Horticulture and Forestry Society of Transylvania

Băcilă I *et al*. (2023)
Notulae Scientia Biologicae
Volume 15, Issue 2, Article number 11549
DOI:10.15835/nsb15211549
Research Article

Notulae Scientia Biologicae

Evaluation of cross-genus transferability of SSR markers from other legumes to two closely related *Onobrychis* (Fabaceae) taxa

Ioan BĂCILĂ[1], Dana ŞUTEU[1,4]*, Ana COSTE[1], Zoltán R. BALÁZS[2,3,4], Gheorghe COLDEA[1]

[1]*National Institute of Research and Development for Biological Sciences, Institute of Biological Research, Department of Experimental Biology, 48 Republicii St., 400015 Cluj-Napoca, Romania; ioan.bacila@icbcluj.ro; dana.suteu@icbcluj.ro; ana.coste@icbcluj.ro; gheorghe.coldea@icbcluj.ro*

[2]*Babeş-Bolyai University, Faculty of Biology and Geology, Department of Molecular Biology and Biotechnology, 1 Kogălniceanu St., 400084 Cluj-Napoca, Romania; zoltan.balazs@ubbcluj.ro*

[3]*Babeş-Bolyai University, Faculty of Biology and Geology, Center for Systematic Biology, Biodiversity and Bioresources - 3B, 1 Kogălniceanu St., 400084, Cluj-Napoca, Romania*

[4]*Babeş-Bolyai University, Doctoral School of Integrative Biology, 1 Kogălniceanu St., 400084 Cluj-Napoca, Romania; dana.suteu@icbcluj.ro (*corresponding author)*

Abstract

Microsatellite markers previously developed for other leguminous species were tested for cross-genus transferability and evaluated for their potential usefulness in providing an improved assessment of the genetic relationships between two closely related taxa belonging to *Onobrychis* genus (Fabaceae). Candidate microsatellite markers were tested for polymorphism and replicability in sixteen populations of *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. and *O. montana*. Out of the 23 SSRs, there were identified seven polymorphic loci. In total 32 alleles were detected and the number of alleles per locus varied from two to six. PIC values ranged from 0.375 to 0.6454, and four SSRs displayed a PIC > 0.5. Relative uniform rates of genetic diversity were obtained. In case of *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. the observed and expected heterozygosity ranged from 0.100 to 0.952 and from 0.219 to 0.525, respectively, while for *O. montana* ranged from 0.166 to 0.750 and from 0.083 to 0.375, respectively. Seven polymorphic SSRs with clear and reproducible amplification were identified. These markers proved to be very efficient for unambiguous population discrimination based on both geographic and taxonomic criteria. Hereafter, these SSR markers can be used as tools for evolutionary studies in *Onobrychis* genus, as well in providing knowledge on patterns of the species phylogeography.

Keywords: cross-genus transferability; leguminous; microsatellite; *Onobrychis*; polymorphism

Received: 13 Apr 2023. Received in revised form: 08 Jun 2023. Accepted: 13 Jun 2023. Published online: 19 Jun 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Introduction

The *Onobrychis* genus includes about 206 species (POWO, 2023), cross-pollinated, diploid (2n = 14, 16) or tetraploid (2n = 28) (Mohsen and Nasab, 2010), perennial or annual herbs or shrubs. The genus extends throughout the Europe (excepting Scandinavia and the British Isles), Central and Eastern Asia, and North Africa (POWO, 2023).

Within the genus, *Onobrychis montana* DC. subsp. *transsilvanica* (Simk.) Jáv. (Ciocârlan, 2009; Sârbu *et al*., 2013) (≡ *Onobrychis transsilvanica* (Nyárády and Nyárády, 1957); ≡ *Onobrychis montana* DC. var. *transsilvanica* (Simk.) Beck (Borza, 1949) is an endemic taxon in the Romanian Carpathian chain. It shares close, yet controversial, taxonomic relationships and a strong morphological resemblance with the allopatric species *Onobrychis montana* DC. Our previous study (Băcilă *et al*., 2015) represented the first attempt to provide some molecular insights for this Carpathian endemic taxon with the use of AFLP and cpDNA markers. Because these markers failed to clearly resolve the distinction between *O. montana* and *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv., other molecular markers, more informative ought to be identified.

