GENETIC STABILITY ASSESSMENT OF *Scilla autumnalis* L. REGENERANTS

BANCIU Cristian, MANOLE Anca

Abstract. The present study concerns the stability assessment after *in vitro* multiplication of *Scilla autumnalis* L. species taken from Comana (Giurgiu county), southern Romania. Seeds from plants grown in the natural habitat have been used for *in vitro* plant regeneration and multiplication. After the multiplication of the regenerated plantlets, molecular studies have been done in order to compare the regenerants from *in vitro* cultures, at genomic DNA level. For assessment, electrophoretic spectra of four nonspecific primers were analysed and compared. The results show identic polymorphic profiles, no matter the primer used, proving that the micromultiplication process did not affect the plant genetic structure.

Keywords: *Scilla autumnalis*, RAPD, ISSR, genetic stability assessment.

Rezumat. Evidenţierea stabilităţii genetice a regeneranţilor speciei *Scilla autumnalis* L. Studiul de faţă prezintă evaluarea stabilităţii genetice după multiplicarea *in vitro* a speciei *Scilla autumnalis* colectată de la Comana (Jud. Giurgiu), din sudul României. Seminţe prelevate de la plante din habitatul natural au fost utilizate pentru regenerarea şi multiplicarea *in vitro* a speciei. După multiplicarea vitroplantelor regenerate, au fost realizate analize moleculare cu scopul comparării regeneranţilor la nivel de ADN genomic. Pentru evaluare au fost utilizate spectrele electroforetice a patru primeri nespecifici. Rezultatele obţinute arată că profilul polimorfic este acelaşi indiferent de primerul utilizat, dovedind astfel că procesul de micromultiplicare nu a afectat structura genetică a regeneranţilor.

Cuvinte cheie: *Scilla autumnalis*, RAPD, ISSR, evaluarea stabilităţii genetice.

INTRODUCTION

Scilla autumnalis L. (*Prospero autumnale* (L.) Speta) is a plant species distributed from Western and Mediterranean Europe to Ukraine, Sardinia, and Turkey. It is a perennial species, with heights of up to 20 cm, flowering from late August to October, with flowers emerging before the leaves have fully developed (CIOCÂRLAN, 2009).

The plant is included in the national Red List of higher plants from Romania (OLTEAN et al., 1994) as vulnerable / rare, in the Red List of Dihoru (DIHORU & DIHORU, 1994) has the status of endangered species and, in the Red List published by the team from Cluj (BOŞCAIU et al., 1994) has the status of rare species. Furthermore, the species is assigned to critically endangered status in the "Red Book of vascular plants in Romania" (DIHORU & NEGREAN, 2009). The species was identified in the Natural Park Comana near the river Neajlov, in few areas of land that are rich in salt in their natural state, most being used in agriculture or fisheries. For these reasons, the species is important for conservation. Furthermore the plant has also ornamental and pharmacological value given that other species of the genus *Scilla* have antioxidant properties with beneficial effects on the digestive, circulatory systems and skin (TRIPATHI et al., 2001; JOSE et. al., 1982; GERACI & SCHICCI, 2002; MCCARTAN & VAN STADEN, 1998).

In vitro cultures are effective ways to preserve endangered plant species both for conservative purposes and for further analyses. Our previous studies show that *S. autumnalis* could be efficiently conserved, *ex situ* by *in vitro* methods (BANCIU et al., 2010). A prerequisite for the success of this approach is the genetic characterization of the regenerated plants to make sure that there is no risk for somaclonal variation incidence during micromultiplication (PĂUNESCU, 2008; SARASAN et. al., 2006). In this respect, we have assessed the regenerants at molecular level to confirm their genetic stability.

MATERIAL AND METHODS

The plant material consists in leaf fragments from completely regenerated plants (annotated P1-P5) originating from a single individual (Fig. 1). Genomic DNA was extracted using the extraction kit from Fermentas and following the entire protocol specified in the instructions of the package. Using the RAPD (Random Amplified Polymorphic DNA) technique for amplification with nonspecific primers, we have selected 3 ISSR primers (17898A, 17898B, and 17899B) and one RAPD (pgs2) that were susceptible to present a polymorphic pattern as there were previously studied on other plant species (BANCIU et al., 2013). The nucleotide sequence of the primers could be followed in Table 1. The RAPD analysis was performed using an EPPENDORF thermocycler, then the samples were migrated in an electrophoresis gel using a BIOMETRA tank and visualized using a hood and a transluminator from SYNGENE.

