RAPD-ANALYSIS OF *CYCLAMEN* SPP. GENOME POLYMORPHISM

Elza Makaradze[1], **Galina Meparishvili**[2], **Natela Varshanidze**[3], **Inga Diasamidze**[4], **Ketevan Dolidze**[5], **Eteri Jakeli**[6], **Nana Zarnadze**[7]

Abstract: Wild plants form the basis of biological resources both for Georgia and the whole world. A strategic task of any country is to preserve the biological diversity of plants. In the territory of Ajara, a large species diversity of plants grows, among which there are rare, endemic and relict plants. In particular, *Cyclamen adzharicum*.

Modern systematics of wild plants in Georgia is based on classical methods of botany. In this regard, it is relevant to conduct genetic studies of species diversity and genetic polymorphism of species and populations using molecular genetic markers, in particular RAPD-PCR methods. The purpose of this study was to identify genetic polymorphism in *Cyclamen* L species using RAPD methods. As a result of the conducted research the 65 RAPD-markers in length from 150 to 1500 BP have been revealed. The number of the amplified fragments DNA varied depending on the primer from 6 (OPA-2) to 11 (OPB-4). The results of grouping *Cyclamen adzharicum* and *C. coum* samples allowed two clusters to be identified. In the first cluster were samples of three populations *Cyclamen adzharicum* and showed a low stubble in the intra-species variability. *Cyclamen coum* was attributed to the second cluster. The used primers gave the opportunity to identify polymorphism between the tested types of cyclamen.

UDC Classification: 582, **DOI**: 10.12955/cbup.v7.1483

Keywords: *Cyclamen adzharicum*, *Cyclamen coum*, RAPD-PCR

Introduction

Wild plant species are biological resources which are spread throughout Georgia and the whole world. A strategic task of any country is to preserve the biological diversity of plants. Many countries allocate large financial resources towards preserving wild flora, but this cannot be said about Georgia. Adjara is located in the south western part of Georgia. It is characterized by warm and humid subtropical climate. The flora of Ajara is unique, diverse and among them are many endemic species (Varshanidze, 2018). The floristic region of Adjara is situated in the north-western part of the western Caucasian corridor of the world-known Caucasian "Hotpoint", which is distinguished with the uniqueness of its relict Colchis flora. In periods of "Ice Age" it represented a shelter for warmth-loving species, the most powerful refugium in Western Europe (Makaradze, 2017). In Eastern Europe it represents (together with Tertiary refugium) a completely distinguished, unique plot with its biodiversity. It should be mentioned that in the initiative – "About 100 Hot Points of European Forests" of the World Wildlife Fund (WWF), the same as 100 plots of the unprotected forests, which should be protected by all means, one of the priorities was given to the unique forests of Adjara Colchis. Adjara florais has rich genetic resources, with its endemic, relict and rare species, many from which were lost as a result of gaining forest resources, trading and collecting successive amounts of plants. The collecting of successive amounts of resources causes reduction in biodiversity. Consequently, the relict, endemic populations faced extinction (Manvelidze, 2009; 2009;Jakeli, 2018).

The genus Cyclamen L, belonging to the group of taxa of the Mediterranean geographical element. The total number of species is more than 20 (Grey-Wilson, 1988, 2005; Compton, 2004, Yesson, 2006), 10 of them grow in Turkey, and five of them are endemic (Curuk, 2015; 2016).The following *Cyclamen* L.5 species are distributed in Georgia: *C. colchicum*, *C. vernum*, *C. coum* Mill., *C. adzharicum*Pobed. and*C. abchasicum* (Flora of Georgia,1985; Dmitrieva, 1990; Varshanidze, 2013).

[1] Batumi Shota Rustaveli State University, Faculty of Natural Sciences and Health Care, Department of Biology, Batumi, Georgia, e.makaradze@gmail.com
[2] Batumi Shota Rustaveli State University, Institute of Phytopathology and Biodiversity, Kobuleti, Georgia, galinameparishvili@yahoo.com
[3] Batumi Shota Rustaveli State University, Faculty of Natural Sciences and Health Care, Department of Biology, Batumi, Georgia, natela.varshanidze@gmail.com
[4] Batumi Shota Rustaveli State University, Faculty of Natural Sciences and Health Care, Department of Biology, Batumi, Georgia, diasamidze.inga@bsu.edu.ge
[5] Batumi Shota Rustaveli State University, Faculty of Natural Sciences and Health Care, Department of Biology, Batumi, Georgia, ketodolidze@yahoo.com
[6] Batumi Shota Rustaveli State University, Faculty of Natural Sciences and Health Care, Department of Pharmacy, Batumi, Georgia, eteri_jakeli@yahoo.com
[7] Batumi Shota Rustaveli State University, Faculty of Natural Sciences and Health Care, Department of Biology, Batumi, Georgia, z_nana@mail.ru

The purpose of this study was to identity genetic polymorphism of the *Cyclamen L* species using Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD–PCR) methods (Williams et al.1990). It is known that this method was used very often to identity polymorphic biodiversity among many wild and agriculture plants species (Deragon, 1992; Weising, 2005; Dugar, 2011; Catana, 2013; Nigmatullina, 2018).

