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POLSKA AKADEMIA NAUK

ACTA BIOLOGICA CRACOVIENSIA Series Botanica 55/2: 29–36 2013
DOI: 10.2478/abcsb-2013-0020

GENETIC DIVERSITY OF *SALIX PURPUREA* L. GENOTYPES AND INTERSPECIFIC HYBRIDS

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Received March 1, 2013; revision accepted July 15, 2013

This study used ISSR markers to assess the genetic diversity of a collection of 15 genotypes of *Salix purpurea* and 6 interspecific hybrids, employing 40 of 60 tested ISSR primers generating polymorphic amplification products. The PCR-ISSR method was adapted for *S. purpurea* by optimizing the annealing temperature for each primer. The polymorphism index of ISSR amplification products was 91.8% for all studied genotypes and 70.4% for *S. purpurea* genotypes. Nei's genetic identity statistics ranged from 0.538 to 0.958. Nei's genetic distance values were used to build a dendrogram (UPGMA) for the investigated genotypes. The dendrogram shows five clusters, and principal coordinate analysis yielded nearly the same genetic relationships among the studied genotypes. The results confirm the usefulness of ISSR markers for determining genetic diversity in *S. purpurea*.

Key words: *Salix purpurea*, hybrids, genetic diversity, ISSR, UPGMA, PCoA.

INTRODUCTION

Purple willow *Salix purpurea* is native to Europe and North Africa (Skvortsov, 1999). In Poland it is a common species found mostly along rivers and streams in, for example, *Salicetum triandro-viminalis* associations (Seneta and Dolatowski, 2003). Purple willow readily forms interspecific hybrids with *S. daphnoides* and *S. viminalis*, and the three have been characterized as sharing the same developmental and geographic type (Neuman, 1981). *S. purpurea* is frequently grown on arable land because its biomass has a variety of commercial applications (Hörandl et al., 2002; Sulima et al., 2006). The bark of purple willow is a particularly valuable herbal material, used for the production of natural aspirin (*EUROPEAN PHARMACOPOEIA*, 2005).

Among willow species, the bark of *S. purpurea* has the highest concentration of salicylic glycosides, known for their analgesic, antipyretic and anti-inflammatory effects (Bisset and Witchtl, 2001; Schmid et al., 2001a). Due to its medicinal properties, willow bark is used in the treatment of colds and rheumatic conditions. The range of effects of the aspirin produced from willow bark is similar to that of synthetic acetylsalicylic acid (Chrubasik et al., 2000, 2001; Schmid et al., 2001b).

The salicylic glycoside content of bark differs between willow species (Pobłocka-Olech et al., 2007), and also varies significantly within species, including *S. purpurea* (Sulima et al., 2006; Förster et al., 2010). Research should focus on selecting *S. purpurea* genotypes and breeding new forms having the highest content of salicylic glycosides. The initial stage of breeding work is aimed at broadening the range of genetic diversity by transgression following crossing of parental forms. Parental forms should be selected to ensure the widest possible genetic variation among the offspring. These imperatives make the genetic diversity of potential parental forms an important consideration.

Molecular techniques can be applied to assess the degree of genetic variation and genetic similarity, identify genotypes, and facilitate marker-assisted selection (MAS). One of the most frequently applied PCR-based techniques employs ISSR (inter-simple sequence repeat) markers (Zietkiewicz et al., 1994). The main advantages of ISSR include its relative simplicity and high reproducibility of results (Bornet and Branchard, 2001; Ning et al., 2007). Another important factor is the presumption that microsatellite markers can be linked to coding regions (Zietkiewicz et al., 1994; Bornet et al., 2002). ISSR markers are highly polymorphic, which

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PL ISSN 0001-5296

TABLE 1. Nucleotide sequences and annealing temperatures of primers generating polymorphic ISSR products from the analyzed genotypes