Microsatellites (or Single Sequence Repeats - SSRs) are codominant markers characterized by high levels of polymorphism, thus being widely recognized as very powerful and informative in both animal and plant species (Ellegren, 2004). The hypervariable nature of SSRs produces allelic variations even among very closely related varieties. Therefore, they are considered the markers of choice for the characterization of core collections and for the management of germplasm collections (Kumar *et al*., 2023). One of the characteristics that make these markers particularly interesting in genetic diversity studies is their high rate of transferability to closely related species (Gupta *et al*., 2003; Simko, 2009). Nevertheless, significantly low values of cross-transferability have been observed for genomic SSRs, which are known to be more polymorphic and located in less conserved regions of the genome (Peakall *et al*., 1998; Sourdille *et al*., 2001).

We selected and tested for transferability and polymorphism 23 expressed sequence tag -EST-SSRs originated from other leguminous species: *Glycine max*, *Medicago sativa*, *Medicago trunculata*, and *Phaseolus vulgaris* (Peakall *et al*., 1998; Yu *et al*., 2000; Gaitán-Solís *et al*., 2002; Julier *et al*., 2003; Gutierrez *et al*., 2005; Zhang *et al*., 2007). Previously, Demdoum *et al*. (2012) successfully cross-amplified 14 of these markers in *O. pyrenaica* Sennen, *O. argentea* Boiss. and *O. viciifolia* Scop., while the remaining nine markers were noted by Avcı *et al*. (2014) as polymorphic in 58 *Onobrychis* species from Turkish flora.

The main purpose of this study was to test the cross-genus transferability of several SSR markers into *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. and *O. montana* and provide a preliminary evaluation of their usefulness for assessing the genetic relationships between the two taxa.

Materials and Methods

Sampling and DNA extraction

Ten populations belonging to *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. and six populations of *O. montana* were sampled from the Alps and the Carpathians Mountains (Table 1). More details on the sampling strategy, on the populations and on the DNA extraction can be found in Băcilă *et al*. (2015).

Table 1. Sampled populations of *O. montana* and *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv.: taxon, numbering, population code, country of origin (Ro – Romania; Fr – France; Po – Poland; Sk – Slovakia; Mne – Montenegro), mountain range, sampling locality, geographic coordinates (partially reproduced from Băcilă *et al*., 2015)

Taxon	No	Population code	Country	Range	Locality/Massif	Coordinates (Longitude °E/ Latitude °N)
O. montana DC. subsp. *transsilvanica* (Simonk.) Jáv.	1	OTRM	Ro	SW Carpathians	Piatra Iorgovanului Peak, Retezat Mts.	45°16'55.96" 22°50'45.09"
	2	OTR	Ro	SW Carpathians	Piule Peak, Retezat Mts.	45°18'25.7" 22°54'31.4"
	3	OTM	Ro	SE Carpathians	Cearcănu Peak, Maramureşului Mts.	47°38'57.96" 24°49'54"
	4	OTCh	Ro	SE Carpathians	Toaca Peak, Ceahlău Mts.	46°59'35.3" 25°57'57.3"
	5	OTGH	Ro	SE Carpathians	Ocsem Peak, Giurgeu-Hăşmaş Mts.	46°40'41" 25°50'11"
	6	OTC	Ro	SE Carpathians	Zăganu Peak, Ciucaş Mts.	45°29'22" 25°58'39.1"
	7	OTPC	Ro	SE Carpathians	Piatra Craiului Mică Peak, Piatra Craiului Mts.	45°33'10.3" 25°15'47.6"
	8	OTB	Ro	SE Carpathians	Caraiman Peak, Bucegi Mts.	45°24'56.7" 25°29'51.71"
	9	OTBV	Ro	SE Carpathians	Postăvaru Peak, Bârsei Mts.	45°33'58.88" 25°33'02.22"
	10	OTF	Ro	SE Carpathians	Jgeabul Văros Peak, Făgăraş Mts.	45°36'20.82" 24°35'37.68"
O. montana	11	OMAC	Fr	Alps	Col d'Izoard, Cottian Alps	44°49'36" 6°43'48"
	12	OMA	Fr	Alps	Col du Lautaret, Dauphiné Alps	45°04'09.13" 6°24'05.23"
	13	OMJ	Fr	Alps	Colomby de Gex, Jura Mts.	46°19'38.75" 6°0'4.35"
	14	OMAD	Mne	Dinaric Alps	Durmitor, Dinaric Alps	43°06'26.75" 19°0.1'10.38"
	15	OMTW	Po	W Carpathians	Wawoz Krakow, High Tatras	49°10'27.24" 20°08'11.97"
	16	OMBT	Sk	W Carpathians	Saddle between Mt. Muran and Mt. Novy, Belianske Tatras	49°14'55" 20°11'00"

