UDC 575:630
DOI: 10.2298/GENSR1501275A
Original scientific paper

DIVERSITY AND GENETIC STRUCTURE OF *Ornithogalum* L. (Hyacinthaceae) POPULATIONS AS REVEALED BY RAPD-PCR MARKERS

Andrijana ANDRIĆ, Nataša KOČIŠ TUBIĆ, Milica RAT, Dragana OBREHT VIDAKOVIĆ

Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad

Andrić A., N. Kočiš Tubić, M. Rat, and Dragana Obreht Vidaković (2015): *Diversity and genetic structure of Ornithogalum L. (hyacinthaceae) populations as revealed by RAPD-PCR markers*.- Genetika, Vol 47, No. 1, 275-288.

Random amplified polymorphic DNA (RAPD) PCR method was used to assess the level of diversity and genetic structure in *Ornithogalum* L. populations from Serbia and Hungary with the main goal of improving the knowledge of this genus in the given region. The material was collected from 19 populations and identified as two morphologically similar and phylogenetically close taxa: *O. umbellatum* L. 1753 and *O. divergens* Boreau 1887. All ten RAPD primers used for the analysis gave PCR products, with length between 3000bp and 300bp. There were 101 amplified fragments in total; number of polymorphic bands per primer varied between seven and 13. Percentage of polymorphic loci was 96% in total and 12% in average in each population. Genetic variation statistics for all loci also showed that genetic diversity for all populations was 0.29 and Shannon index 0.45, while mean values for these parameters calculated for each population were 0.04 and 0.06, respectively. Analysis of molecular variance demonstrated high population genetic differentiation; however Mantel test showed no significant correlation between geographic distances of populations and genetic distances expressed through population pairwise F_{ST}. UPGMA dendrogram based on Jaccard genetic similarity coefficients showed subclustering and principal coordinate analysis based on Nei and Li coefficients of genetic distances indicated grouping. Analysis of populations genetic structure was in accordance with these results and clearly separated populations of *O. umbellatum* from *O. divergens*. RAPDs proved to be a reliable and rapid method suitable for distinguishing genetic differentiation in *Ornithogalum*, thus could be applied as a useful additional tool in resolving taxonomic problems.

Key words: genetic diversity, genetic structure, *Ornithogalum*, RAPD

INTRODUCTION

The genus *Ornithogalum* L. is represented by bulbous monocots with very heterogeneous natural habitats. These geophytes are known as hosts for many insects

Corresponding author: Andrijana Andrić, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia; Telephone: +381641642350, Fax: 021 450 620, E-mail: andrijana.andric@dbe.uns.ac.rs

(HERRMANN, 2002; SPEIGHT, 2012) especially for some hoverflies, whose adults feed on flower pollen and nectar while larvae develop in bulbs (VUJIĆ *et al*., 2012; ANDRIĆ *et al*., 2014a). Certain species of the genus are commercially grown as potted plant crops or cut flowers (JOUNG and ROH, 2004; LITTLEJOHN, 2007), while some are alien in North America (MAIN *et al*., 2004; BROSNAN *et al*., 2010) and treated as weed in Central Europe (HERRMANN, 2002). There are records of *Ornithogalum* as an edible plant, being used in human diet in ancient times (OSBALDESTON, 2000) and also as food for animals (ERTUĞ, 2000). Nevertheless, some parts of certain species were observed to be poisonous (BURROWS and TYRL, 2001); however this is considered to be geographically conditioned (FERTH *et al*., 2001). *Ornithogalum* was used in folk medicine (MULHOLLAND *et al*., 2004; CHEN *et al*., 2010) and its pharmaceutical potential is currently being investigated (CHEN *et al*., 2010; 2012; WAN *et al*., 2012; MULHOLLAND *et al*., 2013).

Genus *Ornithogalum* belongs to family Hyacinthaceae Batsch ex Borckhausen 1797 (Order Asparagales Bromhead, subfamily Ornithogaloideae) (MANNING *et al*., 2009; MARTÍNEZ-AZORÍN *et al*., 2011). There are 34 *Ornithogalum* species recorded in Flora Europaea (ZAHARIADI, 1980), 13 in Flora of Serbia (DIKLIĆ, 1975) and eight for Hungarian Flora (SOÓ, 1973). However, the exact number of belonging species of the genus is somewhat a controversial issue, because definition of taxa within this group has been rather ambiguous (MARTÍNEZ-AZORÍN *et al*., 2011). There were no detailed taxonomic investigations of these taxa in the studied region so far.

Ornithogalum umbellatum L. 1753 is a *type species* of the genus, however its intraspecific systematics is not quite clear. It is widespread in Europe, Southwestern Asia and North Africa and introduced in North America. *Ornithogalum divergens* Boreau 1887 has narrower distribution inclined more towards the south; this primarily Mediterranean taxon is found in Central and Western European ruderal habitats as well as in western Middle East (ww2.bgbm.org; MARTÍNEZ-AZORÍN *et al*., 2009). It is mostly considered as species (GARBARI *et al*., 2003; 2008; MARTÍNEZ-AZORÍN *et al*., 2009) separated from *O. umbellatum* s.s., although its taxonomic position has been interpreted differently. It was considered a synonym, subspecies, variety and a hexaploid form of the polyploid complex of *O. umbellatum* (MARTÍNEZ-AZORÍN *et al*., 2009). *O. umbellatum* and *O. divergens* are presented as separate species (of subgenus *Ornithogalum*) in the Flora Europaea (ZAHARIADI, 1980); however *O. divergens* is described as a subspecies of *O. umbellatum* in both Serbian and Hungarian Flora (SOÓ, 1973; DIKLIĆ, 1975). Many authors realized the need for a revision, especially in *O. umbellatum* s.l. (HERRMANN, 2002; GARBARI *et al*., 2003; 2008; MARTÍNEZ-AZORÍN *et al*., 2010) and they were dealing mostly with morphology and karyology of these taxa. Molecular techniques were used primarily for phylogenetic analyses on higher taxonomical levels, namely molecular phylogenetics of subfamily Ornithogaloideae was done based on nuclear and plastid DNA markers (MANNING *et al*., 2009; MARTÍNEZ-AZORÍN *et al*., 2011).

