14th International Symposium "Prospects for the Third Millennium Agriculture" September 24th-26th 2015 Cluj Napoca, Romania

THE MOLECULAR POLYMORPHISM EVALUATION IN *Salix* sp. ROMANIAN ACCESSIONS – PRELIMINARY RESULTS

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INTRODUCTION

Due to the world energetic crisis the regenerable resources of energy, represented by vegetal biomass from SRC cultures (short rotation coppice) are a sustainable option. The species of *Salix* genus, constitute a promising source in the action for fighting of the environment degradation, and offer remedy for about two third from the all degradation types: erosion, destroyed as result of mining activities, industrial waste dumps, soil degradation, the presence of some ore bad smelling, sewerage works, oil exploitations, petroliferous waste dumps, waste dumps with radioactive waste, a.o. SRC willow genotypes cultivated in Romania exclusively encompasses foreign germplasm. In order to increase adaptability to a changing climate and production, as well, and to start a future breeding program, a collection of progenitors (Romanian accessions) was established.

Aims: Most of the willow genotypes are closely related genetically, due to frequent natural hybridisation in nature. In order to characterize properly local germplasm, beyond morphological criteria, applying molecular marker techniques has to be performed.

Fig. 1. Aspect of the *Salix* progenitors collection in July 2015 (culture established in the end of March 2015)

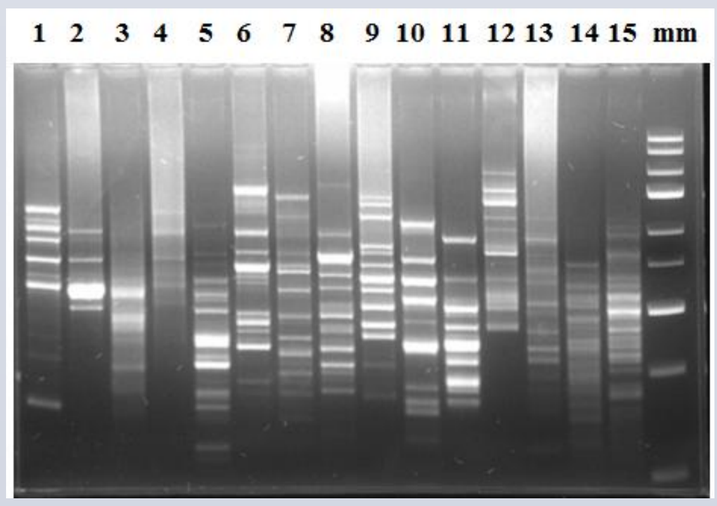
RESULTS AND DISSCUSIONS

In the first step the markers which generated the most complex fingerprints were selected from the 15 ISSR (Inter Simple Sequence Repeats) and 5 DAMD (Direct amplification of minisatellite-region) markers preliminary tested. Both micro and mini-satellites markers were analyzed for a more accurate assessment of genetic variability.

For the tetra nucleotides primers (A2 and A3) the number of generated fragments was smaller compared with the other primers. The primers with degenerated sequences (UBC 884 and UMC 886) generated complex fingerprints, with a high number of fragments of approximately similar length. Therefore their using for willow variability assessment is difficult.

For the preliminary investigations the markers A13 $(GT)_6CC$ și UBC 818 $(CA)_7G$ were used because they amplified a high number of fragments, with different sizes, making them suitable for variability evaluation.

Were used primers with sequences between 11 and 20 nucleotides, originated from the minisatellites fragments present in a high number of species. All the used primers generated fingerprints with a high number of fragments. The primer 5-M13 generated a high number of fragments , with close sizes, making difficult their evaluation. For the following screening the primers 1-URP6R (20 nucleotides) and 4- 14C2 (14 nucleotides) were selected.



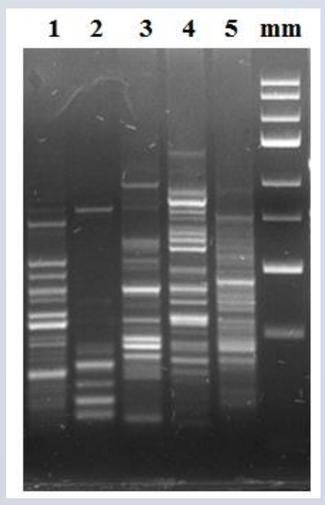


Fig. 2 The agarose gel electrophoresis (2%) of the products generated after the amplification with ISSR (a) and DAMD (b) primers

Further on, 7 *Salix* sp. samples were selected, collected from different locations from the West and South of Romania as follows : 1- *Salix alba* (Agadici), 2- *Salix alba* (Sohodol), 3- *Salix alba/fragilis* (Pocruia), 4- *Salix fragilis* (Sterile dump Pinoasa), 5- *Salix daphnoides* (Agadici), 6- *Salix purpurea* (Sterile dump Farcasesti) and 7- *Salix triandra* (Bobicesti)

Two samples from *Salix alba* were selected because this was the most abundant specie between the collected samples. It was also chosen the sample 3, whose phenotypic identification was not possible, probably being an interspecific hybrid.

MATERIALS AND METHODS

The DNA was extracted from leaf tissues collected from different *Salix* species and locations using the CTAB method (SR EN ISO 21571). The DNA amplification followed the conditions determined by the primers specificity. In a first stage 15 ISSR and 5 DAMD markers were tested, choosing the primers that generated the most complex DNA fingerprints (Table 1). For amplification was used Go Taq Green Master Mix 2x (Promega) kit.

The amplification conditions followed the program: 94°C - 3 min, 45 cycles: denaturation 94°C - 30 sec, primers annealing 54°C - 45 sec, DNA synthesis 72°C - 2 min, final synthesis 72°C - 5 min. The amplification products were separated by agarose gel electrophoresis and visualized with ethidium bromide.