SSR fingerprinting

23 microsatellites (original code names: MtBA01B04R2, MtBA27D09F1, MtBB36F05F1, MtBA04C08R1, MtBB22G10F1, MtBC47B06F1, MtBB44F02R1, AG81, BI74, AL79, BG178, AL46, AW265, AW567861, PV-at001, BM141, MTIC326, MTIC272, MTIC230, MTIC21, BM175, BM152, BM137) developed by Peakall *et al*. (1998), Yu *et al*. (2000), Gaitán-Solís *et al*. (2002), Julier *et al.* (2003), Gutierrez *et al*. (2005), and Zhang *et al.* (2007) for other leguminous species were tested for transferability in *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. and *O. montana*.

Each primer pair had to be optimized, as poor amplification or unspecific bands were otherwise present. Following amplification and analysis of gel patterns, only seven SSR primer pairs were selected, fluorescently dyed (6-FAM) and used in subsequent reactions. For the amplification of these seven microsatellites, four different PCR programs were used in order to obtain a clear and reproductible amplification (Table 2).

Table 2. PCR programs used for SSR amplifications. **PVat001, MtBB22G10, MtBA27D09, AG81, BG178, BM141, and MTIC272** represent the original names of the markers (see also Table 3 for references)

PCR steps	PVat001	MtBB22G10	MtBA27D09, AG81	BG178, BM141, MTIC272
Initial denaturation	94 °C, 2 min	94 °C, 3 min	94 °C, 3 min	95 °C, 5 min
Denaturation	94 °C, 45 sec	94 °C, 45 sec	94 °C, 45 sec	94 °C, 30 sec
Annealing temperature	50 °C, 45 sec	50 °C, 1 min	51 °C, 1 min	50 °C, 30 sec
Elongation	72 °C, 1 min	72 °C, 1.5 min	72 °C, 1.5 min	72 °C, 1 min
Repet steps 2-4	35x	35x	40x	35x
Final elongation	72 °C, 5 min	72 °C, 10 min	72 °C, 10 min	72 °C, 10 min

The PCR products were purified with Sephadex - Sephacryl (1:1) (GE Healthcare Bio-Sciences AB, USA) and then diluted 50 times. 1.5 µL of dilution were added to 10 µL mix of HiDi formamide and GeneScan 500 ROX Size Standard (Applied Biosystems, Thermo Fisher Scientific, USA) and subjected to capillary electrophoresis on an ABI PRISM 3130 Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific, USA). The characteristics of the seven primer pairs are presented in Table 3.

Table 3. Characteristics of seven microsatellite loci used for cross-transferability in *Onobrychis* sp.

Locus	Primer sequence (5'-3')	Allele size range (bp)	Reference
MtBA27D09	F:GAAGAAGAAAAAGAGATAGATCTGTGG R: GGCAGGAACAGATCCTTGAA	100-326	Gutierrez *et al*., 2005
MtBB22G10	F: CCAGTGGCAGCTACGGTACTA R: GAGACGGAGGAGAAGTTGCTT	149-161	Gutierrez *et al*., 2005
AG81	F: ATTTTCCAACTCGAATTGACC R: TCATCAATCTCGACAAAGAATG	134-184	Peakall *et al*., 1998
BG178	F: ACCCACTCAACTCAACACACAC R: TTCTCCTTGACCAACCTTGATT	184-187	Zhang *et al*., 2007
PV-at001	F: GGGAGGGTAGGGAAGCAGTG R: GCGAACCACGTTCATGAATGA	157-266	Yu *et al*., 2000
BM141	F: TGAGGAGGAACAATGGTGGC R: CTCACAAACCACAACGCACC	103-487	Gaitán-Solís *et al*., 2000
MTIC272	F: AGGTGGATGGAGAGAGTCA R: TCATGAATAGTGGCACTCAA	132-210	Julier *et al*., 2003

Data analysis

Alleles scoring was performed with GeneMapper v.4.0 software (Applied Biosystems, Thermo Fisher Scientific, USA).