Methods and materials

Plant material: the leaves and tubers of three populations of *Cyclamen adjaricum* were collected in Adjara, and one population of *C. coum* was sampled in Turkey (Artvin) (Tab. 1).

Table 1: Information about test samples

#	Test species	Samples collected area	Samples data
1	*Cyclamen adzharicum*	v. Tsikhisdziri, Adjara	March 2018
2	*Cyclamen adzharicum*	v. Khala, Adjara	March 2018
3	*Cyclamen adzharicum*	v. Chaisubani, Adjara	March 2018
4	*C. coum*	Artvin, Turkey	April 2018

Source: Makaradze and Varshanidze, 2018

Genomic DNA isolation: Genomic DNA was extracted from leaves and tubers using a protocol the GF-1 DNA Extraction Kit (Vivantis Technologies Sdn. Bhd., Malaysia), which is designed for rapid and efficient purification of genomic DNA from a wide variety of plant tissues without the need for precipitation or organic extractions. According to this optimized protocol, 25 mg of tissue was homogenized. The next step is tissue lysis, for this of Buffer PL is added 280µl to the ground sample followed by mixing thoroughly by vortexing the tube for 30 seconds to obtain a homogeneous solution. Then 20µl of Proteinase K was added and mixed thoroughly by inverting tube and incubated at 65◦ C for 1-2 hours in a shaking water bath and mixed several times during incubation to ensure thorough digestion of the sample. After incubation, the sample was centrifuged at 14,000-16,000 x g for 5 minutes to precipitate any insoluble/undigested materials and the supernatant containing the DNA was transferred into a clean microcentrifuge tube. 2 volumes - 640µl of Buffer PB were added and mixed thoroughly until a homogeneous solution is obtained by inverting the tube several times. The sample was then incubated for 10 minutes at 65◦ C followed by the addition of 200µl absolute ethanol, mixed immediately and thoroughly. Next, the sample was transferred into a column (max. 650µl) and assembled in a clean collection tube which was then centrifuged at 10,000 x g for 1 minute, and the flow through was discarded. This step is repeated for the remaining sample followed by washing the column with 650µl Wash Buffer and Centrifuging at 10,000 x g for 1 minute. The ethanol was removed, and the pellet was dried at 10,000 x g for 1 minute. The flow through was discarded. The Final step was DNA elution, where the column was placed into a clean microcentrifuge tube and 50-100µl of preheated Elution Buffer, TE buffer or sterile water directly into column membrane was added and allowed to stand for 2 minutes. This was followed by centrifuging the sample at 10,000 x g for 1 minute to elute the DNA. DNA was stored at 4◦ C or -20◦ C.

RAPD analysis**:** In this study, the RAPD-PCR method was performed with 18 ten-base primers (Operon Technology) selected on the basis of literature data (Gögmen, 2012) (Table 2). The PCR reaction used was already mixed (Pure Taq Ready – To – Go PCR Beads) in a Termo 412 Thermo cycler. Each reaction was performed in a 25 µl volume containing: 20 - 40 ng/µl DNA, 2.5 mM MgCl2, and 0.2 mM each dNTP, 1 µM primer, 0.2 U Taq DNA polymerase, PCR buffer and sterile distilled water.

The cycling program was as follows**:** initial denaturation stepis at 94˚C for 3 minutes, followed by 45 cycles of denaturation at 95˚C for 1 minute, annealing at 32-37˚C for 1 minute and extension for 2 minutes at 72˚C and a final extension step at 72˚C. The amplification products were separated by electrophoresis in 1.5% agarose gel where 0.8 mcethidium bromide was added. For displaying the DNA fragments a Gel documentation System device was used. Molecular size of the amplification products was estimated by using a 100 bp Plus Blue DNA Ladder.

Data analysis**:** Cluster analysis was based on similarity matrices using the unweighted pair group method analysis (UPGMA) program in the software package MVSP (Version 3.1). The Jaccard coefficient was used for dendrogram construction.