DNA fingerprinting techniques were applied on *Ornithogalum* plants commercially grown, in attempt to identify the parentage of hybrids; particularly RAPD (*Random Amplified Polymorphic DNA*) and AFLP (*Amplified Fragment Length Polymorphism*) analyses were used (JOUNG and ROH, 2004). MARTÍNEZ-AZORÍN (2008) combined analyses of morphological characters with molecular data obtained by AFLP, in order to clarify taxonomy of *Ornithogalum*

in Spain. RAPD markers were used for preliminary analyses of genetic variability in this genus in the studied region (ANDRIĆ *et al*., 2009; 2014b).

Advantages of molecular markers in general, over morphological and biochemical, are that they could be used in any phase of plant life cycle and that environmental factors do not affect them (LISEK *et al*., 2006). Specific asset of RAPD is its universal applicability, since it does not require prior DNA sequence information for designing PCR primers (KOVAČEVIĆ *et al*., 2013). It is widely used for analyzing the diversity of genetically under-researched taxa. Furthermore, compared to similar techniques, such as AFLP, RAPD is less complicated, more economical, faster and needs smaller amounts of DNA (BARDAKCI, 2001). The RAPD technique has found a wide range of applications, especially in population genetics, because of its efficiency and rapidity in revealing the DNA-level genetic variation (BARDAKCI, 2001).

The main goal of this study is to analyze inter- and intrapopulation genetic variability and genetic structure of morphologically similar *Ornithogalum umbellatum* and *O. divergens* from natural habitats in southern and southeastern part of Pannonian Basin, using RAPDs. The knowledge of genetic differentiation may contribute to resolving ambiguities in taxonomy of these insufficiently studied taxa.

MATERIALS AND METHODS

Plant material

Plant sampling was carried out during flowering period, in April and May (2008-2013). Specimens for the analysis were collected from 19 localities in Serbia and Hungary (Fig 1). Ten plants were taken from each population and voucher specimens were deposited in the Herbarium BUNS at the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad. All plants were identified *a priori* based on their morphological characters and determined as *O. divergens* and *O. umbellatum* s.s. (Table 1).

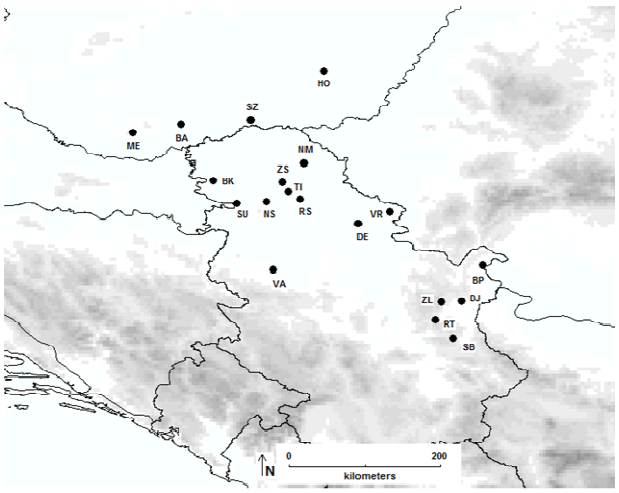

Figure 1. Localities of the analyzed populations in Serbia and Hungary

Table 1. Analyzed populations of Ornithogalum umbellatum and O. divergens

Sampling locality	Abb	Coordinates	Sampling Date	Voucher
Ornithogalum umbellatum:				
Baja	BA	N46°11'47'' E18°47'39''	22.04.2013.	2-1601
Bogojevo-Karavukovo	BK	N45°30'40'' E19°10'60''	07.05.2010.	2-1604
Brza Palanka, Kladovo	BP	N44°29'04'' E22°27'24''	28.04.2010.	2-1606
Deliblatska Peščara	DE	N44°59'16'' E20°56'42''	18.04.2008.	2-1632
Deli Jovan	DJ	N44°02'22'' E22°12'32''	10.05.2009.	2-1652
Mecsek	ME	N46°05'56'' E18°13'07''	21.04.2013.	2-1636
Novo Miloševo	NM	N45°42'60'' E20°17'29''	02.05.2008.	2-1602
Rimski šanac	RS	N45°29'41'' E20°01'52''	23.04.2010.	2-1605
Rtanj, Lukovo	RT	N43°48'35'' E21°53'07''	08.05.2010.	2-1653
Soko Banja	SB	N43°35'43'' E22°05'52''	13.05.2010.	2-1651
Szeged	SZ	N46°15'10'' E19°38'36''	22.04.2013.	2-1637
Valjevo	VA	N44°25'43'' E19°54'35''	29.04.2012.	2-1635
Vršačke Planine	VR	N45°07'22'' E21°19'38''	26.04.2009.	2-1633
Zlotska Klisura	ZL	N44°01'45'' E21°57'28''	10.05.2009.	2-1634
Žabaljske slatine	ZS	N45°22'36'' E20°05'58''	21.04.2010.	2-1603
Ornithogalum divergens:				
Hódmezövásárhely	HO	N46°50'42'' E20°31'47''	22.04.2013.	2-1641
Novi Sad	NS	N45°14'51'' E19°49'43''	26.04.2013.	2-1640
Susek	SU	N45°13'51'' E19°28'38''	19.04.2012.	2-1639
Titelski Breg	TI	N45°17'01'' E20°14'29''	20.04.2012.	2-1638