PowerMarker v.3.25 (Liu and Muse, 2005) was used to calculate the total number of alleles, gene diversity and polymorphism information content (PIC). Descriptive statistics as: number of alleles and observed [Ho] and expected heterozygosities [He], were estimated per population using GenAlEx 6.5 (Peakall and Smouse, 2006). A frequency matrix was generated and subsequently used within SplitsTree v.4.10 (Huson and Bryant, 2006) to compute Unweighted Pair Group Method with Arithmetic Mean (UPGMA)

phylogenetic tree based on the Shared Allele distance and the Neighbor-Net method. Bootstrap values were calculated from 1000 replicates.

Results

23 SSRs were tested in *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. and *O. montana* and consistent amplification was obtained for 18 of them (71.26%), while the rest provided multiple nonspecific bands. However, due to lack of polymorphism and low reproductibility, only seven SSR (Table 3) were selected for the subsequent characterization of the *Onobrychis* sp. populations. A total number of 32 alleles were detected, each SSR amplified 2–6 alleles, and the average number of alleles per SSR was 4.571. PIC values ranged from 0.375 to 0.6454, with an average of 0.5089 (Table 4). Only four SSRs (MtBA27D09, MtBB22G10, PV-at001, and MTIC272) displayed a PIC > 0.5, and therefore were considered informative. Relative uniform rates of genetic diversity were obtained, ranging from the lowest value of 0.5 (AG81, BG178, and BM141) to the highest value of 0.7 (MTIC272). The gene diversity and PIC values pointed out that MTIC272 represented the most informative locus in the two *Onobrychis* species analysed (Table 4).

Table 4. Number of alleles, PIC, and gene diversity values for seven SSR loci analysed in *Onobrychis* sp.

SSR locus	No. of alleles	Gene diversity	PIC
MtBA27D09	6	0.6500	0.5957
MtBB22G10	4	0.6618	0.6033
AG81	4	0.5000	0.3750
BG178	2	0.5000	0.3750
PV-at001	6	0.6486	0.5931
BM141	6	0.5000	0.3750
MTIC272	4	0.7000	0.6454
Mean	***4.571***	***0.5943***	***0.5089***

Ho and He ranged in case of *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. from 0.100 to 0.952, and from 0.219 to 0.525, respectively, while for *O. montana*, they ranged from 0.166 to 0.750 and from 0.083 to 0.375 (Table 5).

Table 5. Genetic characterization of seven polymorphic microsatellite loci tested across sixteen populations of *Onobrychis* sp. Ho = observed heterozygosity; He = expected heterozygosity.

Locus	*O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv.		*Onobrychis montana*	
	H_o	H_e	H_o	H_e
MtBA27D09	0.366	0.183	0.611	0.305
MtBB22G10	0.550	0.275	0.166	0.083
AG81	0.100	0.05	0.333	0.167
BG178	0.952	0.525	0.667	0.333
PV-at001	0.066	0.033	0.222	0.111
BM141	0.433	0.216	0.277	0.139
MTIC272	0.450	0.225	0.750	0.375

The UPGMA analysis (data not shown, manuscript in preparation) managed to clearly differentiate all the 16 populations of *Onobrychis*, exhibiting taxonomic and geographic delineation.

Discussion

The rate of SSR cross-genera transferability was 18 out of 23 tested markers (71.26%). This value was lower than 81%, as previously reported by Demdoum *et al*. (2012), but higher than other related data (Eujayl *et al*., 2004). The intra-genus amplification rate was considered to be around 50% (Peakall *et al*., 1998), but this value quickly declined inter-genera. Zhang *et al*. (2007) found 18-22% transferability from *Medicago* to *Trifolium*, while Peakall *et al*. (1998) reported only 1-3% transferability of *Glycine*'s SSR to other leguminous genera.