Primer	Nucleotide sequence (5'-3')	Annealing temperature (°C)	Primer	Nucleotide sequence (5'-3')	Annealing temperature (°C)
ISSR-1	GGGACAACAACAACAACA	50	ISSR-57	GTCCACCACCACCACCAC	60
ISSR-2	GGTACAACAACAACAACA	50	ISSR-59	AACCACCACCACCACCAC	60
ISSR-3	GGCACAACAACAACAACA	50	ISSR-64	GAGCGACGACGACGACGA	61
ISSR-4	GAGACAACAACAACAACA	50	ISSR-67	AAGCGACGACGACGACGA	60
ISSR-5	GATACAACAACAACAACA	50	ISSR-68	AATCGACGACGACGACGA	60
ISSR-6	GACACAACAACAACAACA	60	ISSR-91	GAGGTGTGTGTGTGTGTG	61
ISSR-7	GTGACAACAACAACAACA	50	ISSR-92	GAAGTGTGTGTGTGTGTG	57
ISSR-8	GTTACAACAACAACAACA	50	ISSR-93	GACGTGTGTGTGTGTGTG	63
ISSR-9	GTCACAACAACAACAACA	49.5	ISSR-94	AAGGTGTGTGTGTGTGTG	57
ISSR-11	TGTACAACAACAACAACA	48	ISSR-95	AAAGTGTGTGTGTGTGTG	53
ISSR-12	TGCACAACAACAACAACA	49.5	ISSR-97	CAGGTGTGTGTGTGTGTG	63
ISSR-14	TATACAACAACAACAACA	49.5	ISSR-98	CAAGTGTGTGTGTGTGTG	65
ISSR-28	GGGCACCACCACCACCAC	60	ISSR-137	GCAACACACACACACAC	65
ISSR-29	GGTCACCACCACCACCAC	60	ISSR-139	ACGACACACACACACAC	65
ISSR-32	GATCACCACCACCACCAC	60	ISSR-141	ACTACACACACACACAC	61
ISSR-33	GACCACCACCACCACCAC	58	ISSR-142	TCGACACACACACACAC	63
ISSR-34	GTGCACCACCACCACCAC	58	ISSR-213	GATAGATAGATAGATA	42
ISSR-39	TGCCACCACCACCACCAC	61	ISSR-214	GACAGACAGACAGACA	52
ISSR-55	GGCCACCACCACCACCAC	60	ISSR-215	CAGCAGCAGCAGCAG	61
ISSR-56	GACCACCACCACCACCAC	60	ISSR-216	CAACAACAACAACAA	53

makes them a useful tool in studies of genetic diversity. They can also be used as a supplementary technique for gene mapping. The high repeatability of ISSR markers (Fang and Roose, 1997; Moreno et al., 1998) is due mostly to the use of longer primers (16–25 bp) and higher annealing temperatures (45–65°C) (Reddy et al., 2002). Annealing temperature is determined largely by the GC content of the applied primers (Reddy et al., 2002). These specific properties make ISSR markers a highly effective method for investigating genetic diversity.

In this study we used ISSR markers to analyze the genetic diversity of collected material of *S. purpurea* and its interspecific hybrids.

MATERIALS AND METHODS

PLANT MATERIAL

The experimental material comprised 21 genotypes, including 15 of the species *S. purpurea* (UWM 062, UWM 072, UWM 142, UWM 143, UWM 147, UWM 164, UWM 165, UWM 166, UWM 167, UWM 168, UWM 169, UWM 170, UWM 173, UWM 174, UWM 199) and six hybrid forms (*S. purpurea* × *S. daphnoides* – UWM 029, UWM 193; *S. viminalis* × *S. purpurea* – UWM 033, UWM 065, UWM 086, UWM 093). All of the studied genotypes were selected from the collection of the Department of Plant Breeding and Seed Production of the University of Warmia and Mazury in Olsztyn (Poland). Previous studies of these genotypes examined their productivity and the content of pharmacologically active compounds in bark, as a source of herbal material (Sulima et al., 2006; Sulima, unpub. data). The *S. purpurea* genotypes and natural hybrids of this species will be used for the production of clones (varieties) with high salicylic glycoside content in the bark and for development of a mapping population.

