Ann. For. Res. 58(2): 347-355, 2015
DOI: 10.15287/afr.2015.389

ANNALS OF FOREST RESEARCH
www.afrjournal.org

Genetic diversity within a newly identified population of *Adenophora liliifolia* (L.) A.DC. in Romania: implications for conservation

A. Manole, C. Banciu, A. Indreica

Manole A., Banciu C., Indreica A., 2015. Genetic diversity within a newly identified population of *Adenophora liliifolia* (L.) A.DC. in Romania: implications for conservation. Ann. For. Res. 58(2): 347-355.

Abstract. *Adenophora liliifolia* is a relict plant species, endangered at European level. Its occurrence in Romania is limited to a very few sites. The amount of genetic diversity of plant species is a valuable indicator of population, being the baseline in developing proper strategies for their conservation. Inter-simple sequence repeats (ISSRs) markers were used to analyze polymorphism in *A. liliifolia* genome and to evaluate the genetic diversity and accordingly, the state of a recently identified population. Five ISSR primers, specially designed for plants, and containing different simple sequence repeat motifs were tested. A total of 52 ISSR fragments were generated of which 41 were polymorphic (78.84%) and 32 (61.53%) specific to *Adenophora* genus. The value of Shannon's index of genotypic diversity was 0.812. Jaccard similarity coefficient was calculated for pair wise comparisons among all individuals and ranged from 0.17 to 0.83. The genetic variability between individuals was 78.84% which suggests a relative high genetic differentiation. Although the level of genetic variability is moderate to high, the population is declining and exposed to demographic stochasticity. A possible cause is species germination requirements hampered by modification in vegetation structure and abundance. The population survival and reinforcement is conditioned by urgent measures for forest management in order to reduce herbaceous and shrubby vegetation and to limit mowing and grazing. *Ex situ* conservation measures are also proposed.
Keywords *Adenophora liliifolia*, ISSR markers, genetic diversity, conservation

Authors. Anca Manole (anca.manole@ibiol.ro), Cristian Banciu - Institute of Biology Bucharest, 296 Splaiul Independenţei, 060031, Bucharest, Romania; Adrian Indreica - Transilvania University of Braşov, 1 Şirul Beethoven, 500123 Braşov, Romania.
Manuscript received February 02 2015; revised May 12, 2015; accepted May 20, 2015; online first May 21, 2015.

Introduction

Assessment of the level and distribution of genetic diversity within endangered species is critical to their management and conservation (Ge et al. 1999). *Adenophora liliifolia* (L.) A.DC. (*Campanulaceae*) is a continental-eurasiatic plant species being the only species of genus *Adenophora* identified in Romania. As listed in Annex II (b) of the Habitats Directive (Anonymous1992) is considered a plant species of European Community interest whose conservation requires designation of special areas of conservation (*Natura 2000* sites). Moreover, it is considered species indicator of thermophilous forest hotspots signaling remnant pools of biodiversity (Kiedrzyński & Jakubowska-Gabara 2014). Species conservation status at European level was assessed as "Unfavourable bad", for both reporting periods (2001-2006 and 2007-2012) under article 17 of Habitats Directive (European Topic Centre on Biological Diversity).

The main habitats for the species are open woodlands and fields with damp light (sandy) to medium (loamy) acid, neutral or basic (alkaline) soils, namely wet meadows of *Molinion*, dry grassland of *Festuco-Brometea* and forests of *Quercetalia pubescenti-petraea* (Ghişa 1964, Ciosek 2006). Even though this species was not included in any national red list/book in Romania, it is obvious that natural sites with *A. liliifolia* dramatically decreased over the last 50 years. More precisely, out of the 34 sites with *A. liliifolia* listed by E.V.Ghişa in 1964, only a number of 6 were reconfirmed after the nineties (Sârbu 2006). Moreover, in the last 5 years we have reconfirmed only one site out of the 6 reported, where only one small size population was identified (unpublished data). Apart from the previously reported sites, two new additional sites were identified in Transylvania but unfortunately, only one was recently confirmed (Indreica 2011). This new site was identified near Herculian village (Covasna County) and encloses a small population of *A. liliifolia* in a dry-mesic oak forest.

