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APPLICABILITY OF MICROSATELLITE LOCI DESIGNED FOR COSMOPOLITAN SPECIES FOR THE INVESTIGATION OF ENDEMIC SPECIES: A CASE STUDY OF *SILENE SENDTNERI* BOISS.

MOGUĆNOST PRIMJENE MIKROSATELITNIH LOKUSA KREIRANIH ZA KOSMOPOLITSKE VRSTE ZA ISTRAŽIVANJE ENDEMSKIH VRSTA S POSEBNIM OSVRTOM NA *SILENE SENDTNERI* BOISS.

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Abstract

Silene sendtneri Boiss. (Caryophyllaceae) is the Dinaric endemic plant species with white, decorative and scented flowers. Previous studies on this endemic species were based on morphology and effects on seed germination after the treatment with salicylic acid. However, no molecular genetic studies have been conducted on this species so far. This paper presents preliminary results of the usefulness of microsatellite loci created for cosmopolitan species in assessing the genetic diversity of endemic plant species. A total of 100 specimens were collected from 18 localities in the mountain regions of Treskavica, Igman, Bjelašnica and Ozren in Bosnia and Herzegovina. No *S. sendtneri* individuals were found at the mountain Trebević. We tested cross-amplification success and a polymorphism level for the set of microsatellite markers (Sil01, Sil03, Sil16, Sil31, Sil35) designed for the cosmopolitan species *Silene nutans*. In 100 analyzed individuals of *S. sendtneri*, Sil31 and Sil35 did not amplify, Sil01 was monomorphic and the remaining two loci showed a high level of allelic diversity. Our findings suggest that caution should therefore be exercised in selecting microsatellite markers designed for cosmopolitan plant species in the analyses of endemic species of the same genus since different genetic factors affect the amplification success and polymorphism of the given loci. Attention should be given to the number of detected and effective alleles and their ratio, the success of locus amplification concerning the complete set of markers used, and the ratio of polymorphs to the total number of observed loci.

Keywords: cosmopolitan species, diversity, endemic species, microsatellite, *Silene sendtneri*

1. INTRODUCTION / UVOD

The genus *Silene* L. (Caryophyllaceae Juss.) includes approximately 700 species (Oxelman et al., 2001), about half of which occur in the Mediterranean area. Within this genus, there are both cosmopolitan species widespread and distributed on all continents and many islands, as well as endemic species that can only be found in a confined region. Some species of *Silene*, with a history of genetic and ecological studies dating back to Mendel and Darwin, have served

as important model plants (Bernasconi et al., 2009). *Silene sendtneri* Boiss. is the Dinaric endemic plant species, found only in Bosnia and Herzegovina, Serbia, Montenegro, and Macedonia, with locus classicus described in Bosnia (leg. Sendtner 0., Boissier P. E. 1867; Šilić, 1990). Previous studies on *S. sendtneri* were based on a large morphological variability of all plant parts (Slavnić, 1969), HPLC profiles (Zibareva et al., 2009) and the evaluation of different effects after the treatment with salicylic acid (Kukuljac et al., 2016).

Genetic diversity studies are primarily based on mitochondrial and nuclear markers such as mtDNA genes, microsatellites and SNPs. In most cases, it is necessary to use a number of diverse techniques to avoid mistaken interpretations and conclusions. The main goal of such an approach is to assess the genetic flow rate among populations to determine their fragmentation and/or isolation pattern in terms of further management. Furthermore, species natural distribution can be determined by using genetic markers for genetic structure, diversity and differentiation analysis (Arif et al., 2010). Changes in genetic diversity are common in a small population, and it is important to rapidly identify them by using molecular markers like microsatellites, single nucleotide polymorphisms (SNPs), or whole-genome fingerprinting. Microsatellites are two to 20 times more informative on a per-locus basis than SNPs and provide an appropriate information density at substantially lower costs than genome-based approaches (Arthofer et al., 2018). Godé et al. (2014) developed 24 microsatellite markers to analyze the population structure and mating system of *Silene nutans*, and tested them for cross-amplification in eight additional *Silene* species (i.e. *S. acaulis, S. italica, S. latifolia, S. noctiflora, S. otites, S. paradoxa*, *S. vulgaris, S. scouleri*). These markers exhibited a high level of amplification success which ranged from 7 to 19 loci, depending on the taxa. The authors also recorded a high level of polymorphism, with *S. italica* displaying the largest observed number of alleles across the tested loci. Thus, the availability of informative markers specific for a given genus/species facilitates paternity analyses, as well as fine-scale and large-scale population-genetic studies. However, the most adequate number of microsatellite loci required for a discriminative genetic diversity study cannot be universally set, since it depends on the origin of the markers used (species or genus-designed) and the investigated species (ploidy level, life scenario, etc.) (Pfeiffer et al., 2011). This study aimed to assess the applicable value of microsatellite loci created for cosmopolitan species on the endemic species *S. sendtneri* for which no previous genetic data exist.