Table 2: The names of Primers used in this study

#	Primers name	Sequence 5'...3'	#	Primers name	Sequence 5'... 3'
1	OPA-2	TGCCGAGCTG	**10**	OPI-2	GGAGGAGAGG
2	OPB-4	GGACTGGAGT	**11**	OPI-7	CAGCGACAAG
3	OPC-9	CTCACCGTCC	**12**	OPJ-2	CCCGTTGGGA
4	OPE-2	GGTGCGGGAA	**13**	OPK-6	CACCTTTCCC
5	OPF-1	ACGGATCCTG	**14**	OPK-7	AGCGAGCAAG
6	OPF-10	GGAAGCTTGG	**15**	OPL-6	GAGGGAAGAG
7	OPG-3	GAGCCCTCCA	**16**	OPM-7	CCGTGACTCA
8	OPG-10	AGGGCCGTCT	**17**	OPP-8	ACATCGCCCA
9	OPH-3	AGACGTCCAC	**18**	OPQ-1	GGGACGATGG

Source: Gögmen, 2012

Results and their review

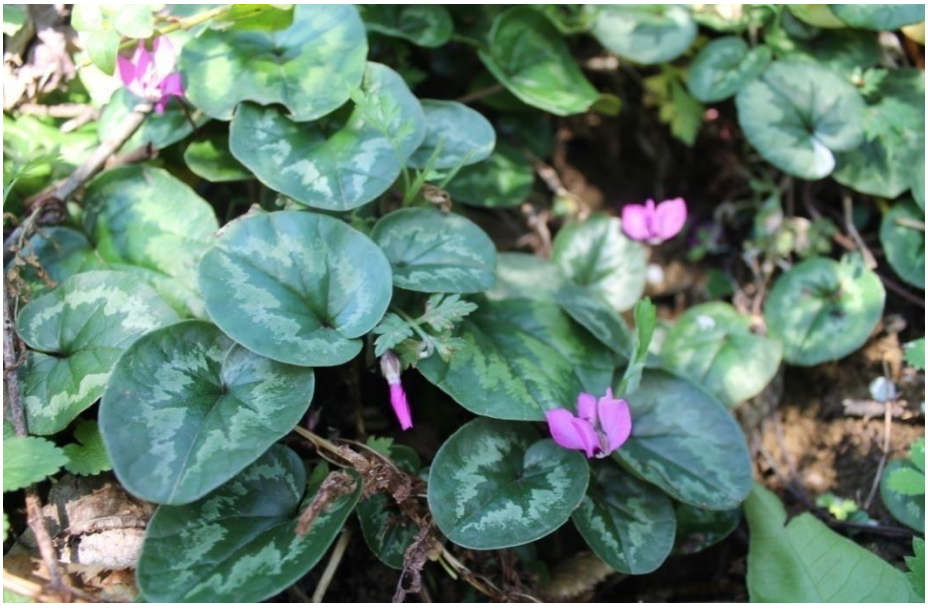
Cyclamen adzharicum Pobed are considered as a highly local endemics in Adjara (Memiadze, 2004; Varshanidze, 2013; 2015) and it is Red list category plant in regard to the IUCN guidelines (IUCN 2006). But this species is found in the literature with a different taxonomic status - *Cyclamen coum ssp. caucasicum* (Zernov, 2013).

According to our observations, the species of *C. adzharicum* and *C. coum* are different amongst themselves through some morphological characteristic (unpublished data of the authors) (Fig. 1; 2). We attempted to detect these differences at the genetic level using RAPD-PCR by the random sequence decanucleotide primers (Williams, 1990).

As a result of the conducted researches it was revealed that 65 RAPD-markers were selected from 150 to 1500 BP in length. The number of the amplified fragments DNA varied depending on the primer from 6 (OPA-2) to 11 (OPB-4). The obtained data were presented in the form of a binary matrix to study the quantification of the polymorphism degree of the species, which the presence of the component was designated as 1, the absence - as 0. Based on this matrix, a similarity matrix was calculated of the species using the Jaccard coefficient. We carried out a hierarchical cluster analysis (UPGMA) and have constructed a dendrogram (Fig. 3).