Table 1. Primers used for amplification and their nucleotide sequence.

	Primer	Sequence
1	17898A	[CA]6AC
2	17899B	[CA]6 GG
3	17898B	[CA]6GT
4	pgs2	GTTTCGCTCC

The amplification steps were performed using the Mastercycler gradient from Eppendorf equipment, respecting the following program: initial denaturation: 94°C for 2 minutes, denaturation: 94°C for 30 seconds, annealing: 44°C for 45 seconds, extension: 72°C for 1 minute and 30 seconds and final extension: 72°C for 10 minutes. The 2-4 steps were repeated for 35 cycles. The concentration of DNA in the sample was adjusted to 20ng/µl. The PCR amplificons were visualized on agarose gel on 1.5% concentration at 120V with bromophenol blue as dye on UV transluminator with digital camera from Syngene.

Figure 1. *In vitro* regenerated *S. autumnalis* - scale bar – 2cm (original).

RESULTS AND DISCUSSIONS

Establishing the optimal conditions for the short and medium term conservation by *in vitro* micromultiplication, of endangered species, cannot be achieved without a complex analysis of regeneration and development. In this context, regenerated material from *in vitro* cultures must be the subject of molecular analysis meant to highlight that experimental conditions did not affect the normal pattern of their normal development, neither their genetic structure.

The genetic variability of natural populations ensures species long term perpetuation and plasticity to adapt in a changing environmental condition. Thus, preserving genetic structure of the origin material is prerequisite to a successful *in vitro* conservation approach.

Our results shows that *in vitro* regenerated individuals has the same RAPD profiles, the same polymorphic bands being generated with all the tested primers, although there are some small differences in the intensity of bands amplified for data quality (Figs. 2-5). The identical bands on the same locus for a particular primer amplified, means that the nucleotide sequence on the DNA sample is similar on all regenerants tested, and they were not subject to any genetic alteration induced by artificial culture conditions. This suggests that there is no genetic variability between the regenerated individuals and their genetic structure is the same with the parental individuals from the natural population.

CONCLUSIONS

The protocol elaborated for *in vitro* multiplication of *S. autumnalis* was able to maintain the genetic structure of the initial parental plants. The molecular analyses showed that the regenerants were genetically identical and were no subject to genetic variations. This protocol is thus able to be used for *in vitro* conservation of other Liliaceae plant species, with very low risk of somaclonal variations.

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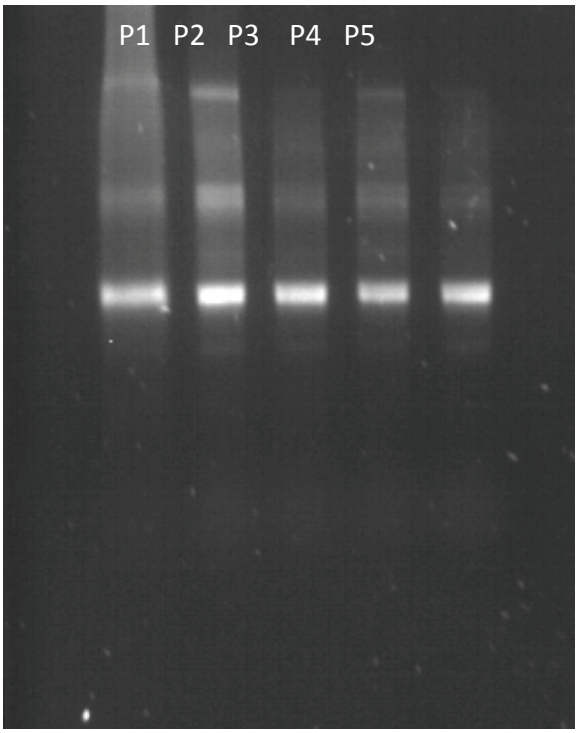

Figure 2. P1-P5- DNA samples amplified with the primer ISSR 17898A.