However, a narrow proportion of microsatellites was found to be polymorphic in *Onobrychis* (38.8% out of the 18 transferable SSRs). Several markers showed multiple bands that could not be eliminated by calibrating the PCR conditions. The generation of multiple products during cross-species amplification may occur by mutation, rearrangements and duplications in the flanking region and/or changes in the number of repeats (Peakall *et al*., 1998), similar results being reported by Gutierrez *et al*. (2005) in their study of EST-SSR in leguminous. Eventually, only seven SSR loci were selected on the base of polymorphism and reproducibility and they were subsequently used for characterization and genetic diversity evaluation of 16 populations of *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. and *O. montana*. These seven markers showed medium PIC values (average 0.5089) (Table 3). The number of alleles per locus ranged from 2 to 6 (Table 3), lower than previously reported by other studies (4-14) (Falahati-Anbaran *et al*., 2007). Since the studied *Onobrychis* species are diploid or tetraploid species (*O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. 2n=14, Löve, 1975; and respectively *O. montana* 2n=28; Löve, 1984), the number of detected alleles seemed to be low. A possible explanation is the PCR amplification bias, which could cause the loss of the less frequent alleles and predominant detection of the most common alleles, therefore leading to an under estimation of the number of alleles per loci in each population (Peakall *et al*., 1998). Although the level of polymorphism exhibited by the seven employed microsatellites was relatively low and only four of them (MtBA27D09, MtBB22G10, PV-at001, and MTIC272) were informative (PIC > 0.5), it was possible to differentiate all the analysed populations by taxonomic and even geographic criteria.

Conclusions

Within the studied group represented by 16 populations of *O. montana* DC. subsp. *transsilvanica* (Simonk.) Jáv. and *O. montana* the rate of SSR cross-genera transferability was 18 out of 23 tested markers (71.26%). Subsequently, only seven SSR loci were selected on the base of polymorphism and reproducibility. A total number of 32 alleles were detected, the average number of alleles per SSR being 4.571. Relative uniform rates for PIC and genetic diversity were obtained, pointing out that MTIC272 represented the most informative microsatellite. Although the level of polymorphism of the seven analysed microsatellites was relatively low, they managed to clearly differentiate all the analysed populations based on taxonomic and geographic criteria.

Authors' Contributions

The contributions of authors to the manuscript are as follows: conceptualization: IB, GC; field work: GC; data curation: IB, AC, ZRB, DȘ; formal analysis: IB and DȘ; funding acquisition: IB; investigation: IB; methodology: IB; project administration: IB; writing - original draft: IB; writing - review and editing: IB, AC, ZRB, GC and DȘ. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

We are grateful to Andreas Tribsch, Nadir Alvarez, Rolland Douzet, Zbigniew Mirek, Liviu Filipaş, Mihai Puşcaş, Adrian Ilie Stoica, and Tudor Ursu for their valuable help in collecting the plant material.

This work was financially supported by a grant from the Romanian National Authority for Scientific Research, CNDI–UEFISCDI, project number PN–II–RU–PD–2012–3–0005; 15/26.04.2013, as well as by the Core Project BIORESGREEN, subproject BioClimpact no. 7N/03.01.2023, code 23020401.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Avcı S, Ilhan E, Erayman M, Sancak C (2014). Analysis of *Onobrychis* genetic diversity using SSR markers from related legume species. Journal of Animal and Plant Sciences 24(2):556-566.

Băcilă I, Şuteu D, Coldea G (2015). Genetic divergence and phylogeography of the alpine plant taxon *Onobrychis transsilvanica* (Fabaceae). Botany 93:257-266. *https://doi.org/10.1139/cjb-2014-0175*

Borza A (1949). Conspectus florae Romaniae regionumque affinium, Vol. II. Cartea Românească Press, Cluj.

Ciocârlan V (2009). Flora ilustrată a României – Pteridophyta et Spermatophyta. Ceres Press, Bucharest.

Demdoum S, Muñoz F, Delgado I, Valderrábano J, Wünsch A (2012). EST-SSR cross-amplification and genetic similarity in *Onobrychis* genus. Genetic Resources and Crop Evolution 59:253-260. *https://doi.org/10.1007/s10722-011-9681-x*

Ellegren H (2004). Microsatellites: Simple sequences with complex evolution. Nature Reviews Genetics 5:435-445. *https://doi.org/10.1038/nrg1348*

Eujayl I, Sledge MK, Wang L, May GD, Chekhovskiy K, Zwonitzer JC, Mian MAR (2004) *Medicago truncatula* EST-SSRs reveal cross-species genetic markers for *Medicago* spp. Theoretical and Applied Genetics 108:414-422. *https://doi.org/10.1007/s00122-003-1450-6*

Falahati-Anbaran M, Habashi AA, Esfahany M, Mohammadi SA, Ghareyazie B (2007). Population genetic structure based on SSR markers in alfalfa (*Medicago sativa* L.) from various regions contiguous to the centres of origin of the species. Journal of Genetics 86(1):59-63. *https://doi.org/10.1007/s12041-007-0008-9*