To date, worldwide there is a little information available on the genetic variability of this species. Although the critical conservation status of the species was known since nineties, at European level are no studies upon the level of genetic variation among or within populations. The only studies upon population genetics in *A. liliifolia* were made by Boronikova and refer to four populations from Ural region (Perm) (Boronnikova 2009). The same author developed also four IRAP (Inter-Retrotransposons Amplified Polymorphisms) markers as tools for future genetic assessment of *A. liifolia* populations (Boronikova & Kalendar 2010). Moreover, the lack of information about genetic variability concerns the entire genus *Adenophora* where except Boronikova's studies there are only a few and refers to *A. lobophylla*, *A. potaninii* (Ge et al. 1999), *A. pallustris* (Masumoto et al. 2011) and *A. grandiflora* (Chung & Epperson 1999).

Taking into account the lack of studies upon genetic variability within *Adenophora* genus in general and *A. liliifolia* in particular, the present work was focused on genetic characterization of a newly identified population in order to assess its state. We have considered for assessment only the new indentified population because in the last 10 years, at national level was not identified another population stable, in a favorable conservation status, or with more individuals. The assessment was made at DNA level using the Inter-simple sequence repeat (ISSR) method. ISSR amplification uses PCR amplification of DNA to identify the genetic differences between repeated motives of microsatellite sequences occurring within coding regions, both centromeric and telomeric, that are highly polymorphic (Ziekiewicz et al. 1994). The primers anneal to simple-sequence repeats that are abundant throughout the eukaryotic genome (Tautz & Renz 1984) and this method does not require prior knowledge of DNA sequence for primer design. Compared with RAPD, ISSRs can be highly variable

within species and have the advantage in longer primers, allowing for more precise annealing temperatures and generating a much higher number of polymorphic fragments (Bornet & Branchard 2001). Accordingly, ISSR have been widely used to detect polymorphism and to evaluate genetic diversity in many plant species including *A. liliifolia* (Boronnikova 2009).

This is the first study upon the genetic structure of a natural population of *A. liliifolia* at European level. The aim of the present study was to reveal the level of genetic diversity and to assess the population status, in order to understand the causes of population decline and accordingly to suggest some forest management measures and conservation strategies. The data gained may contribute particularly to the population survival and reinforcement, and further for species conservation.

Materials and methods

The plant material was collected in late July 2014, from a natural population recently identified near Herculian village (Covasna county) (Indreica 2011). The population was small consisting of 12 individuals scattered over an area of about 2500 square meters. Fresh young leaves from each individual were dried in silica gel and stored at -20˚C until processing. In order to evaluate the level of genus specificity of the primers an outlier sample of *Campanula rapunculoides* L. was added to those of *Adenophora*.

DNA extraction and PCR amplification were performed simultaneous directly from plant samples without DNA purification, using a Thermo Scientific Phire Plant Direct PCR Kit. This method was based on the activity of Phire Hot Start II DNA Polymerase, a specially engineered enzyme with a DNA binding domain which was recognised as tolerant of many PCR inhibitors present in plant material. Following an initial screen of 25 primers - others than those tested by Boronnikova (2009) - only 5 were selected as suitable for *Adenophora* species. Reactions were carried out in a total volume of 20 µl consisting of 9.4 µl nuclease free water, 10 µl Phire Plant PCR Buffer, 0.4 µl Phire Hot Start II Polymerase, 0.2 µl primer (0.5 µM), and 0.5mm punch of plant sample. PCR was performed using an Eppendorf thermal cycler (Mastercycler Gradient). Amplifications were performed using the following programme: pre-denaturation for 2 min at 94°C, 35 cycles of 30 s at 94°C, 45 s at annealing temperature (44°C), 30 s at 72°C, and 10 min at 72°C for final extension. PCR products were electrophoretically separated in 1.5 % agarose gels buffered with 1 X TBE containing ethidium bromide (0.5µg x ml^{-1}) at constant voltage of 120V for one hour. O'GeneRuler™ 50bp (1000 – 50 bp) DNA Ladder (Fermentas) was used as size marker. PCR products were visualized and analyzed under GENi Gel Documentation System from SynGene.

Band scoring and data analyses were performed using Gene Tools software (SynGene, Cambridge, UK, version 4.02). Each amplification fragment was considered as a dominant allele for a given locus. The fingerprint pattern of the bands was transformed into a binary character matrix with 1 for presence and 0 for absence of a band of a particular position in a lane. For each polymorphic profile were generated similarity matrices. Genetic similarities among individuals were quantified with the Jaccard similarity coefficient and visualized using a cluster analysis (unweighted pair-group method with arithmetic averages, UPGMA) and illustrated in a dendrogram. Genetic variability within population was estimated as percentage of polymorphic loci. For more accuracy, the degree of within-population genetic diversity was quantified also, using Shannon's index of diversity, estimated as $-\Sigma\, p_i \times ln\, p_i$, where p_i was the relative frequency of i^{th} allele (Lewontin 1972).