ISOLATION OF GENOMIC DNA AND PRIMER SELECTION

DNA was isolated from fresh tissue of young leaves by Milligan's (1998) method, with modifications (Sulima et al., 2009). Primers were selected based on reference data (Hantula et al., 1996; McGregor et al., 2000). Sixty primers were tested. Gradient PCR was used to determine the optimum annealing temperature for each primer. Following preliminary screening, 40 primers generating polymorphic amplification products with stable band patterns were selected for the study (Tab. 1, Fig. 1).

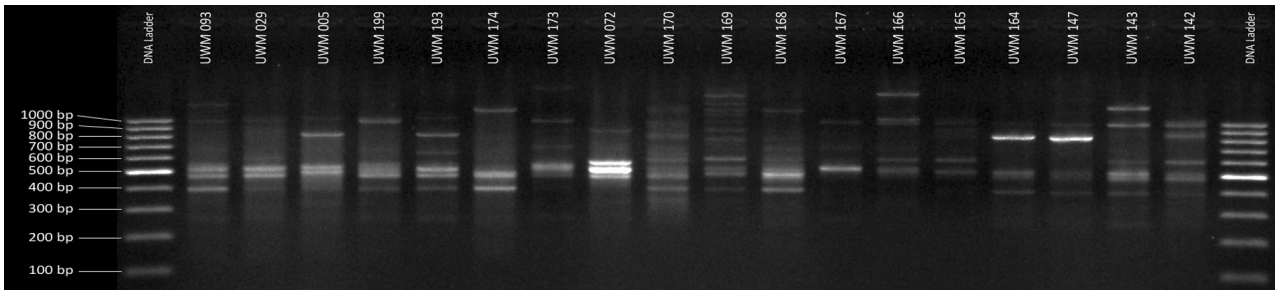

Fig. 1. Amplification products using ISSR-214 for eighteen analyzed genotypes of *Salix purpurea*.

PCR CONDITIONS

PCR-ISSR reactions (Zietkiewicz et al., 1994) were performed in a peqSTAR 96 Universal Gradient thermocycler (Peqlab Biotechnologie GmbH). The reaction mixture (25 l) contained 2.5 µl 10x Taq Buffer with KCl (Fermentas), 2 mM $MgCl_2$ (Fermentas), 0.6 mM dNTP (Sigma), 0.2 mM primer, DNA (~10 ng), 1.0 U thermostable DNA polymerase (Fermentas) and sterile distilled water. The reaction was performed in 37 cycles with initial denaturation at 94°C for 1 min and final elongation at 72°C for 8 min. The following thermal cycling profile was applied: I – denaturation at 94°C for 1 min, II – annealing (42–66°C) for 2 min, III – elongation at 72°C for 2 min. The optimum annealing temperature was determined for each primer in test PCR-ISSR reactions along a temperature gradient. Amplification products were separated on 1% agarose gel in TBE buffer. DNA was stained with ethidium bromide and visualized under UV with a Biogenet DIGIDOC gel imaging system. Every genotype was analyzed twice. The size of the obtained products was determined with TotalLab 2003 software. The applied standard was a Fermentas GeneRuler^{TM} 100 bp DNA Ladder (100–1000 bp).

STATISTICAL ANALYSIS

The products of the PCR-ISSR reactions were processed in a binary system where 1 denoted the presence and 0 the absence of particular amplification products. Data were entered in a Microsoft EXCEL spreadsheet to create a binary matrix. The number of polymorphic and monomorphic amplification products generated by each primer and the degree of homology of the investigated genotypes was determined for each primer. The binary matrix was analyzed and the basic parameters of genetic diversity were calculated with the POPGENE application (Yeh et al., 1997). Analyses were performed for two groups: the first involved all studied genotypes, and the other involved genotypes of *S. purpurea*. The polymorphism index of the amplification products (P), number of observed alleles (*na*), mean number of effective alleles (*ne*) (Hartl and Clark, 1989), mean Nei's gene diversity index (*h*) (Nei, 1973) and Shannon index (*I*) (Shannon and Weaver, 1949) were determined for both groups. A matrix of Nei's genetic identity (IS) and Nei's genetic distance (D_S) between all investigated genotypes (Nei, 1972, 1978) was also developed using POPGENE software. Based on Nei's genetic distance statistics, a dendrogram was built with MEGA 5 software (Tamura et al., 2011). The dendrogram was developed by UPGMA cluster analysis (Sneath and Sokal, 1973). Principal coordinate analysis (PCoA) based on Nei's genetic distance was done to analyze the genetic diversity of the studied genotypes, using GenAlEx 6.5 (Peakall and Smouse, 2006, 2012).