2. MATERIAL AND METHODS / MATERIJAL I METODE

Plant material of 100 sampled individuals of *S. sendtneri* Boiss. was collected from 18 different localities in four mountain regions (Treskavica (19), Igman (39), Bjelašnica (10), and Ozren (32)) near Sarajevo during July and August 2015 (Fig.1). Field research at Trebević mountain resulted in no sampled individuals of *S. sendtneri*. Herbarium vouchers were deposited in the Institute for Genetic Engineering and Biotechnology, University of Sarajevo. The collected fresh plant material was stored in individual paper bags at -20˚C until total genomic DNA isolation was performed using CTAB protocol (Doyle & Doyle, 1987) that included minor modifications by adding vitamin C to the cell lysis buffer (0.2% of the total volume). Five microsatellite loci, Sil01, Sil03, Sil16, Sil31, and Sil35 (Tab. 1), were tested for PCR amplification according to Godé et al. (2014). The amplification was performed in 10 µl reactions containing 1 µl of template DNA, 1.5 mM $MgCl_2$, 1.5 x PCR buffer, 0.2 mM dNTPs, 0.1 µM of each primer, and 0.05 U/µl of True start Taq polymerase (Thermo Scientific). After the initial activation, thermal conditions were: 30 s denaturation at 94°C, 1 min annealing at 55°C, 1 min 15 s extension at 72°C in 32 cycles, followed by a final extension at 72°C for 10 min. The detection of SSR products was performed on ABI PRISM™ 3500 Genetic Analyzer (Applied Biosystems). GeneMapper Software ID v5 was used for allele scoring.

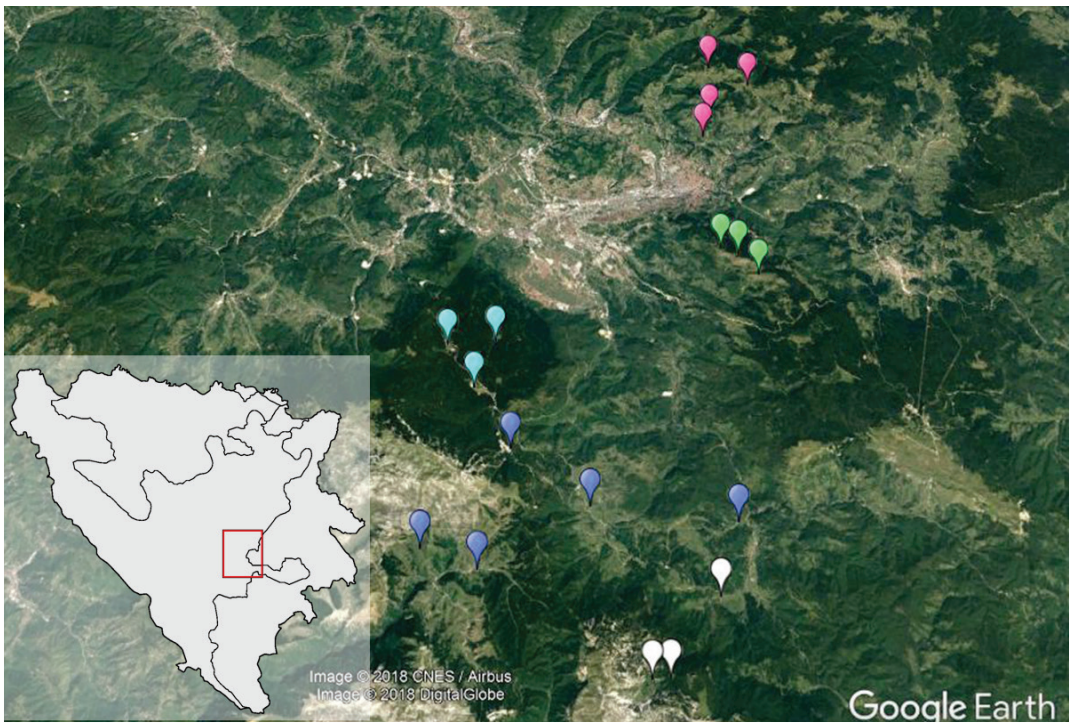

Figure 1. Sampling sites at four mountain regions: Ozren (purple marks); Trebević (light green marks); Igman (light blue marks); Bjelašnica (blue marks); Treskavica (white marks) based on Google Earth platform / **Slika 1**. Mjesta uzorkovanja na četiri planinska regiona: Ozren (ljubičasta oznaka); Trebević (svijetlozelena oznaka); Igman (svijetloplava oznaka); Bjelašnica (plava oznaka); Treskavica (bijela oznaka) prikazana na osnovi Google Earth platforme