DNA isolation and PCR amplification

Leaves were sampled, one for each specimen, and stored at +4°C till DNA extraction. Total genomic DNA was isolated (2 cm of leaf; 50 – 100 mg) using the modified CTAB method (PADMALATHA and PRASAD, 2006). RAPD – PCR marker system was used for genome polymorphism detection. Ten RAPD primers (Operon Technologies Inc., USA) gave clear reproducible banding patterns (Table 2). Preliminary experiments were carried out to optimize the PCR conditions leading to clear amplification products. RAPD – PCR reactions were done in a volume of 25 μl, containing 1U Dream Taq polymerase (Thermo Scientific), 1× Dream Taq Buffer (with 2 mM $MgCl_2$), 0.2 mM dNTPs, 10 pmoles primer and 100 ng genomic DNA. Amplifications were carried out in a Mastercycler personal (Eppendorf) in 35 cycles using following program: initial denaturation step at 94°C for 5 min followed by denaturation at 94°C for 1 min, primer annealing 36°C for 1 min, polymerization 72°C for 90 sec and a final extension step at 72°C for 10 min. The amplified products were separated by horizontal electrophoresis in 1.5% agarose gels. The gels were photographed under UV light by BioDocAnalyze (Biometra). Product sizes were determined using DNA Ladders: 1Kbp (SERVA) and 100bp (Thermo Scientific). Clear reproducible bands were scored and used for the statistical analysis.

Data analysis

DNA banding patterns were scored for presence (1) or absence (0) of the fragment in each sample and the binary matrix was made. Data were statistically processed using Free Tree

(PAVLICEK, 1999) and Treeview (PAGE, 1996) software packages. Genetic similarity among populations was calculated by pairwise comparisons in accordance with JACCARD (1908). These coefficients were used to analyze the relations among populations and to construct UPGMA (*Unweighted Pair Group Method with Arithmetic mean*) dendrogram. Bootstrap analysis, with 5,000 repetition value, was used to assess the tree topology robustness. PCoA (*Principal Coordinate Analysis*) was also performed in order to graphically represent relations among populations, based on NEI and LI (1979) coefficients of genetic distances, in XLSTAT ver. 2014.5.02 (MS Excel. Addinsoft). POPGENE ver. 1.32 (YEH *et al.,* 1999) was used for calculation of genetic diversity parameters: h - Nei's (NEI, 1973) gene diversity; I - Shannon's (1949) information index and p - the percentage of polymorphic loci. Analysis of molecular variance (AMOVA) was conducted in Arlequin ver. 3.5.1.2 (EXCOFFIER *et al*., 2005) with 10,000 permutations. The same software package was employed for testing the correlation between genetic distances (F_{ST}) and geographic distances among sampling localities using Mantel test with 1000 permutations.

Genetic structure among populations was assessed using software Structure ver. 2.3.4. (PRITCHARD *et al*., 2000; FALUSH *et al*., 2007). Genotype classes were considered to consist of haploid alleles (OLIVEIRA *et al*., 2010; PINHEIRO *et al*., 2012). The ancestry model of admixture was followed, as well as correlated allele frequencies model. For each value of K, the number of clusters, ranging from one to 10, the Monte Carlo Markov chain (MCMC) simulation was run five times for 20,000 iterations and 20,000 burn-in period. Selection of the most probable number of genetic clusters (K) was made using ΔK methods (EVANO *et al*., 2005). For the graphical representation of genetic structure programs CLUMPP 1.1.2 (JAKOBSSON and ROSENBERG, 2007) and Distruct 1.1 (ROSENBERG, 2004) were used.

RESULTS AND DISCUSSION

Genetic structure and genetic diversity within and among the populations of two *Ornithogalum* taxa were analyzed. Populations TI, SU, HO and NS (Table 1) were *a priori* identified as *O. divergens*, based on some morphological characters which separated them from other populations determined as *O. umbellatum* s.s.

The RAPD analyses of 190 *Ornithogalum* plants from 19 populations with ten different primers resulted in 101 amplified fragments, 91% of them were polymorphic. The number of detected polymorphic loci varied between primers from seven (OPB06, K15, M02) to 13 (OPC10). Total number of bands per primer ranged from eight to 14 (Table 2). RAPD-PCR reaction products varied in size ranging 300bp to 3kb.

The total number of amplified fragments by population was between 42 (ZL, HO) and 63 (RT), 51 in average. Parameters of genetic diversity were calculated for each population and for all populations in total (Table 3). The percentage of polymorphic loci was 96% in total and 12% in average in each population. The genetic variation statistics for all loci also showed that genetic diversity for all populations was 0.29 and Shannon index 0.45, while mean values for these parameters calculated for each population were 0.04 and 0.06, respectively. These values of genetic diversity parameters were in accordance with the results of the previous study of subgenus *Ornithogalum* by MARTÍNEZ-AZORÍN (2008) using molecular data obtained by AFLP.