RESULTS

ISSR POLYMORPHISM AND BASIC GENETIC DIVERSITY PARAMETERS

After test PCR-ISSR reactions involving 60 primers, 40 primers were selected for assessing the genetic diversity of *S. purpurea* (Tab. 1). The other 20 tested primers did not generate any amplification products or polymorphic products. The selected primers generated 331 amplification products, only 8% of which were monomorphic (Tab. 2). The total genotyping error rate for all ISSR markers used in this study was 5.73%. The number of products amplified with single primers ranged from 4 to 14 (average 8.15). The ISSR products ranged in size from 200 to 2000 bp. Average homology between genotypes for each primer was 44.5% (Tab. 2). Homology was lowest for primer ISSR-92 (26.32%) and highest for primer ISSR-55 (89.68%). The polymorphism of all amplification products (*P*) was 91.84% for all investigated genotypes and 70.39% for *S. purpurea* genotypes (Tab. 3). The mean number of effective alleles (*ne*) per locus was 1.49 for all genotypes and 1.42 for *S. purpurea*. The values for Nei's gene diversity index and the Shannon index were higher for all investigated genotypes (h=0.29, I=0.44) than for *S. purpurea* genotypes alone (h=0.24, I=0.37).

TABLE 2. Number of amplification products and degree of homology between the analyzed genotypes for the primers used in the study

Primer	Number of amplification products			Degree of homology of genotypes [%]	
	Total	Monomorphic	Polymorphic	Mean	Range
ISSR-1	7	0	7	44.90	9.5 – 95.2
ISSR-2	8	1	7	41.67	9.5 – 100
ISSR-3	8	0	8	49.40	19.05 – 95.2
ISSR-4	8	0	8	47.02	9.5 – 90.5
ISSR-5	9	1	8	41.80	19.05 – 100
ISSR-6	8	0	8	36.31	4.8 – 90.5
ISSR-7	10	0	10	31.90	4.8 – 85.7
ISSR-8	11	0	11	29.87	4.8 – 85.7
ISSR-9	7	0	7	43.54	9.5 – 85.7
ISSR-11	8	0	8	35.12	4.8 – 95.2
ISSR-12	9	0	9	44.97	4.7 – 90.5
ISSR-14	5	0	5	39.05	9.5 – 95.2
ISSR-28	7	1	6	41.50	9.5 – 100
ISSR-29	8	1	7	51.19	9.5 – 100
ISSR-32	11	1	10	48.48	4.8 – 100
ISSR-33	8	3	5	57.03	6.3 – 100
ISSR-34	7	2	5	57.14	11.8 – 100
ISSR-39	8	1	7	52.98	9.5 – 100
ISSR-55	6	3	3	89.68	66.7 – 100
ISSR-56	6	2	4	78.57	4.8 – 100
ISSR-57	7	3	4	71.43	4.8 – 100
ISSR-59	4	2	2	79.76	23.8 – 100
ISSR-64	6	1	5	36.67	5.0 – 100
ISSR-67	8	2	6	36.31	4.8 – 100
ISSR-68	4	1	3	41.67	4.8 – 100
ISSR-91	11	0	11	28.14	14.3 – 57.1
ISSR-92	14	0	14	26.32	5.3 – 68.4
ISSR-93	13	0	13	35.90	4.8 – 95.2
ISSR-94	9	0	9	33.86	4.8 – 66.7
ISSR-95	10	0	10	33.33	14.3 – 66.7
ISSR-97	10	0	10	51.90	4.8 – 90.5
ISSR-98	6	0	6	46.83	9.5 – 81.0
ISSR-137	11	0	11	40.19	10.5 – 78.9
ISSR-139	9	0	9	31.22	4.8 – 90.5
ISSR-141	5	0	5	38.10	4.8 – 66.7
ISSR-142	8	0	8	26.79	4.8 – 66.7
ISSR-213	10	0	10	26.67	9.5 – 81.0
ISSR-214	10	0	10	34.76	4.8 – 95.2
ISSR-215	9	2	7	57.22	5.0 – 100
ISSR-216	8	0	8	40.63	5.0 – 95.0
Sum	331	27	204	Mean: 44.50	

