC 827	5'(AC)8G-3'	13	13	100
UBC 842	5'(GA)8CG-3'	9	9	100
UBC 847	5'(CA)8AC-3'	20	20	100
UBC 848	5'(CA)8AGC-3'	23	23	100
UBC 888	5'GAG(CA)7-3'	9	5	55.5
UBC 889	5'AGT(AC)7-3'	17	17	100
UBC 891	5'ACG(TG)7-3'	16	13	81.2
Total		152	145	-
Mean		15.2	14.5	93.67

average proportion of loci expected to be heterozygous was 0.15. Shannon diversity index (*I*) ranged from 0.19 (*S. triandra*) to 0.30 (*S. excelsa*) with the mean of 0.25 showing the low level of diversity. The total genetic diversity (H_T) estimated 0.20 and the proportion of total diversity residing among populations (G_{ST}) obtained 0.20.

The assessment of the genetic similarities at both the intra and inter-specific levels of *Salix* gives useful information for breeding programs and germplasm resource management. DNA markers are functional in crop breeding, especially in genetic diversity and gene mapping studies. So far, very few

Table 2. The genetic diversity parameters studied

Population name	Sample size	Na[a]	Ne[b]	H[c]	I[d]	NPL[e]	PPL[f]
S. acmophylla	6	1.5	1.25	0.16	0.25	83	54.61
S. excelsa	12	1.80	1.28	0.18	0.30	122	80.26
S. alba	9	1.67	1.27	0.17	0.28	103	67.76
S. triandra	3	1.36	1.19	0.12	0.19	56	36.84
Total	30	6.33	4.99	0.63	1.02	364	239.4
Mean	-	1.58	1.24	0.15	0.25	91	59.86

a = Observed number of alleles; b = Effective number of alleles; c = Expected heterozygosity; d = Shannon's information index; e = Number of polymorphic loc and f = Percentage of polymorphic loci

studies have been conducted for investigating the genetic diversity of the genus *Salix* and its species.In this study, ten selected ISSR primers generated 93.67% polymorphism implying effectiveness of ISSR marker in investigation of genetic diversity in *Salix*. In our study, effective alleles accounted for 78.48% of total number of alleles, which obtained higher than the value reported by Przyborowski and Sulima (2010) analyzing *Salix viminalis* genotypes (76.3%) based on RAPD marker. However, ten selected markers generated 93.67% polymorphism and 145 polymorphic bands, which appeared to be higher than the value reported earlier by Sulima and Przyborowski (2013) among 15 genotypes of *S. purpurea* (70.40%) employing 40 ISSR primers. The difference in polymorphic value reflects the diverse material and the inadequate number of ISSR markers in present study. The average Nei's gene diversity (H_E) estimated 0.15 and average Shannon index (*I*) obtained 0.25 (Table 2). This level of genetic variation is lower than

reported by Sulima et al. (2009) estimating values of 0.24 for H_E and 0.36 using RAPD primers in *Salix*, probably due to different marker system used. However, the obtained G_{ST} value (0.20) in this study, indicates little genetic differentiation among clones. It is necessary for future breeding programs to determine genetic diversity and relationships among *Salix* species using different molecular markers.

Authors' contribution

Conceptualization of research (FR); Designing of the experiments (FR); Contribution of experimental materials (AK); Execution of field/lab experiments and data collection (MG); Analysis of data and interpretation (MG, FR); Preparation of manuscript (MG, FR).

Declaration

The authors declare no conflict of interest.

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