Table 2. Attributes of the RAPD primers with number of fragments amplified (N) and level of polymorphism (shown as P – number of polymorphic fragments, and % of polymorphism)

primer	nucleotide sequence (5'-3')	N	P	%
OPA01	CAGGCCCTTC	10	10	100.0
OPA02	TGCCGAGCTG	11	11	100.0
OPA08	GTGACGTAGG	11	9	81.82
OPB06	TGCTCTGCCC	8	7	87.5
OPB11	GTAGACCCGT	11	11	100.0
OPB12	CCTTGACGCA	10	9	90.0
OPC10	TGTCTGGGTG	14	13	92.9
K01	CATTCGAGCC	9	8	88.9
K15	CTCCTGCCAA	9	7	77.8
M02	ACAACGCCTC	8	7	87.5
Total		101	92	91.1

Table 3. Genetic variation statistics per populations for all loci

Population	h	I	p (%)	N	J
BA	0.0527	0.0782	13.86	54	0.8596
BK	0.0225	0.0319	04.95	47	0.9554
BP	0.1024	0.1509	26.73	61	0.7792
DE	0.0310	0.0482	09.90	49	0.8999
DJ	0.0323	0.0470	07.92	50	0.9246
HO	0.0043	0.0062	00.99	42	0.9967
ME	0.0411	0.0613	10.89	57	0.9082
NM	0.0450	0.0639	09.90	48	0.9066
NS	0.0436	0.0640	10.89	51	0.8833
RS	0.0312	0.0459	07.92	47	0.9272
RT	0.1124	0.1677	30.69	63	0.7446
SB	0.0489	0.0764	15.84	51	0.8582
SU	0.0119	0.0174	02.97	45	0.9753
SZ	0.0317	0.0478	08.91	54	0.9223
TI	0.0035	0.0053	00.99	46	0.9872
VA	0.0201	0.0293	04.95	45	0.9466
VR	0.0332	0.0517	10.89	48	0.8823
ZL	0.0432	0.0689	14.85	42	0.8016
ZS	0.1044	0.1564	28.71	61	0.7794
Average	0.0429	0.0641	11.72	51	0.8915
Total	0.2910	0.4462	95.50	-	-

h – Nei's gene diversity; I – Shannon's Information index; p – The percentage of polymorphic loci. N – Number of fragments amplified in each population for all ten primers; J – Jaccard similarity coefficient (average) among genotypes

Coefficients of the genetic similarity (JACCARD, 1908) of combined data from ten primers were calculated on intrapopulation level (Table 3) and the average between genotypes within populations of all localities was 0.891. These coefficients did not differ much among localities; however, higher values were detected in some of the populations determined as *O. divergens* (the highest value in HO – 0.997), as opposed to *O. umbellatum* (the lowest value in RT – 0.745). This could be related to reproductive biology features, considering variation and mixed preferences in respect with different infrageneric taxa belonging to *O. umbellatum* complex. Namely, *O. divergens* plants are mostly characterized by vegetative propagation by bulbils, while *O. umbellatum* s.s. are considered to be insect-pollinated as well, therefore partially outcrossing. Furthermore, selfing species are known for having higher levels of genetic differentiation among populations and lower levels of diversity within, than genetically more variable allogamous plants (NYBOM and BARTISH, 2000; MA *et al*., 2012; PINHEIRO *et al*., 2012).

Results in accordance with these were also shown using AMOVA to calculate proportions of genetic variability for the two analyzed taxa separately; 77% of total variance was found in *O. umbellatum* (23% within populations), while 91% (only 9% intrapopulation) in *O. divergens* (Table 4). Overall, AMOVA showed that considerable level, 65%, of the genetic variability is found among analyzed *Ornithogalum* populations; 20% between *O. umbellatum* and *O. divergens* groups and 15% within the populations of the certain localities (Table 4). Some studies have indicated that regional and widespread taxa such as these show significantly higher between-population diversity than the ones with narrow geographic range or endemic (NYBOM and BARTISH, 2000).

Table 4. Analysis of molecular variance (AMOVA) of 190 genotypes of Ornithogalum using RAPD markers

Source of variation	Degrees of freedom (d.f.)	Sum of squares	Variance components	Percentage of variation (%)
Among populations	17	2030.01	11.67	64.62
Among *a priori* identified taxa	1	350.36	3.66	20.25
Within populations	171	467.20	2.73	15.13
Among *O.umbellatum* pop.	14	1713.21	11.89	77.24
Within *O.umbellatum* pop.	135	472.80	3.50	22.76
Among *O.divergens* pop.	3	287.25	9.48	91.33
Within *O.divergens* pop.	36	32.40	0.90	8.67

Significance level $P < 0.0001$

However, Mantel test between geographic and genetic distances among studied populations has shown that these two matrices are not correlated, as the computed P value was 0.840 (>0.05) (correlation coefficient r=-0.1385). This lack of significant correlation indicates no distinct geographic trend in the disposition of genetic variability (MA *et al*., 2012), which is presumably due to the large distribution area of the species.

Values of genetic similarity between 19 populations, obtained by pairwise comparisons (JACCARD, 1908) of combined data based on 101 amplified fragments (data not shown), ranged

from 0.74 (SZ-BA) to 0.36 (NS-ZS). The highest values were recorded between pairwise SZ, BA and ME, populations of *O. umbellatum* from relatively adjacent Hungarian localities, and also for pairwise TI, NS, HO and SU, previously determined as *O. divergens*. The UPGMA dendrogram based on Jaccard genetic similarity coefficients (Fig 2) showed subclustering, with one cluster consisting of *O. divergens* populations, with a bootstrap value of 100%. The PCo analysis also gave visual representation of the genetic distance and potential grouping of the analyzed populations. The first two principal coordinates have explained 37.37% (24.10% and 13.27%) of total variability. This analysis also shows that *O. divergens* populations form distinct group, separated by both axes (Fig 3).