DENDROGRAM AND PRINCIPAL COORDINATE ANALYSIS

Nei's genetic identity index ranged from 0.538 (between *S. viminalis* × *S. purpurea* UWM 065 and *S. purpurea* × *S. daphnoides* UWM 029) to 0.958 (between *S. purpurea* × *S. daphnoides* UWM 029 and *S. purpurea* × *S. daphnoides* UWM 193) (Tab. 4). Genetic similarity between *S. purpurea* genotypes ranged from 0.631 to 0.940. In Figure 2,

TABLE 3. Main genetic diversity parameters

Parameter	All genotypes	*S. purpurea*
The number of observed alleles *(na)*	1.92	1.70
The mean number of effective alleles *(ne)*	1.49	1.42
The mean Nei's gene diversity index *(h)*	0.29	0.24
Shannon index *(I)*	0.44	0.37
The polymorphism of amplification products *(P)*	91.84	70.39

genetic distance between the analyzed genotypes is given on a UPGMA dendrogram, and Figure 3 shows genetic distance as found by principal coordinate analysis. The dendrogram distinguishes five clusters among the 21 studied genotypes. The first and largest cluster groups 13 *S. purpurea* genotypes (UWM 062, UWM 072, UWM 142, UWM 164, UWM 165, UWM 166, UWM 167, UWM 168, UWM 169, UWM 170, UWM 173, UWM 174, UWM 199). The second, 0.2 units distant from the first group, comprises two *S. purpurea* × *S. daphnoides* genotypes (UWM 029, UWM 193). Genetic distance is highest (0.23 units) between those two groups and the other three clusters. The third group contains two *S. viminalis* × *S. purpurea* genotypes (UWM 086, UWM 093). The remaining two *S. purpurea* genotypes (UWM 143, UWM 147) form the fourth group, 0.2 units distant from the third group. These genotypes differed most from the other forms of *S. purpurea*. Principal coordinate analysis (Fig. 3) yielded four clusters. One cluster consists of four genotypes of *S. viminalis* × *S. purpurea* hybrids, revealing more genetic similarity between these hybrids than the dendrogram did.

DISCUSSION

The literature provides little information on the use of molecular markers for evaluating the genetic diversity of *Salix purpurea*, and gives no information on the use of ISSR markers for this purpose, except for a short report on the genus *Salix* (Park et al., 2011). A few authors have used SSR markers to study the genetic structure of *Salix purpurea* populations (Barker et al., 2003; Lin et al., 2009). Differences in the character of these markers make it difficult to compare results based on SSR with those based on RAPD or ISSR, the latter two of which share more similarities. Our present findings can be compared with RAPD results for *Salix* and ISSR results for related genera such as *Populus*.

The percentage shares of polymorphic amplification products reported in this study correspond with other findings. In previous work using RAPD markers to assess genetic diversity (Sulima et al., 2009) we reported somewhat different polymor-

TABLE 4. Genetic identity (IS) (above diagonal) and genetic distance (DS) (below diagonal) statistics for the analyzed genotypes of *S. purpurea* and their hybrids

