Selection of DNA Isolation Method and PCR Protocol for the Study of Endemic *Astragalus exscapus* L. ssp. *Transsilvanicus* (Schur) Nyár

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BulletinUASVM Horticulture 72(1) / 2015

Print ISSN 1843-5254, Electronic ISSN 1843-5394

DOI:10.15835/buasvmcn-hort:11056

ABSTRACT

Astragalus exscapus (Fabaceae) is a melliferous, perennial plant, which fits within a rarity type characterized by having small populations of relatively high habitat specificity. *Astragalus exscapus* L. ssp. *transsilvanicus* (Schur) Nyár., the endemic subspecies that we inspect, is considered vulnerable and needs special protection. In order to establish conservation strategies and to characterize polymorphism of the threatened subspecies, genetic and morphologic analysis will be implemented. Our study describes the application and development of SRAP procedures to *A. exscapus* ssp. *transsilvanicus*, including sampling, DNA extraction, PCR amplification and electrophoresis. Sequence Related Amplified Polymorphism (SRAP) molecular marker system is going to be applied to samples from eight different populations of *A. exscapus* ssp. *transsilvanicus* to identify the degree of polymorphism and to determine the survival potential of the subspecies. The results confirmed the suitability of the DNA extraction method to be applied and the optimal PCR protocol of the SRAP technique for large-scale studies we are performing on this vulnerable subspecies.

Keywords: *Astragalus exscapus*, DNA isolation, endemic species, SRAP

INTRODUCTION

Astragalus exscapus (Fabaceae) is a rare perennial plant originating from Eurasia. In Europe it is restricted to dry and semiarid regions because of its relatively high habitat specificity. *Astragalus exscapus* L. ssp. *transsilvanicus* (Schur) Nyár. is an endemic subspecies (Podlech, 1988) with only 24 populations localized in the Transylvanian basin. The population number has been lost by 85 per cent in the last 200 years and it is still decreasing. Therefore this subspecies is considered to be vulnerable and requires efforts for preservation (Bădăraş et al. 2000, 2001).

AIM AND OBJECTIVES

The aim of our study is to evaluate the genetic diversity and population structure among and within populations of *A. exscapus* ssp. *transsilvanicus* with the view to provide conservation planning. For this purpose, a SRAP molecular marker system (Li and Quiros, 2001) will be employed for detecting genetic variation in the open reading frames (ORFs) of plant genomes. The procedure requires isolation of genomic DNA, quantification of the purified DNA, polymerase chain reaction and electrophoresis, followed by statistical analysis of the data. For the application of the protocol to a new species like *A. exscapus* ssp. *transsilvanicus*

all these steps have to be optimized and the SRAP primers had to be preliminary tested to allow the large-scale implementation of the procedure to numerous samples.

MATERIALS AND METHODS

Plant samples were collected from eight natural populations located in the Transylvanian basin. From these, 22 individuals (2 or 3 from each population) were randomly chosen for DNA isolation. DNA extraction was performed using the CTAB method (Pop et al., 2008). An initial grinding stage with liquid nitrogen was employed to break down cell wall material and allow access to DNA. We implemented this step of the protocol in two different ways:

1. using the TissueLyser II – Qiagen for 30 seconds,
2. the classic method with mortar grinding.

The quality and quantity of the isolated genomic DNA were determined by spectrophotometry, using the NanoDrop1000 instrument. DNA amplification with SRAP primers was carried out in Corbett-Research PalmCycler under specific conditions. Each 15 µl PCR reaction mixture consisted of 1×Green buffer, 1,5 mM MgCl₂, 200 µM of dNTPs, 0,3 µM of both forward (Me1) and reverse primer (Em2), 1 U of Taq DNA polymerase (Promega) and approximately 50 ng of template DNA. The PCR was also tested in a 20 µl