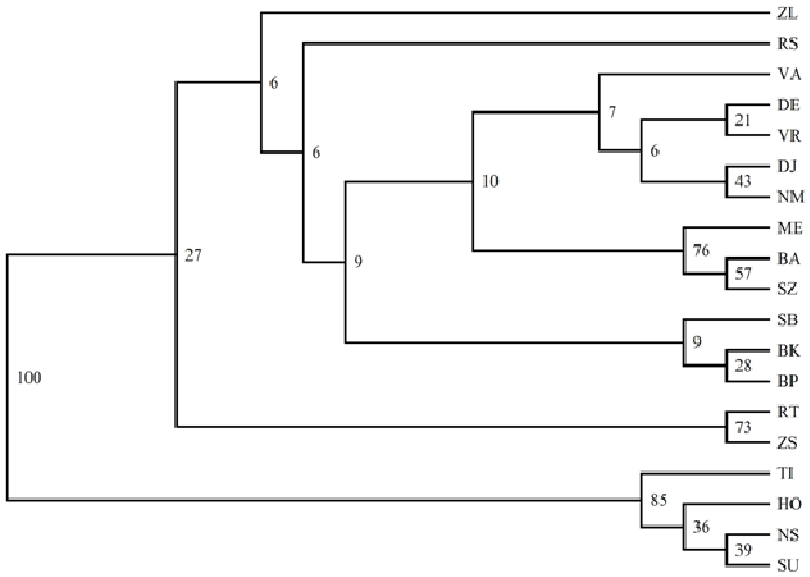

Figure 2. UPGMA dendrogram of *Ornithogalum* populations based on Jaccard genetic similarity coefficients. The numbers indicate bootstrap values in percentages (calculated with 5000 repetitions)

In order to determine genetic structuring among *Ornithogalum* genotypes, Bayesian clustering analysis was performed. ΔK implied that two is the most probable number of genetic clusters to explain the structuring of analyzed populations (Fig 4A). This approach assigned all examined individuals to two groups and populations were clearly separated. One group consisted of HO, NS, SU and TI, while all other populations formed the second group, represented graphically in different color (Fig 4B). These results, which are in accordance with UPGMA and PCoA, undoubtedly divided *O. divergens* plants from *O. umbellatum*.

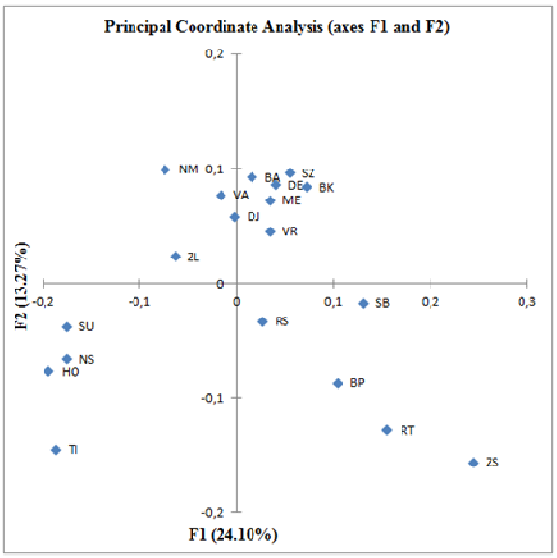

Figure 3. PCo analysis of *Ornithogalum* populations based on RAPD data

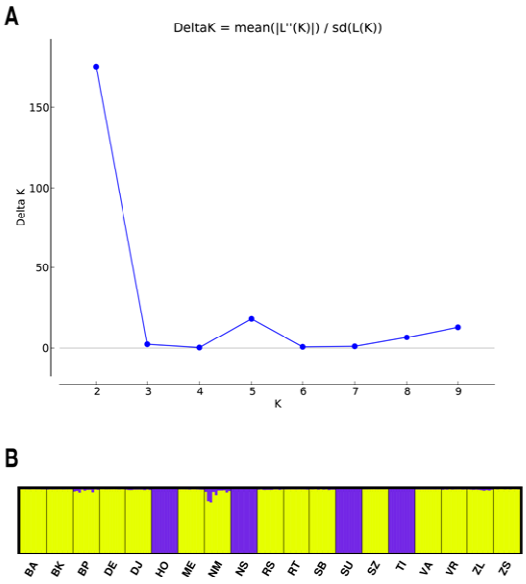

Figure 4. Genetic structure among 19 *Ornithogalum* populations based on RAPD data. A) ΔK: function of the number of clusters (K); B) The graphical display of genetic structure for K=2

GARBARI *et al*. (2003; 2008), and MARTÍNEZ-AZORÍN *et al*. (2009; 2010) after them, showed substantial morphological differences and different ploidy levels between *O. divergens* and *O. umbellatum* taxa. Results of our outgoing analyses suggest that populations TI, SU, HO and NS are different in some of their morphological characteristics which classify them as *O. divergens,* and furthermore a study dealing with anatomy of these plants has also shown these populations distinguished from those determined as *O. umbellatum* (ANDRIĆ *et al*., in prep.). Although type of research presented here is insufficiently dependable for precise separation of *O. divergens* from *O. umbellatum*, RAPDs proved to be a reliable and rapid method suitable for distinguishing genetic variation in *Ornithogalum*, thus could be applied as a useful additional tool in unraveling taxonomic problems.