Results

The assessed population consisted of 12 mature individuals, in flowering and fructification stage, up to 80 cm tall, some of them visible grazed. Inflorescences were well developed bearing up to 40 flowers.

The five ISSR primers produced a total of 52 clear and distinct bands that could be scored. Of these bands 41 (78.84%) were polymorphic and 32 (61.53%) specific to genus *Adenophora* compared with *Campanula*. The total number of scored bands varied from 15 for primer HB15 to 6 for primer 17899B with a mean of 10.4 bands per primer (Table 1).

Shannon index of diversity was calculated as 0.812 and shows a relative high level of genetic diversity between the individuals of the population. This value was sustained by the percentage of the polymorphic loci (78.84%). The similarity matrix showing Jaccard's coefficient for similarity ranged from 0.173349 (for pair 8 - 2) to 0.839 (for pair 6 - 10). Comparisons with sample 13 were excluded, because this was considered outlier and corresponds to genus *Campanula* (Table 2).

The ISSR-based UPGMA dendrogram showed that the individuals were grouped in three main clusters: cluster I, small and tight, made by two individuals (4 and 8), cluster II, small but not so tight, made by three individuals (7, 2 and 11), and cluster III the largest made by six individuals (9, 3, 6, 10, 1 and 5). A relative distinct branch, but with affinities with both cluster II and cluster III, leaded to individual 12. A completely separate branch is represented by individual 13 (Figure 1).

Discussion

In conservation genetics was statuated that genetic variability is beneficial, hence worth to be preserved as a primary concern (Pertoldi et al. 2007). Even under permissive conditions, populations with very low genetic diversity demonstrated reduced fitness relative to high diversity populations. Our data revealed a relatively high genetic diversity within a population with a very low number of individuals. Even though the level of genetic diversity could sustain population stability, the low number of individuals is far from enough to ensure population survival. As shown in the reports of EU countries issued under article 17 of Habitats Directive, a population of *A. liliifolia* could be considered stable if the number of individuals are between 3500 - 4200 while between 2700 - 3000 is considered "Unfavourable –inadequate" and between 1950 - 2000 "Unfavourable-bad" (European Topic Centre on Biological Diversity). The size of Romanian population of *A. liliifolia* is far away from these figures. The level of genetic diversity within population may be explained by sexual reproduction and gene flow when the population size was much larger than the current one. The very limited number of mature individuals and the absence of young plants showed that there are some factors that significantly reduced the propagation through

Table 1 Primers sequence and number of loci scored

Primer		No.of loci	No.of polymorphic loci	No.of genus specific loci
Code	Sequence 5' - 3'			
17899A	$(CA)_6AG$	14	10	11
17898A	$(CA)_6AC$	7	6	4
17898B	$(CA)_6GT$	10	6	3
17899B	$(CA)_6GG$	6	6	4
HB15	$(CA)_6GC$	15	13	10
Total		52	41	32
Mean		10.4	8.2	6.4

Table 2 Synthetic Similarity matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1	0.644381	0.754396	0.568812	0.721223	0.684212	0.182470	0.370209	0.601675	0.676723	0.492618	0.492194	0.219487
2	0.644381	1	0.555464	0.316304	0.577605	0.492882	0.436201	0.173349	0.698915	0.489369	0.739199	0.590062	0.215968
3	0.754396	0.555464	1	0.596439	0.746154	0.793820	0.271868	0.386260	0.688468	0.778022	0.483776	0.559148	0.266017
4	0.568812	0.316304	0.596439	1	0.604941	0.575011	0.309083	0.826167	0.483691	0.494153	0.313068	0.352514	0.179020
5	0.721223	0.577605	0.746154	0.604941	1	0.706809	0.252119	0.407963	0.705784	0.675998	0.494285	0.527225	0.259837
6	0.684212	0.492882	0.793820	0.575011	0.706809	1	0.417228	0.361991	0.768483	0.839429	0.475451	0.623097	0.355044
7	0.182470	0.436201	0.271868	0.309083	0.252119	0.417228	1	0.301796	0.466442	0.319061	0.674686	0.288183	0.113135
8	0.370209	0.173349	0.38626	0.826167	0.407963	0.361991	0.301796	1	0.265981	0.268933	0.187591	0.190498	0.171704
9	0.601675	0.698915	0.688468	0.483691	0.705784	0.768483	0.466442	0.265981	1	0.732584	0.672482	0.683431	0.271163
10	0.676723	0.489369	0.778022	0.494153	0.675998	0.839429	0.319061	0.268933	0.732584	1	0.483104	0.632119	0.290462
11	0.492618	0.739199	0.483776	0.313068	0.494285	0.475451	0.674686	0.187591	0.672482	0.483104	1	0.510662	0.221798
12	0.492194	0.590062	0.559148	0.352514	0.527225	0.623097	0.288183	0.190498	0.683431	0.632119	0.510662	1	0.321853
13	0.219487	0.215968	0.266017	0.17902	0.259837	0.355044	0.113135	0.171704	0.271163	0.290462	0.221798	0.321853	1