Gaitán-Solís E, Duque MC, Edwards KJ, Tohme J (2002). Microsatellite repeats in common bean (*Phaseolus vulgaris*) isolation, characterization, and cross-species amplification in *Phaseolus* ssp. Crop Science 42(6):2128-2136. *https://doi.org/10.2135/cropsci2002.2128*

Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003). Transferable EST-SSR markers for the study of polymorphism and genetic diversity in bread wheat. Molecular Genetics and Genomics 270:315-323. *https://doi.org/10.1007/s00438-003-0921-4*

Gutierrez MV, Vaz Patto MC, Huguet T, Cubero JI, Moreno MT, Torres AM (2005). Cross-species amplification of *Medicago truncatula* microsatellites across three major pulse crops. Theoretical and Applied Genetics 110:1210-1217. *https://doi.org/10.1007/s00122-005-1951-6*

Huson DH, Bryant D (2006). Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23(2):254-267. *https://doi.org/10.1093/molbev/msj030*

Julier B, Flajoulot S, Barre P, Cardinet G, Santoni S, Huguet T, Huyghe C (2003). Construction of two genetic linkage maps in cultivated tetraploid alfalfa (*Medicago sativa*) using microsatellite and AFLP markers. BMC Plant Biology 3:1-19. *https://doi.org/10.1186/1471-2229-3-9*

Kumar GP, Pathania P, Goyal N, Gupta N, Parimalan R, Radhamani J, ... Rajkumar S (2023). Genetic diversity and population structure analysis to construct a core collection from safflower (*Carthamus tinctorius* L.) germplasm through SSR markers. Agriculture 13(4):836. *https://doi.org/10.3390/agriculture13040836*

Liu K, Muse SV (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21(9):2128-2129. *https://doi.org/10.1093/bioinformatics/bti282*

Löve Á (1975). IOPB chromosome number reports XLIX. Taxon 24(4):501-516. *https://doi.org/10.1002/j.1996-8175.1975.tb00341.x*

Löve Á (1984). Chromosome number reports LXXXII. Taxon 33(1):126-134. *https://doi.org/10.1002/j.1996-8175.1984.tb02474.x*

Mohsen HHS, Nasab MZ (2010). Cytotaxonomy of some *Onobrychis* (Fabaceae) species and populations in Iran. Caryologia 63(1):8-31. *https://doi.org/10.1080/00087114.2010.589705*

Nyárády A, Nyárády EI (1957). *Onobrychis*. In: Săvulescu T (Ed). Flora Republicii Populare Române, Vol. V. Academiei Republicii Populare Române Press, Bucharest pp 341-345.

Peakall R, Gilmore S, Keys W, Morgante M, Rafalski A (1998). Cross-species amplification of soybean (*Glycine max*) simple sequence repeats (SSRs) within the genus and other legume genera: implications for the transferability of SSRs in plants. Molecular Biology and Evolution 15(10):1275-1287. *https://doi.org/10.1093/oxfordjournals.molbev.a025856*

Peakall ROD, Smouse PE (2006). GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6(1):288-295. *https://doi.org/10.1093/bioinformatics/bts460*

POWO (2023). Plants of the World Online. Retrieved 2023 May 31 from: *http://www.plantsoftheworldonline.org/*

Sârbu I, Ştefan N, Oprea A (2013). Plante vasculare din România, Determinator ilustrat de teren. Victor B. Victor Press, Bucharest.

Simko I (2009). Development of EST-SSR markers for the study of population structure in lettuce (*Lactuca sativa* L.). Journal of Heredity 100(2):256-262. *https://doi.org/10.1093/jhered/esn072*

Sourdille P, Tavaud M, Charmet G, Bernard M (2001). Transferability of wheat microsatellites to diploid Triticeae species carrying the A., B and D genomes. Theoretical and Applied Genetics 103:346-352. *https://doi.org/10.1007/s00122-001-0542-4*

Yu K, Park SJ, Poysa V, Gepts P (2000). Integration of simple sequence repeat (SSR) markers into a molecular linkage map of common bean (*Phaseolus vulgaris* L.). Journal of Heredity 91(6):429-434. *https://doi.org/10.1093/jhered/91.6.429*

Zhang Y, Sledge MK, Bouton JH (2007). Genome mapping of white clover (*Trifolium repens* L.) and comparative analysis within the Trifolieae using cross-species SSR markers. Theoretical and Applied Genetics 114:1367-1378. *https://doi.org/10.1007/s00122-007-0523-3*

- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.