	UWM 093	UWM 029	UWM 065	UWM 199	UWM 193	UWM 174	UWM 173	UWM 072	UWM 170	UWM 169	UWM 168	UWM 167	UWM 166	UWM 165	UWM 164	UWM 147	UWM 143	UWM 142
UWM 093	****	0.7432	0.6949	0.7462	0.7492	0.7553	0.7432	0.7674	0.7644	0.7432	0.7130	0.8097	0.6918	0.6798	0.7341	0.6103	0.6224	0.5559
UWM 029	0.2968	****	0.7946	0.8036	0.6737	0.6677	0.6858	0.8187	0.7069	0.6858	0.6737	0.6495	0.6526	0.6707	0.6828	0.6073	0.6375	0.5831
UWM 065	0.3640	0.2300	****	0.6828	0.7825	0.6798	0.8066	0.6979	0.7190	0.6798	0.7281	0.6314	0.6526	0.6647	0.6405	0.6073	0.6677	0.6133
UWM 199	0.2927	0.2186	0.3816	****	0.7432	0.7251	0.7553	0.9063	0.7039	0.7251	0.6949	0.7190	0.6798	0.7281	0.5861	0.5619	0.5740	
UWM 193	0.2887	0.3949	0.2453	0.2968	****	0.7644	0.9396	0.7523	0.7190	0.7039	0.7644	0.7402	0.6767	0.7070	0.7070	0.5710	0.6012	0.5770
UWM 174	0.2807	0.4040	0.3860	0.3215	0.2687	****	0.7644	0.7402	0.7251	0.7523	0.6979	0.7341	0.7311	0.6767	0.7069	0.5589	0.5952	0.5589
UWM 173	0.2968	0.3772	0.2149	0.2807	0.0623	0.2687	****	0.7523	0.7311	0.7644	0.7341	0.6949	0.6767	0.6888	0.5952	0.6314	0.5952	
UWM 072	0.2648	0.2000	0.3597	0.0983	0.2847	0.3009	0.2847	****	0.7553	0.7462	0.7402	0.7462	0.7009	0.7190	0.7251	0.5952	0.5891	0.5952
UWM 170	0.2687	0.3468	0.3298	0.3511	0.3298	0.3215	0.3132	0.2807	****	0.7372	0.7432	0.7674	0.6858	0.6500	0.7160	0.6042	0.6405	0.5982
UWM 169	0.2968	0.3772	0.3860	0.3215	0.3511	0.2847	0.3257	0.2927	0.3050	****	0.7704	0.7221	0.7553	0.6647	0.7130	0.6193	0.6677	0.6073
UWM 168	0.3383	0.3949	0.3172	0.3640	0.2687	0.3597	0.2687	0.3009	0.2968	0.2609	****	0.7221	0.7553	0.7130	0.7009	0.5650	0.6495	0.6012
UWM 167	0.2111	0.4315	0.4598	0.3298	0.3009	0.3091	0.3091	0.2927	0.2648	0.3257	****	0.7372	0.6647	0.7855	0.5770	0.5952	0.5589	
UWM 166	0.3684	0.4268	0.4268	0.3860	0.3905	0.3132	0.3640	0.3554	0.3772	0.2807	0.2807	0.3050	****	0.7583	0.6858	0.5982	0.7009	0.6707
UWM 165	0.3860	0.3994	0.4085	0.3860	0.3994	0.3905	0.3905	0.3298	0.4315	0.4085	0.3383	0.4085	0.2767	****	0.7341	0.6586	0.6888	0.6767
UWM 164	0.3091	0.3816	0.4455	0.3173	0.3994	0.3468	0.3728	0.3215	0.3341	0.3383	0.3554	0.2414	0.3772	0.3091	****	0.6224	0.6284	0.6103
UWM 147	0.4939	0.4988	0.4988	0.5343	0.5604	0.5818	0.5189	0.5189	0.5038	0.4791	0.5710	0.5498	0.5139	0.4176	0.4742	****	0.7281	0.6798
UWM 143	0.4742	0.4503	0.4040	0.5764	0.5088	0.5189	0.4598	0.5291	0.4455	0.4040	0.4315	0.5189	0.3554	0.3728	0.4646	0.3173	****	0.7221
UWM 142	0.5872	0.5394	0.4889	0.5551	0.5498	0.5818	0.5189	0.5189	0.5139	0.4988	0.5088	0.5818	0.3994	0.3905	0.4939	0.3860	0.3257	****