Note. 1-12 *Adenophora* samples, 13 – *Campanula rapunculoides* sample

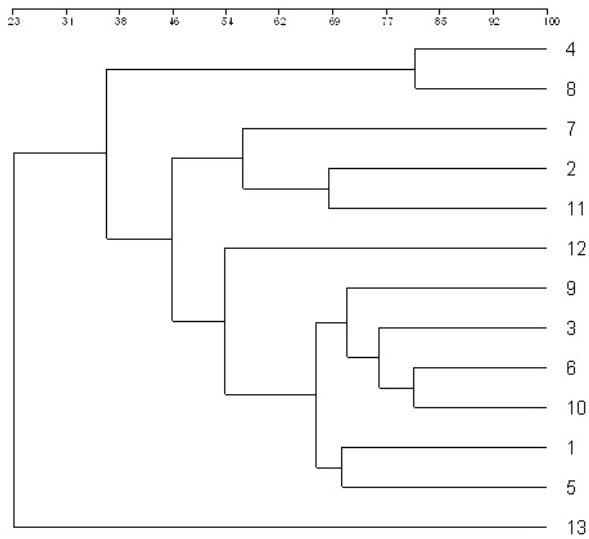

Figure 1 ISSR-based UPGMA dendrogram for entire *Adenophora liifolia* population (1-12 samples from *Adenophora* individuals, 13- sample from *Campanula rapunculoides*)

seeds and the actual population is probably the remnant of a much wider population, currently fragmented and declining. Given its current narrow distribution *A. liliifolia* appears to be extremely vulnerable to habitat reduction and modifications in habitat structure. According to a report published by European Environment Agency (13 July 2009), upon conservation status at European level of *A. liliifolia,* the species is threatened by overgrowth from shrubby vegetation and also by inappropriate forest management (Anonymous 2009). The pathway of how overgrowth of vegetation impedes the increase of *Adenophora* populations is still unknown. A reasonable explanation could reside in germination requirements. A recent study revealed that seed germination requires light in all members of *Campanulaceae* family, especially smaller seeded taxa including *Adenophora* species (Koutsovoulou et al. 2014). Horticultural practice with *A. liliifolia* confirms that the seed should be surface sowed to germinate (Cullen et al. 2011). The inhibitory effect of darkness could be enhanced by decreased oxygen and increased carbon dioxide levels into a shaded soil litter (Baskin & Baskin 2001). Increases in shadow level decreased the percent of seed germination and reduced dramatically population number and consequently the variability, which is ensured by sexual propagation. As a result, population declined in both number and fitness. Studies upon spatial distribution of individuals within two populations of *A. grandiflora,* which reproduced both vegetatively and sexual, showed that clonal reproduction is very localized, with most ramets occurring within less than 10 meters (Chung & Epperson 1999). If we assume that *A. liliifolia* should exhibit a similar reproductive behaviour, the individuals located at distance of more than 10 m could not result from clonal propagation. Field data collected by us showed that the individuals were solitary and scattered over the habitat at distances which could not result from vegetative propagation (more than 20 m). Also, the three main clusters from the UPGMA tree clearly shows that the individuals are not clones and suggest that some individuals originate from same source of seed (parent) such as the pairs 4 - 8, 2 - 11, 6 - 10, and 1 - 5. The dendrogram shows also, different levels of relatedness within all the individuals (except individual 13, which belong to *Campanula* genus, configuration

that sustain the accuracy of obtained data). In summary, spatial distribution of the individuals within population, incomplete age pattern, and the heterogeneous genetic structure, confirms the assumption that all the population originate from seeds. We can state also, that currently both propagation types through seeds and vegetatively, are absent, since young plants were lacking and the numbers of individuals were the same as recorded in 2009 (Indreica 2011). Besides, the absence of the propagation seems to be correlated with the well developed and in expanding herbaceous and shrubby vegetation.