Since the morphology of *Ornithogalum* plants is considered inadequately connected with variations in karyotype, this genus is recognized as particularly complicated from the standpoint of systematics (MERIÇ *et al*., 2011). Therefore, any new data could be very valuable, and this is especially true for new information on *Ornithogalum* taxa from incompletely investigated regions. There are no previous comprehensive studies regarding taxonomy of these plants in the area studied in the present paper. This area is particularly important regarding distribution of these taxa, being part of the Balkan Peninsula and Pannonian Plain regions, which represent geographical connection between Mediterranean populations and those of Central and Western Europe. Mediterranean is considered to be the center of diversity for this group of taxa, from where spreading continued to the Central Europe where currently they primarily occupy anthropogenic habitats (DIKLIĆ, 1975; HERRMANN, 2002; MARTÍNEZ-AZORÍN *et al*., 2010).

Survival of the taxa, in terms of adaptation to changing environment, depends upon genetic variation of populations and this richness can be estimated by different genetic diversity parameters (MA *et al*., 2012). On a broader ecological level, information on polymorphism and population structure could resolve uncertainties related to specific plant–insect interactions, such as connectivity between species diversity and pollination and phytophagy, as well as complex spatial and biogeographical relations. By providing an insight into intra- and interpopulation genetic variability of *Ornithogalum* species, studies like this will contribute to appropriate design of the biodiversity conservation strategies, as well as management of these food plants and insect species which are related to them.

ACKNOWLEDGEMENT

The study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Grant No. 173002.

Received December 20th, 2014
Accepted February 25th, 2015

REFERENCES

ANDRIĆ, A., D. OBREHT, M. RAT, P. BOZA, A. VUJIĆ (2009): Analysis of genetic variability in *Ornithogalum* L. using RAPD markers. IV Congres of the Serbian Genetic Society, Tara. Book of abstracts, 264.

ANDRIĆ, A., B. ŠIKOPARIJA, D. OBREHT, M. ĐAN, J. PRERADOVIĆ, S. RADENKOVIĆ, C. PÉREZ – BAÑÓN, A. VUJIĆ (2014a): DNA barcoding applied: identification of the larva of *Merodon avidus* (Diptera: Syrphidae). Acta Entomol. Mus. Nat. Pragae, *54*(2), 741–757.

ANDRIĆ, A., M. RAT, D. OBREHT (2014b): Analysis of genetic variability in two *Ornithogalum* L. (Hyacinthaceae) taxa from Serbia and Hungary using RAPD markers. V Congres of the Serbian Genetic Society, Belgrade. Book of abstracts, 190.

BARDAKCI, F. (2001): Random amplified polymorphic DNA (RAPD) markers. Turk. J. Biol., *25*, 185–196.

BROSNAN, J. T., G. R. ARMEL, W. E. KLINGEMAN, G. K. BREEDEN, J. J. VARGAS, P. C. FLANAGAN (2010): Selective Star-of-Bethlehem Control with Sulfentrazone and Mixtures of Mesotrione and Topramezone with Bromoxynil and Bentazon in Cool-season Turfgrass. Hort. Technology, *20* (2), 315–318.

BURROWS, G. E., R. J. TYRL (2001): Toxic Plants of North America. Iowa State University Press.

CHEN, R., F. MENG, Z. LIU, R. CHEN, M. ZHANG (2010): Antitumor activities of different fractions of polysaccharide purified from *Ornithogalum caudatum* Ait. Carbohyd. Polym., *80*, 845–851.

CHEN, R., Z. LI, H. DONG, Z. LIU, S. LI, S. YANG, H. LI (2012): Optimization of ultrasonic extraction process of polysaccharides from *Ornithogalum caudatum* Ait and evaluation of its biological activities. Ultrason. Sonochem., *19*, 1160–1168.

DIKLIĆ, N. (1975): Rod *Ornithogalum* (Liliales – Liliaceae). In: Josifović M. (ed) Flora SR Srbije, VII, Serbian Academy of Sciences and Arts, Belgrade, pp. 544–559.

ERTUĞ, F. (2000): An Ethnobotanical Study in Central Anatolia (Turkey). Econ. Bot., *54* (2), 155–182.

EVANNO, G., S. REGNAUT, J. GOUDET (2005): Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol., *14*, 2611–2620.

EXCOFFIER, L., G. LAVAL, S. SCHNEIDER (2005): Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evol. Bioinform. Online *1*, 47–50.

FALUSH, D., M. STEPHENS, J. K. PRITCHARD (2007): Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes, *7*, 574–578.

FERTH, R., F. SPETA, B. KOPP (2001): Beitrag der Cardenolide zur Taxonomie der *Ornithogalum umbellatum* – Verwandtschaft (Hyacinthaceae). Stapfia, *164*, 121–138.

GARBARI, F., A. GIORDANI, R. MARCUCCI, N. TORNADORE (2003): The genus *Ornithogalum* L. (Hyacinthaceae) in Italy, XIV: towards a redefinition of infrageneric taxa, with new proposals. Bocconea, *16*, 269–281.

GARBARI, F., L. PERUZZI, N. TORNADORE (2008): *Ornithogalum* L. (Hyacinthaceae Batsch) e generi correlati (subfam. Ornithogaloideae Speta) in Italia. Atti Soc. Tosc. Sci. nat., Mem., Serie B *114*, 35–44.