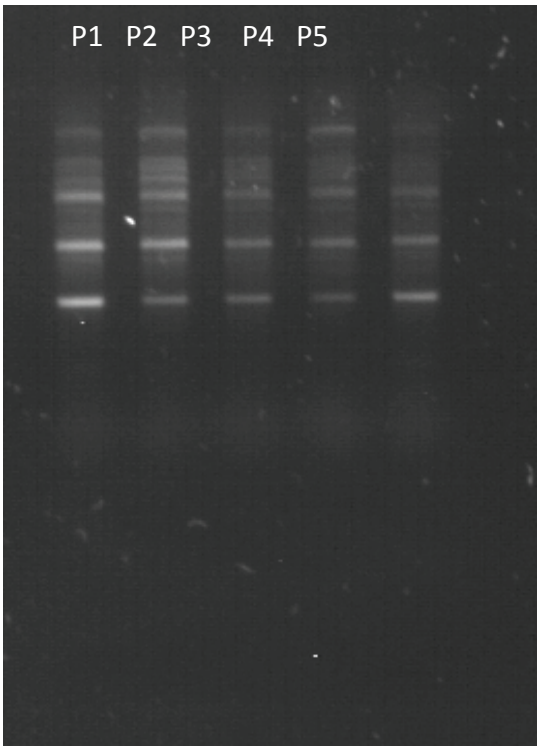

Figure 3. P1-P5- DNA samples amplified with the primer ISSR 17899B.

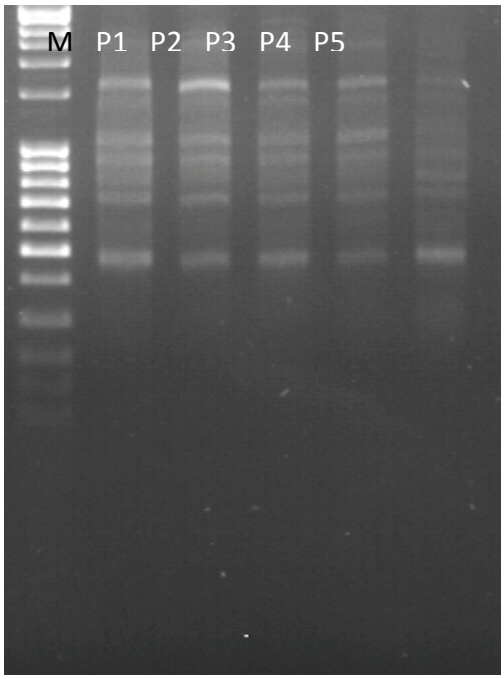

Figure 4. P1-P5- DNA samples amplified with the primer ISSR 17898B (M-Weight marker).

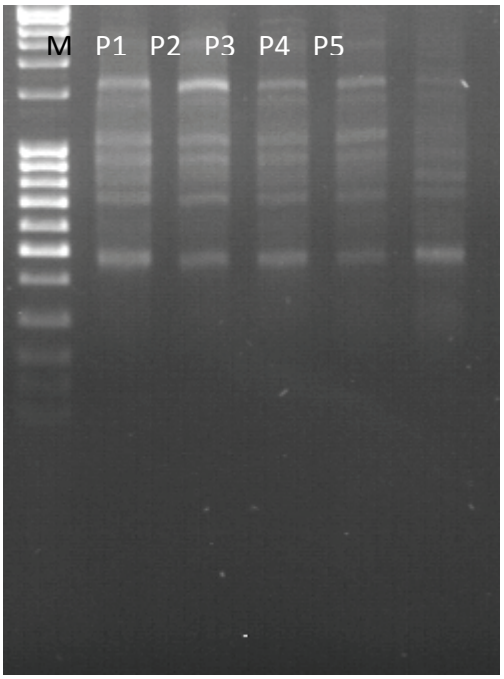

Figure 5. P1-P5- DNA samples amplified with the primer pgs2 (M-Weight marker).

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