Tab. 1

Sample no	Sample ID	DNA obtained with the TissueLyser				DNA obtained by mortar grinding			
		DNA ng/μl	Absorbance			DNA ng/μl	Absorbance		
			A260	A280	A260/A280		A260	A280	A260/A280
1	B1	173.32	3.466	1.866	1.86	672.06	13.441	5.859	2.29
2	B2	175.16	3.503	1.859	1.88	590.35	11.807	5.170	2.28
3	B3	144.92	2.898	1.571	1.85	648.03	12.966	5.610	2.31
4	G1	161.50	3.230	1.719	1.88	707.07	14.141	6.207	2.28
5	G2	285.19	5.704	3.086	1.85	1025.68	20.514	8.819	2.33
6	M1	218.07	4.361	2.371	1.84	2795.74	55.915	27.949	2.00
7	M2	596.31	11.926	5.926	2.00	984.71	19.694	9.399	2.01
8	S1	1165.14	23.303	11.134	2.09	-11.16	-0.223	-0.482	0.46
9	S2	1116.83	22.337	10.997	2.03	564.63	11.293	4.839	2.33
10	S3	2159.26	43.185	20.788	2.08	556.17	11.123	4.930	2.26
11	SG1	226.16	4.523	2.301	1.97	549.51	10.990	4.965	2.21
12	SG2	218.77	4.375	2.395	1.83	582.22	11.644	5.152	2.26
13	SG3	537.92	10.758	5.678	1.89	233.57	4.671	5.792	0.81
14	SI1	895.52	17.910	8.976	2.00	-34.56	-0.691	-0.702	0.98
15	SI2	594.69	11.894	5.986	1.99	899.44	17.989	7.836	2.30
16	SI3	330.71	6.614	3.318	1.99	765.82	15.316	6.621	2.31
17	VF1	333.58	6.672	3.489	1.91	1231.60	24.632	11.102	2.22
18	VF2	857.01	17.140	8.530	2.01	407.27	8.145	3.428	2.38
19	VF3	1612.23	32.245	15.601	2.07	1613.25	32.265	14.815	2.18
20	V1	1158.89	23.178	10.930	1.92	1264.21	25.284	11.534	2.19
21	V2	218.07	4.361	2.371	1.84	1440.86	28.817	12.965	2.22
22	V3	144.92	2.898	1.571	1.85	2843.47	56.869	26.757	2.13
Standard deviation					0.19	6.05			
Average		605.644	12.112	6.021	1.947	924.100	18.481	8.571	2.033

volume, adding 1 μl polyvinylpyrrolidone (PVP, m.w. 25000-30000, Merck) as an antioxidant.

RESULTS AND DISCUSSION

After DNA isolation we measured the quantity of the extracted DNA (ng/μl) and the quality of it. The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA. If the ratio 260/280 of the two readings has values between 1.7 and 2.0, DNA is considered pure enough for further analysis. The spectrometry measurements are represented in Table 1. Although the average DNA quantity obtained by classical grinding is higher, the TissueLyser provided sufficient amount of DNA with satisfactory purity. As it can be noticed, standard deviation for the first method is significantly smaller comparatively with the second method. This is a good indicator of the fact that even if the average values of the ratio A260/A280 are almost equal indeed there are qualitative differences between the two methods applied on the samples. Moreover, the TissueLyser method is faster and uses less amount of liquid nitrogen, which justifies further use in larger number of samples.

CONCLUSION

Regarding the optimal volume for the polymerase chain reaction, there were no significant differences between the

two alternatives (15 or 20 μl volume). In conclusion, for further analysis we will employ the PCR protocol with a 15 μl reaction volume and TissueLyser method to extract DNA for the large-scale implementation of the procedure.

Acknowledgement. This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

REFERENCES

1. Bădăraș Al. S., Dezsi St., Comes O., (2000), Cercetări biogeografice asupra speciilor stepice -silvostepice de *Astragalus L.* din depresiunea Transilvaniei, Studia universitatis Babes-Bolyai, Geographia, XLV, 2.
2. Bădăraș Al. S., Dezsi St., Man T., (2001), Cercetări biogeografice asupra speciilor stepice -silvostepice de *Astragalus L.* din depresiunea Transilvaniei (II), Studia universitatis Babes-Bolyai, Geographia, XLVI, 1.
3. Li G. and Quiros C. F., (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet., 103:455-461.
4. Pop Rodica, (2008) Studiul variabilității somaclonale la vița de vie cu ajutorul markerilor molecular Editura Bioflux, Cluj-Napoca