HERRMANN, N. (2002): Biological Flora of Central Europe: „*Ornithogalum angustifolium*“ nom. prov., Syn. p.p. *O. orthophyllum* ssp. *kochii* = *O. kochii* Parl., *O. gussonei* Ten. Flora, *197*, 409–428.

JACCARD, P. (1908): Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat., *44*, 223–270.

JAKOBSSON, M., N. A. ROSENBERG (2007): *CLUMPP*: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, *23*, 1801–1806.

JOUNG, Y. H., M. S. ROH (2004): Paternity determination of *Ornithogalum* seedlings using DNA markers. J. Hortic. Sci. Biotech., *79* (2), 316–321.

KOVAČEVIĆ, D., B. NIKOLIĆ, S. MLADENOVIĆ DRINIĆ, S. BOJOVIĆ, T. DODOŠ, N. RAJČEVIĆ, P. D. MARIN (2013): Genetic relationships among some *Pinus, Picea* and *Abies* species revealed by RAPD markers. Genetika, *45* (2), 493–502.

LISEK, A., M. KORBIN, E. ROZPARA (2006): Using simply generated RAPD markers to distinguish between sweet cherry (*Prunus avium* L.) cultivars. J. Fruit Ornam. Plant Res. *14* (Suppl. 1), 53–59.

LITTLEJOHN, G. M. (2007): Star of Bethlehem *Ornithogalum.* In: Anderson N.O. (ed.) Flower breeding and genetics. Springer, Netherlands, pp 741–754.

MA, X., S.-Y. CHEN, S.-Q. BAI, X.-Q. ZHANG, D.-X. LI, C.-B. ZHANG, J.-J.YAN (2012): RAPD analysis of genetic diversity and population structure of *Elymus sibiricus* (Poaceae) native to the southeastern Qinghai-Tibet Plateau, China. Genet. Mol. Res., *11* (3), 2708–2718.

MAIN, C. L., D. K. ROBINSON, T. C. TEUTON, T. C. MUELLER (2004): Star-of-Bethlehem (*Ornithogalum umbellatum*) control with postemergence herbicides in dormant bermudagrass (*Cynodoń dactylon*) turf. Weed Technol., *18* (4), 1117–1119.

MANNING, J. C., F. FOREST, D. S. DEVEY, M. F. FAY, P. GOLDBLATT (2009): A molecular phylogeny and a revised classification of Ornithogaloideae (Hyacinthaceae) based on an analysis of four plastid DNA regions. Taxon, *58*, 77–107.

MARTÍNEZ-AZORÍN, M. (2008): Sistemática del género *Ornithogalum* L. (Hyacinthaceae) en el Mediterráneo occidental: implicaciones taxonómicas, filogenéticas y biogeográficas. Tesis doctoral. Universidad de Alicante, España.

MARTÍNEZ-AZORÍN, M., M. B. CRESPO, A. JUAN (2009): Nomenclature and taxonomy of *Ornithogalum divergens* Boreau (Hyacinthaceae) and related taxa of the polyploidy complex of *Ornithogalum umbellatum* L. Candollea, *64*, 163–169.

MARTÍNEZ-AZORÍN, M., M. B. CRESPO, A. JUAN (2010): Taxonomic revision of *Ornithogalum* subg. *Ornithogalum* (Hyacinthaceae) in the Iberian Peninsula and the Balearic Islands. Plant Syst. Evol., *289*, 181–211.

MARTÍNEZ-AZORÍN, M., M. B. CRESPO, A. JUAN, M. F. FAY (2011): Molecular phylogenetics of subfamily Ornithogaloideae (Hyacinthaceae) based on nuclear and plastid DNA regions, including a new taxonomic arrangement. Ann. Bot. – London, *107*, 1–37.

MERIÇ, C., Ö. AKSOY, F. DANE (2011): Morphological and anatomical contributions to the taxonomical identification of two *Ornithogalum* taxa (*O. nutans* and *O. boucheanum*) from Flora of Turkey. Biologia, *66*, 68–75.

MULHOLLAND, D. A., N. R. CROUCH, T. L. POHL, E. NDLOVU (2004): A homoisoflavanone from *Ornithogalum longibracteatum* (Ornithogaloideae: Hyacinthaceae). Biochem. Syst. Ecol., *32*, 499–502.

MULHOLLAND, D. A., S. L. SCHWIKKARD, N. R. CROUCH (2013): The chemistry and biological activity of the Hyacinthaceae. Nat. Prod. Rep., *30*, 1165–1210.

NEI, M. (1973): Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA, *70*, 3321–3323.

NEI, M., W. H. LI (1979): Mathematical model for styding genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Science USA, *76*, 5269–5273.

NYBOM, H., I. V. BARTISH (2000): Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Plant Ecol. Evol. Syst., *3* (2), 93–114.

OLIVEIRA, L. O., B. A.VENTURINI, A. A. B. ROSSI, S. S. HASTENREITER (2010): Clonal diversity and conservation genetics of the medicinal plant *Carapichea ipecacuanha* (Rubiaceae). Genet. Mol. Biol., *33*, 86–93.

OSBALDESTON, T. A. (2000): De Materia Medica: Being an Herbal with many other medicinal materials. Ibidis Press, Johannesburg.

PADMALATHA, K., V. PRASAD (2006): Optimization of DNA isolation and PCR protocol for RAPD analysis of selected medicinal and aromatic plants of conservation concern from Peninsular India. Afr. J. Biotechnol., *5* (3), 230–234.