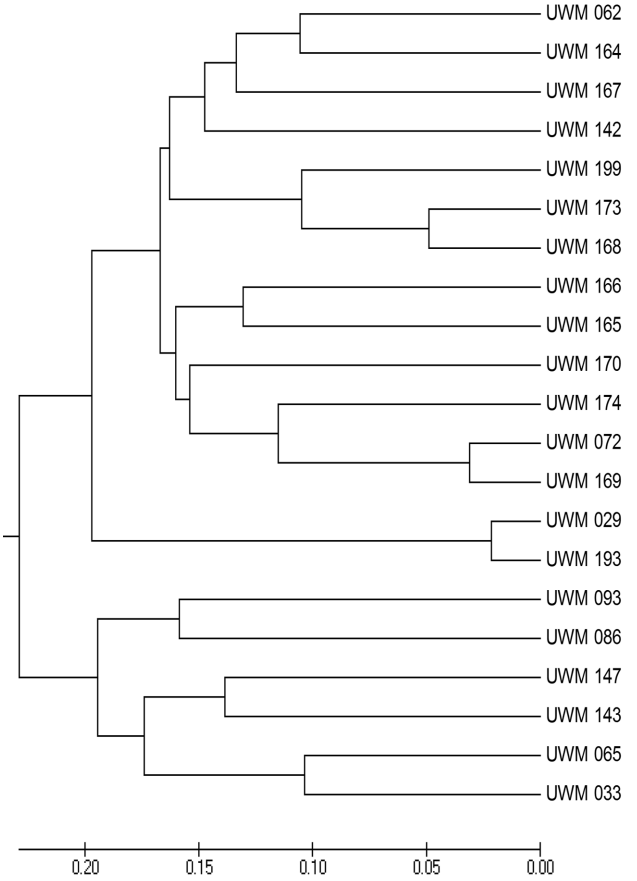

Fig. 2. UPGMA dendrogram of Nei's genetic distance between the analyzed genotypes of *Salix purpurea* and their hybrids based on ISSR markers.

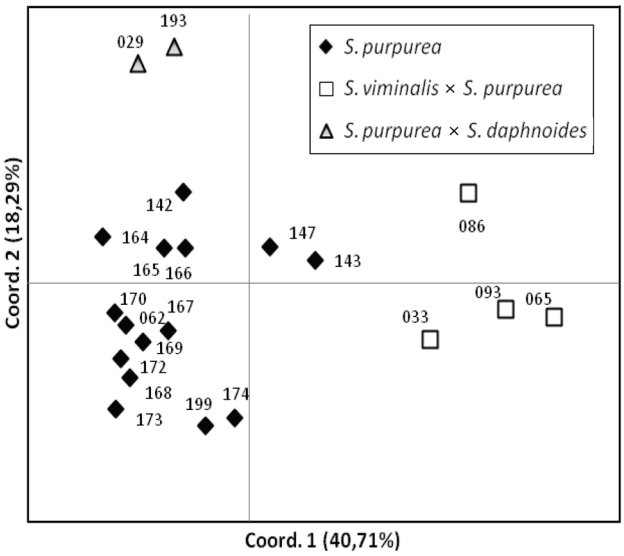

Fig. 3. Principal coordinate analysis (PCoA) of 21 genotypes of *Salix purpurea* and their hybrids based on ISSR markers.

phism values; the polymorphism of the amplification products was lower for all studied genotypes (88.8%) but higher for *S. purpurea* genotypes (75.5%). The differences in polymorphism values may be due to the use of a different set of genotypes. For *S. purpurea* genotypes the greater polymorphism was due to treating genotype UWM 172 as *S. purpurea*. In that study, genetic identity between UWM 172 and the other representatives of the species was low. UWM 172, incorrectly identified as pure *S. purpurea*, was later found to be a hybrid, most probably with *S. viminalis* (Sulima, unpub. data). Thus the polymorphism of the RAPD and ISSR amplification products can be taken as comparable. We stress that the ISSR primers generated many more amplification products (331 products, avg. 8.3 products per primer) than RAPD primers did (224 products, 5.7 products per primer). ISSR markers clearly are the better choice for determining genetic diversity in *S. purpurea*.