Sequence of the primers used for variability evaluation

Table 1

ISSR Markers			DAMD Markers		
No.	Designation	Sequence (5'-3')	No.	Designation	Sequence (5'-3')
1	A2	$(ACTG)_4$	1	URP6R	GGCAAGCTGGTGGGAGGTAC
2	A3	$(GACA)_4$	2	URP9F	ATGTGTGCGATCAGTTGCTG
3	A7	$(AG)_{10}T$	3	33.6	GGAGGTGGGCA
4	A10	$(CT)_{10}T$	4	14C2	GGCAGGATTGAAGC
5	A12	$(GA)_6CC$	5	M13	GAGGGTGGCGGCTCT
6	A13	$(GT)_6CC$			
7	A17	$(GTG)_5GC$			
8	A21	$(CA)_6AC$			
9	UBC 818	$(CA)_7G$			
10	UBC810	$(GA)_8T$			
11	UBC811	$(GA)_8C$			
12	UBC814	$(CT)_8A$			
13	UBC816	$(CA)_8T$			
14	UBC884	$HBH(AG)_7$			
15	UBC886	$VDV(CT)_7$			

where B = (non A), D = (non C), V = (non T), H = (non G)

RESULTS AND DISSCUSIONS

The analysis of the both agarose gels pointed out a high degree of intraspecific (samples 1 and 2) and interspecific polymorphism (samples 1-7). It says so that ISSR markers, as well as DAMD markers can be successfully used for variability evaluation in *Salix* sp.

These findings are in accord with the ones obtained in *Populus* sp. from China by Zheng et.al (2003) and Gao et al. (2006), who concluded that ISSR markers could generate abundant polymorphism in this specie. Smulders et al. (2008) reported similar results for European *Populus nigra* populations.

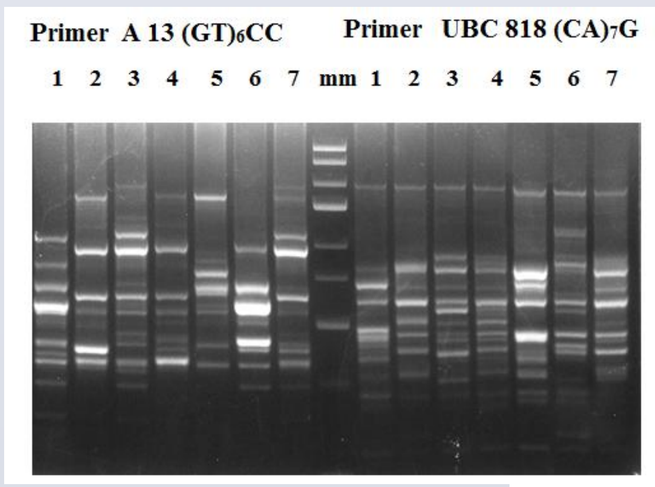
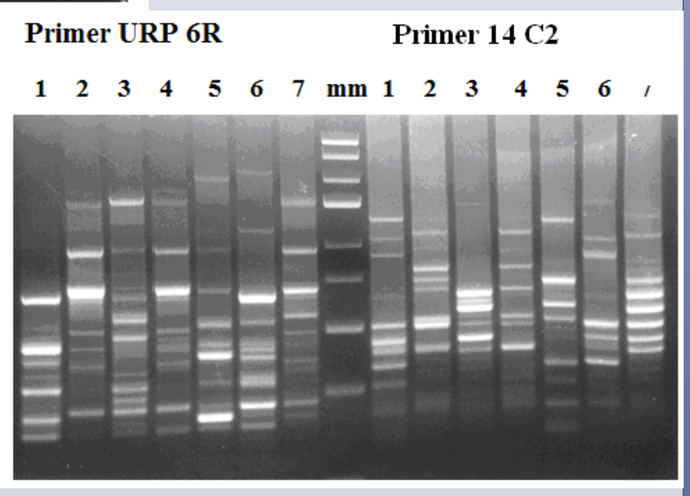


Fig. 3 The agarose gel electrophoresis (2%) of the products generated by the amplification with the ISSR primers A 13 si UBC 818 for the *Salix* sp. samples: 1- *Salix alba* (Agadici), 2- *Salix alba* (Sohodol), 3- *Salix alba/fragilis* (Pocruia), 4- *Salix fragilis* (Sterile dump Pinoasa), 5- *Salix daphnoides* (Agadici), 6- *Salix purpurea* (Sterile dump Farcasesti) and 7- *Salix triandra* (Bobicesti)

Fig. 4 The agarose gel electrophoresis (2%) of the products generated by the amplification with the DAMD primers URP 6R si 14 C2 for the *Salix* sp. samples: 1- *Salix alba* (Agadici), 2- *Salix alba* (Sohodol), 3- *Salix alba/fragilis* (Pocruia), 4- *Salix fragilis* (Sterile dump Pinoasa), 5- *Salix daphnoides* (Agadici), 6- *Salix purpurea* (Sterile dump Farcasesti) and 7- *Salix triandra* (Bobicesti)



CONCLUSIONS

- The fingerprints analysis pointed out a high level of polymorphism both within different accessions from a specie and between the analyzed species.
- The ISSR and DAMD markers could be successfully used for the molecular polymorphism evaluation of different *Salix* sp. accessions.
- The high degree of polymorphism at minisatellite sequences, as well as microsatellite sequences level, emphasized the necessity of increasing markers number and statistic analysis of obtained fragments. These will permit to determine the degree of relationships between collected genotypes.

Acknowledgements. This research was funded by the 111/2014 SAROSWE grant, by the Education and Research Ministry, UEFISCDI, Bucharest, Romania.

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