Some studies summarised by Hamrick & Godt (1994) have argued that populations decline and species extinction are more often due to ecological and demographic causes rather than the lack of genetic variation. This is more likely the case of *A. liliifolia*. Although the assessed level of genetic variability could sustain species survival the population is endangered by the extremely reduce number of individuals being highly vulnerable to demographic stochasticity. Population could survive only if the number of individuals will increase significantly. This would be possible only if appropriate measures for forest management and conservation are taken. The forest management measures should be conducted in order to limit herbaceous (mostly the invasive species as *Urtica dioica*) and shrubby vegetation spreading and excessive development, allowing the ground seed to be exposed to light and to germinate. In addition, should be taken some measure to limit mowing and grazing (goats graze *Adenophora* inflorescences). Taking into account that from seed to a mature individual able to produce flowers and seeds takes 2 years in *A. liliifolia* (Shulkina et al. 2003), if only ten seeds from each individual succeed to germinate and develop, in only 6 years this small and endangered population could be significantly reinforced. Furthermore, consecutive with the forest management measures a periodic genetic survey of the population should be taken, the data gained by the present study being the baseline for a genetic monitoring of this population in future decades. Genetic monitoring by quantifying the temporal changes in population genetics is a useful tool for evaluating the cumulative effects of habitat fragmentation (Schwartz et al. 2007). Also, *ex situ* conservation measures need to be undertaken to preserve this new identified pool of species variability. As *ex situ* conservation measure we suggest collection of mature seeds and preservation in seed bank, following the Polish example (Puchalski et al. 2014). Another efficient conservation measure could be cultivation in botanical gardens, parks, private gardens, etc., the species being very decorative, fragrant and easy to grow (Cullen et al. 2011). Cultivation in private gardens is practised abroad relative frequent, but unfortunately most of the seeds sold and traded as from *A.liliifolia* are actually from *Campanula rapunculoides,* therefore for horticultural practices a trusted source is required. At national level, to our knowledge, no botanical gardens cultivate *A. liliifolia,* probably due to the lack of autochthonous seeds. This newly identified population could be a valuable seed source for *ex situ* cultivation.

Conlusions

The present study indicated that the *A. liliifolia* population is probably the remnant of a much wider population. The level of genetic diversity may sustain population stability if the conditions for seed dispersal and germination are fulfilled and growth and development of new individuals is sustained. One of possible causes for population decline is the overgrowth of shrubby and herbaceous vegetation that impede seed germination. Population could be reinforced through a range of *in situ* measures, e.g. clearings to create areas of exposed ground where seed could germinate, removal of invasive species, impeding mowing and grazing, etc. *In situ* measures are

also important to preserve the specific habitat for *A. liliifolia,* which is one with high conservative value as it encloses remaining of old natural forests which preserve pools of relict species. In addition, some *ex situ* conservation measures should be taken to preserve species germplasm as seeds (in seed banks) or by cultivation. Genetic data revealed by this study are important as the baseline for a future genetic monitoring of the population and also to contribute to understanding and stopping the populations decline currently generalised in whole Europe. Further investigations upon species ecology, especially on germination are required to better understand the whole synergic factors influencing population decline. Germination specific requirements for *A. liliifolia* conjugated with habitat fragmentation and habitat structure alteration, may explain the decline of this species at European level.

Acknowledgments

The study was funded by project no RO1567-IBB06/2014 supported by Romanian Academy. We thank an anonymous reviewer for constructive comments on the manuscript.

References

Anonymous, 1992. Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora. Annex IIb (Animal and plant species of community interest whose conservation requires the designation of special areas of conservation).

Anonymous, 2009. Habitats Directive Article 17 Reporting. Web: http://forum.eionet.europa.eu/…/adenophora_lilifoliapdf/. Accesed 01.2015.

Anonymous. European Topic Centre on Biological Diversity. Web: http://biodiversity.eionet.europa.eu/article 17- accessed on 12.01.2015.

Baskin C.C., Baskin J.M., 2001. Seeds: Ecology biogeography and evolution of dormancy and germination. Academic Press, San Diego, CA, 666 p.

Bornet B., Branchard M., 2001. Non-anchored inter-simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Molecular Biology Reporter 22: 427–432.