In ten *S. koreensis* populations, Park et al. (2011) reported 48% average ISSR marker polymorphism. Using a RAPD-based method to assess the genetic diversity of 19 *S. viminalis* genotypes, Przyborowski and Sulima (2010) reported higher polymorphism (94.29%) in 210 amplification products, confirming high genetic diversity within *S. viminalis*. Barker et al. (1999) studied genetic variation in fast-growing willow clones (mostly *S. viminalis* and its hybrids), finding that 88% of the 194 amplified RAPD products were polymorphic. Sabatti et al. (2001) reported 80% polymorphism in 71 RAPD markers for 53 *Populus alba* genotypes. In a study of *Populus* × *canadensis* hybrids, Rajora and Rahman (2003) found that 76% of 248 RAPD products were polymorphic. They used only 26 primers, each of which generated 9.54 products on average, to reveal such a high number of loci. Lu et al. (2006) used ISSR primers to study seven natural populations of *Populus cathayana* (161 genotypes) and reported 98.7% polymorphism. In another study, ISSR marker polymorphism was a little lower (84%) among 28 genotypes of various *Populus* species (Jianming et al., 2006). These various studies confirm the high genetic diversity of *Salix* and *Populus* species.

The remaining parameters determined in this study also indicate high genetic diversity in *S. purpurea* (Tab. 3). The data are in accord with Sulima et al. (2009), who found *ne* to be 1.46 in all studied genotypes and 1.42 in *S. purpurea*. Przyborowski and Sulima (2010) reported *ne* of 1.48 in *S. viminalis* genotypes. Lu et al. (2006) gave numbers of effective alleles ranging from 1.228 to 1.390 in natural populations of *Populus cathayana*. The values for Nei's gene diversity index and the Shannon index

from the present study are almost identical with those found using RAPD (Sulima et al., 2009). Przyborowski and Sulima (2010) gave $h = 0.29$ and $I = 0.45$ for *S. viminalis*, higher than those we calculated for *S. purpurea*.

The values for Nei's genetic identity index determined for *S. purpurea* genotypes using RAPD technique (0.580–0.950) (Sulima et al., 2009) were similar to those we found by ISSR technique. Przyborowski and Sulima (2010) gave slightly lower values (0.519–0.891) for *S. viminalis*, indicating more distant relationships between the studied forms. Five *Populus euphratica* populations gave values for Nei's gene diversity index ranging from 0.059 to 0.212 (Saito et al., 2002). Lu et al. (2006) reported similar values from natural populations of poplar. Such a wide range of genetic variation may be associated with dioecism and the mechanism of generative reproduction observed in both *Salix* and *Populus* species, as well as their ease of interspecific hybridization.

Two *S. purpurea* genotypes (UWM 143, UWM 147) differed most from the other forms of *S. purpurea*. A previous RAPD analysis (Sulima et al., 2009) also found genetic similarity to be lowest between those two *S. purpurea* genotypes and the other genotypes of that species. Molecular biology techniques are commonly applied to analyze the genetic diversity of living organisms at various development stages, regardless of environmental impacts. They provide highly reproducible results and are easy to use. Molecular techniques are applied at different stages in breeding new high-yielding varieties. They can also be used for taxonomic identification, an important consideration in heterogeneous genera such as *Salix* (Argus, 1997). Molecular techniques also are useful for determining the identity of genotypes (Rajora and Rahman, 2003), which in the genus *Salix* is difficult to do based solely on morphological characters (Neumann, 1981; Skvortsov, 1999). In our previous work the genetic diversity of *S. purpurea* and *S. viminalis* was assessed by RAPD technique. Due to its simplicity and repeatability (Fang and Roose, 1997; Moreno et al., 1998), ISSR-based methods are increasingly applied to evaluate genetic diversity in various organisms.

CONCLUSIONS

This study confirmed the usefulness of ISSR markers for determining the genetic diversity of *S. purpurea*. ISSR markers provided more information than RAPD markers and used fewer primers, reducing analysis time. We successfully adapted the ISSR method for analyzing genetic material of *S. purpurea* by optimizing the annealing temperature for each primer. Our results support the notion that microsatellite regions are subject to evolutionary change, leading to higher polymorphism (Bornet et al., 2002; Hu et al., 2011).

ACKNOWLEDGEMENTS

This work was financed in part by the Polish Ministry of Science and Higher Education (project No. N N310 088337) and by the Department of Plant Breeding and Seed Production, University of Warmia and Mazury (Olsztyn, Poland).

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