PAGE, R. D. M. (1996): TreeView: An application to display phylogenetic trees on personal computers. Comput. Appl. Biosci., *12*, 357–358.

PAVLICEK, A., S. HRDA, J. FLEGR (1999): Free Tree - Freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of the genus *Frenkelia*. Folia Biol., *45*, 97–99.

PINHEIRO, L. R., A. R. CARREGOSA RABBANI, A. V. C. DA SILVA, A. DA SILVA LEDO, K. L. GARCIA PEREIRA, L. E. CARDAMONE DINIZ (2012): Genetic diversity and population structure in the Brazilian *Cattleya labiata* (Orchidaceae) using RAPD and ISSR markers. Plant Syst. Evol., *298*, 1815–1825.

PRITCHARD, J. K., M. STEPHENS, P. DONNELLY (2000): Inference of population structure using multilocus genotype data. Genetics, *155*, 945–959.

ROSENBERG, N. A. (2004): Distruct: a program for the graphical display of population structure. Mol. Ecol. Notes, *4*, 137–138.

SHANNON, C. E., W. WEAVER (1949): The mathematical theory of communication. Univ. of Illinois Press, Urbana.

SOÓ, R. (1973): A Magyar flóra és vegetáció rendszertani-növényföldrajzi kézikönyve V. Akadémiai Kiadó, Budapest.

SPEIGHT, M. C. D. (2012): Species accounts of European Syrphidae (Diptera). In: *Syrph the Net, the database of European Syrphidae*, Dublin: Syrph the Net publications *69*, 296 pp.

VUJIĆ, A., S. RADENKOVIĆ, G. STÅHLS, J. AČANSKI, A. STEFANOVIĆ, S. VESELIĆ, A. ANDRIĆ, R. HAYAT (2012): Systematics and taxonomy of the *ruficornis* group of genus *Merodon* Meigen (Diptera: Syrphidae). Syst. Entomol., *37*, 578–602.

WAN, Y., Y. WU, L. LIAN, J. NAN (2012): Protective effect of *Ornithogalum saundersiae* Ait (Liliaceae) against acetaminophen-induced acute liver injury via CYP2E1 and HIF-1α. Chin. J. Nat. Med., *10* (3), 177–184.

XLSTAT Version. 2014.5.02, MS Excel. Addinsoft, NY, USA. Available at http://www.xlstat.com (accessed 17 November 2014)

YEH, F. C., R. C. YANG, T. BOYLE (1999): POPGENE Version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis. University of Alberta, Edmonton, Canada.

ZAHARIADI, C. (1980): *Ornithogalum* L. In: Tutin T. G., Heywood V. H., Burges N. A., Moore D. M., Valentine D. H., Walters S. M., Webb D. A., (eds) Flora Europaea, Cambridge University Press, pp 35–40.

http://ww2.bgbm.org/EuroPlusMed/PTaxonDetail.asp?NameCache=Ornithogalum%20divergens&PTRefFk=8000000 (accessed 3 December 2014)

DIVERZITET I GENETIČKA STRUKTURA POPULACIJA *Ornithogalum* L. (Hyacinthaceae) - EVALUACIJA PRIMENOM RAPD-PCR MARKERA

Andrijana ANDRIĆ, Nataša KOČIŠ TUBIĆ, Milica RAT, Dragana OBREHT VIDAKOVIĆ

Departman za biologiju i ekologiju, Prirodno-matematicki fakultet, Univerzitet u Novom Sadu

Izvod

U cilju procene nivoa genetičkog diverziteta i strukture populacija roda *Ornithogalum* L. u Srbiji i Mađarskoj, korišćena je RAPD (*Random amplified polymorphic DNA*) PCR metoda, radi što boljeg upoznavanja ovog taksona u datom području. Materijal je uzorkovan iz 19 prirodnih populacija, među kojima su identifikovana dva morfološki veoma slična i filogenetski bliska taksona: *O. umbellatum* L. 1753 i *O. divergens* Boreau 1887. Svih deset primenjenih RAPD prajmera dali su PCR produkte, dužine između 3000bp i 300bp i ukupno je amplifikovan 101 fragment. Broj polimorfnih traka po prajmeru varirao je između sedam i 13. Ukupan procenat polimorfnih lokusa bio je 96%, a prosečan po populaciji 12%. Genetički diverzitet za celokupan uzorak iznosio je 0,29 a Shannon indeks 0,45, dok su srednje vrednosti ovih parametara po populacijama iznosile 0,04 odnosno 0,06. Visok nivo diferencijacije između populacija, u odnosu na intrapopulacionu genetičku varijabilnost, ustanovljen je uz pomoć AMOVA analize, dok je Mantel test pokazao da ne postoji značajna korelacija između geografskih udaljenosti populacija i genetičkih distanci izraženih kroz $F_{ST.}$ UPGMA dendrogram zasnovan na Jaccard koeficijentima genetičke sličnosti prikazuje grupisanje u klastere kao i analiza glavnih koordinata (PCoA) urađena na osnovu Nei i Li koeficijenata genetičke udaljenosti. Analiza genetičke strukture populacija (STRUCTURE) potvrdila je razdvajanje populacija *O. divergens* od *O. umbellatum*. RAPD-PCR se pokazala kao pouzdana i brza tehnika pogodna za detekciju genetičke diferencijacije u okviru roda *Ornithogalum*, stoga se može primenjivati kao dodatni diferencijalni karakter u cilju rešavanja postojećih taksonomskih problema.

Primljeno 20. XII. 2014.
Odobreno 25 II 2015.