Boronnikova S.V., 2009. Genetic variation in Ural populations of the rare plant species *Adenophora lilifolia* (L.) DC. on the basis of analysis of polymorphism of ISSR markers. Russian Journal of Genetics 45(5): 571-574. DOI: 10.1134/S1022795409050081.

Boronikova S.V., Kalendar R.N., 2010. Using IRAP markers for analysis of genetic variability in populations of resource and rare species of plants. Russian Journal of Genetics 46(1): 36-42. DOI: 10.1134/S1022795410010060.

Chung M.G., Epperson B.K., 1999. Spatial genetic structure of clonal and sexual reproduction in populations of *Adenophora grandiflora* (Campanulaceae). Evolution 53(4): 1068-1078. DOI: 10.2307/2640812.

Ciosek M., 2006. The ladybells *Adenophora liliifolia* (L.) Besser in forests near Kisielany (Siedlce Upland, E Poland). Biodiversity: Research and Conservation 3-4: 324-328.

Cullen J., Knees S.G., Cubey S.H., 2011. The European garden flora, V Boraginaceae to Compositae. Second edition, Cambridge University Press, Cambridge, 652 p.

Ge S., Wang K.Q., Hong D.Y., Zhang W.H., Zu Y.G., 1999. Comparison of genetic diversity in the endangered *Adenophora lobophylla* and its widespread congener *A. potaninii*. Conservation Biology 13(3): 509-513. DOI: 10.1046/j.1523-1739.1999.98136.x.

Ghişa E., 1964. Genul *Adenophora* Fisch. In: Săvulescu T. (ed.), Flora R.P. Române (IX). Ed. Academiei Române. Bucureşti, pp. 123-128.

Hamrick J.L., Godt J.W., 1996. Conservation genetics of endemic plant species. In: Avise J.C., Hamrick J.L., (eds.) Conservation Genetics. Chapman & Hall, New York, pp. 281-304. DOI: 10.1007/978-1-4757-2504-9_9.

Indreica A., 2011. Forest habitats with *Adenophora liliifolia* from SE Transylvania. In: Anonymous (ed.), Proceedings of the Biennial International Symposium Forest and sustainable development, 15-16 October 2010, Braşov, Romania, Transilvania University Press, Braşov, pp. 283-288.

Kiedrzyński M., Jakubowska-Gabara J., 2014. The detection of thermophilous forest hotspots in Poland using geostatistical interpolation of plant richness. Acta Societatis Botanicorum Poloniae 83(3): 183-189. DOI: 10.5586/asbp.2014.019.

Koutsovoulou K., Dawsand M.I., Thanos C.A., 2014. Campanulaceae: a family with small seeds that require light for germination. Annals of Botany 113: 135–143. DOI: 10.1093/ aob/mct250.

Lewontin R., 1972. The apportiortionment of human diversity. Evolutionary Biology 6: 381-398.

Masumoto I., Kaneko S., Otake K., Isagi Y., 2011. Development of microsatellite markers for *Adenophora pallustris* (Campanulaceae), a critically endangered wetland plant species in Japan. Conservation Genetics Resources 3(1): 163-165. DOI: 10.1007/s12686-010-9314-2.

Pertoldi C., Bijlsma R., Loeschcke V., 2007. Conservation genetics in a globally changed environment: present problems, paradoxes and future changes. Biodiversity and Conservation 16(14): 4147-4163. DOI: 10.1007/ s10531-007-9212-4.

Puchalski J., Niemczyk M., Walerowski P., Podyma W., Kapler A., 2014. Seed banking of Polish endangered plants – the FlorNatur Project. Biodiversity Research and Conservation 34: 65-72. DOI: 10.2478/biorc-2014-0005.

Sârbu A., 2006. Important areas for plants in Romania. Preinventory for a draft list of Natura 2000 sites (SCIs) for plant species. Final report, 254 p.

Schwartz M.K., Luikart G., Waples R.S., 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology & Evolution 22(1): 25-33. DOI: 10.1016/ j.tree.2006.08.009.

Shulkina T.V., Gaskin J.F., Eddie W.M., 2003. Morphological studies toward an improved classification of Campanulaceae s. str. Annals of the Missouri Botanical Garden 90(4): 576-591. DOI: 10.2307/3298543.

Tautz D., Renz M., 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Research 12: 4127–4138. DOI: 10.1093/ nar/12.10.4127.

Ziekiewicz E., Rafalski A., Labuda A., 1994. Genome fingerprinting by simple sequence repeat (SSR) anchored polymerase chain reaction amplification. Genomics 20: 178–183.