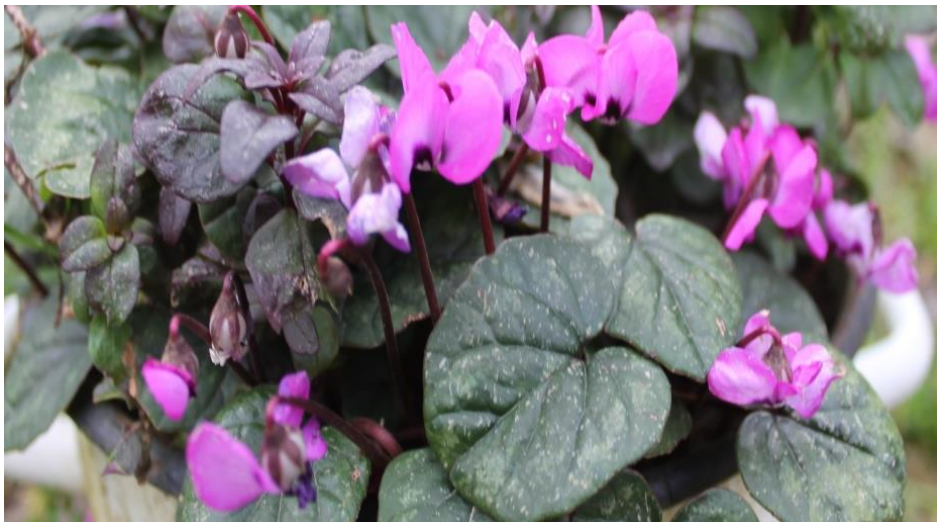
Analysis of the dendrogram shows a zero degree of intraspecific variability among the populations of C. adzharicum, which means the absence of genetic polymorphism between these three populations. However, between *C. adzharicum* and *C. coum*, the percent similarity amounted to 33.3%.

Figure 1: *Cyclamen adzharicum*(Adjara)



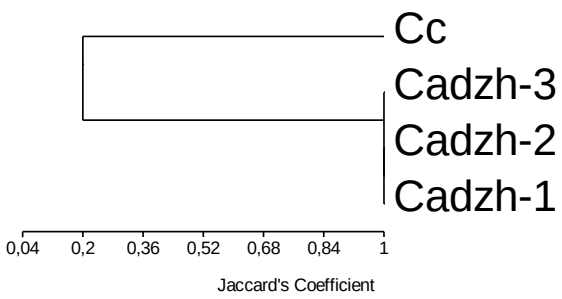
Source: Makaradze, 2018

Figure 1: *Cyclamen coum*(Turkey)



Source: Makaradze, 2018

Figure 3:UPGMA diagram based on Jaccard"s Coefficient



Source: Mepharishvili, 2018.

Conclusion

This is the first attempt to use molecular markers to study the genetic link of *Cyclamen* species in Georgia. On the basis of the conducted analysis it is possible to make a conclusion about the suitability of RAPD-analysis both for interspecies, and intra-species differentiation in *Cyclamen* L, and also for other wild plants, to identify the biodiversity of the species.

An analysis of the dendrogram showed that the analyzed genotypes were divided into two classes. In the first case were the three were of C. adzharicum, wherease C. coum - belonged to the second cluster. From the dendrogram, it is evident that there is the law of the variability between the types of *C. adzharicum*, which means that there are no genetic polymorphism between these three populations. However, there is the law similarity between *C. adzharicum* and *C. Coum.*. However, the variability between C. adzharicum and C. coum species is 33.3% .

Acknowledgement

Thanks to Temel Göktürk, Artvin Coruh University Faculty of Forestry Department and Forest Engineering

References

Catană, R., Mitoi, M., Ion, R. (2013). The RAPD techniques used to assess the genetic diversity in Drabadorneri, a critically endangered plants species. Advances in Bioscience and Biotechnology, 4, 164-169. http://dx.doi.org/10.4236/abb.2013.42024

Compton, J.A., Clennett, J.C.B., Culham, A. (2004). Nomenclature in the dock. Verclassification leads to instability: a case study in the horticulturally important genus Cyclamen (Myrsinaceae). Bot. J. Linn. Soc., 146, 339–349.

Curuk, P., Sogut, Z., Izgu, T., Sevindik, B., Tagipur, E.M., Teixeira da Silva, J.A. (2015). Morphological characterization of Cyclamen sp. grown naturally in Turkey: Part I. South African Journal of Botany 100, 7–15

Curuk, P., Sogut, Z., Izgu, T., Sevindik, B., Tagipur, E.M., Teixeira da Silva, J.A., Serce, S., Solmaz, I., Kacar, Y.A., Mendi, N.Y.Y. (2016). Morphological characterization of Cyclamen sp. grown naturally in Turkey: Part II. Acta Sci. Pol. HortorumCultus, 15(5), 205–224.

Deragon, J-M., Landry, B. (1992). RAPD and other PCR-based analyses of plant genomes using DNA extracted from small leaf disks. PCR Methods and Applications

Dmitrieva, A. (1990). Key to flora of Adjara. Vol. II. Tbilisi: Metsniereba. (In Russian)

Dugar, Yu., Popov, V. (2011). RAPD analysis of Ukrainian red clover (Trifoliumpratense L.) cultivars of different ecology-geographical origin. Vestnik Khar'kovskogo natsional'nogo universiteta imeni V.N. Karazina. Seriya: biologiya. 13 (947), 81-86.

Flora of Georgia, Families Pyrolaceae -Verbenaceae (1985) [in Russian], 2nd Ed., Nauka, Tbilisi, 90–96.

Grey-Wilson, C. (1988). The genus Cyclamen. Christopher Helm/ Timber, London, UK

Grey-Wilson, C. (2002). Cyclamen: A guide for gardeners, horticulturists and botanists. Batsford, London, UK.

IUCN. (2006). Guidelines for using the IUCN Red List categories and criteria. Version 6.2. www.redlist.org/info/categories criteria.html

Gocmen T., Vardareli N., Dogac E., Mammadov R., Taskin V. (2012) Genetic diversity of natural Cyclamen alpinum populations. Turk J Biol, V.36 pp. 413-422. © Tubitak. https://doi.org/10.3906/biy-1111-9

Jakeli, E., Varshanidze,N., Diasamidze I., Dolidze K., Zarnadze N. (2018, September). Biodiversity of medical plants of wild flora in Ajara-South Colchis and their usage in folk medicine. 3 rd International Science Symposium “New Horizons in Science”, Proceeding Book., At Pristina, Cosovo. 80-96.

Makaradze E. (2017). Diversity of Genus Cyclamen in Georgia. The 3rd International Symposium on Euro Asian Biodiversity, Minsk,Belarus, 55. http://aves.istanbul.edu.tr/YayinGoster.aspx?ID=42057&NO=28

Manvelidze, Z., Eminağaoğlu, Ö., Memiadze,N., Charazishvili, D. (2009). Species Diversity and Conservation Priorities for Endemic Plants of Georgian-Turkish Trans-boundary Zone in the West Lesser Caucasus Corridor. https://nanopdf.com/download/species-diversity-and-conservation-priorities-for-endemic-plants-of_pdf

Manvelidze, Z., Memiadze, N., Kharazishvili, D., Varshanidze N. (2010). Diversity of floral area of Adjara. (List of wild grown plants species). Annalis of Agrarian science, 8 (2), 93-164.

Memiadze, N. (2004). Botanical and geographical survey of the endemics of Ajara-Lazeti flora. Bull. Georg. Acad. Sci. 169 (2), 341-343.

Nigmatullina N.V., Kuluev A.R., Kuluev B.R. (2018). Molecular markers used to determine the genetic diversity and species identification of wild plants. Biomics. V 10(3). P. 290-318. https://doi.org/10.31301/2221-6197.bmcs.2018-39

Varshanidze N. Dolidze K, Turmanidze N, zarnadze N. Chitanava J. (2015). Protected species of adjarian flora. Proceedings ICAE International Conference "Applied Ecology: Problems, Innovations" . Tbilisi, ISBN 978-9941-0-7644-2; 250-254.

Varshanidze, N., Jakeli, E., Turmanidze, N. (2013). Wild medical plants diversity in Adjara National Parks. International Caucasian forestry Symposium. Artvin. Turkey. Retrieved from https://ekonferans.artvin.edu.tr/index.php/ICFS/ICFS/paper/view/343

Varshanidze, N., Turmanidze, N., Dolidze, K., Zarnadze, N., Diasamidze,I., Epitashvili, T., Katcharava, T. (2018). Biodiversity of Medicinal Plants Containing Essential Oil and Their Spreading in Adjara. Universal Journal of Agricultural Research 6(3), 99-104 https://doi.org/10.13189/ujar.2018.060301

Weising, K., Nybom, H., Wolff, K., Kahl, G. (2005). Finger printing in Plants: Principle, Methods, and Applications. CRC Press. Boca Raton, FL, USA.

William,s J., Kubelik, A.R., Livak, K.J. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acid Res. 18: 6531-6535. https://doi.org/10.1093/nar/18.22.6531

Yesson, C., Culham, A. (2006). A phyloclimatic study of Cyclamen. BMC Evol. Biol., 6, 72.

Zernov, A., Tyuvetskaya M. (2013). Taxonomy and nomenclature of the caucasian Cyclamen L. species. Bulletin of Moscow Society of Naturalists, Biological Series, Volume 118 (3), pp.28-36. http://herba.msu.ru/russian/journals/bmsn/archive/moip_2013_118_3.pdf