Table 1. Name, primer sequence (5'- 3'), allelic size range (bp), and the source of five used microsatellite loci / **Tabela 1**. Naziv, prajmerska sekvenca, veličinski raspon alela i izvor pet korištenih mikrosatelitnih lokusa

Locus name	Primer sequences	Allelic size range	Source
Sil01	F: CATAAGGCAGCAAGTTTGGC R: GCCAATAAAATTCTGGTGATTAGG	167-209	Godé et al. (2014)
Sil03	F: AAGCTTCATCAAATGAAATCGG R: GGTGGAGGAGAAGACCACAG	208-214	Godé et al. (2014)
Sil16	F: GCCAAAACTAACAAGCAGCC R: TTTTGGGATTAAGGCTGTGA	121-147	Godé et al. (2014)
Sil31	F: TTGCCCTATTCTTTTACCCAA R: CGGACTTGTAAGGCCTGAAT	155-212	Godé et al. (2014)
Sil35	F: TCTGTGAATCTGTGATACTAACTGC R: ACCTCTATCCCACCATGTCA	98-140	Godé et al. (2014)

The differences between various measures of heterogeneity were observed in this study, as well as the differences in amplification and polymorphic ratio. The microsatellite-based population-genetic analysis included the assessment of observed (H_O) and expected (H_E) heterozygosity according to Nei (1987). These two parameters, as well as allelic diversity, the effective number of alleles and inbreeding coefficient (f), were calculated in GenAlex v6.5 (Peakall & Smouse, 2012). Polymorphic information content (PIC) was calculated in Powermarker v3.25 (Liu &

Muse, 2005). ALRATIO – R script (Pojskić, 2019) was used for calculating the ratio of the effective and detected number of alleles (Pojskic & Kalamujic, 2015) and its statistical significance. The considered level of statistical significance was p<0.01, following Pojskić (2019). Chi-squared proportion test was used for the comparison of amplification ratio (the number of successfully amplified relative to the observed number of loci) as well as a polymorphic ratio (the number of polymorphic relative to the observed number of loci) between *S. sendtneri* and other nine observed species of genus *Silene*. The above-mentioned test was calculated within MedCalc software v.16.4.3. After applying the Bonferroni correction, the considered level of statistical significance was p<0.01. However, Bonferroni correction was not applied for the comparison of average ratio values of the amplified and polymorphic loci, as this is the case of comparing two groups. Therefore, the level of statistical significance was considered to be p<0.05.

3. RESULTS AND DISCUSSION / REZULTATI I DISKUSIJA

A common limiting factor in the genetic analysis of plants is the extraction of good-quality DNA with a high yield (Abdel-Latif & Osman, 2017). Modification of CTAB protocol through the addition of vitamin C resulted in successful isolation of DNA from all 100 sampled individuals of *S. sendtneri* Boiss., even though it is known that species of this genus produce a high diversity of secondary metabolites with more than 450 isolated compounds (phytoecdysteroids, triterpene saponins, volatiles, terpenoids, and phenolics) (Mamadalieva et al., 2014).

Out of the microsatellites that Godé et al. (2014) designed for *Silene nutans*, five markers (Sil01, Sil03, Sil16, Sil31, and Sil35) for cross-amplification in S. *sendtneri* were tested to assess the species population diversity. These five loci were selected under the assumption that all loci would be amplified and polymorphic. However, the amplification was successful for only three SSR loci (Sil01, Sil03, and Sil16). The number of alleles detected by Godé et al. (2014) for five examined loci in nine other species from genus *Silene*, as well as the results of molecular genetic detection for *S. sendtneri,* are shown in Tab. 2.

Regarding *S. sendtneri*, the highest number of alleles was detected at Sil03 locus (15), while Godé et al. (2014) reported only four alleles at that locus for *S. nutans* (Tab. 2). Sil16 locus in both studies showed the same number of alleles (12). However, Sil01 locus was monomorphic in our study, while in *S. nutans* was highly polymorphic (18). The mean value of allelic diversity for three amplified loci in our study was 9.3333 (Tab. 3). Two loci that were not amplified successfully in *S. sendtneri*, namely Sil31 and Sil35, showed

Table 2. Results of cross-amplification of the five observed microsatellite loci in 9 *Silene* species from Godé et al. (2014) and *S. sendtneri* / **Tabela 2**. Rezultati unakrsne amplifikacije pet promatranih mikrosatelitnih lokusa kod 9 vrsta roda *Silene* prema Godé et al. (2014) i *S. sendtneri*

Locus	*Silene acaulis (4)*	*Silene italica (16)*	*Silene latifolia (8)*	*Silene noctiflora (1)*	*Silene otites (6)*	*Silene paradoxa (8)*	*Silene vulgaris (8)*	*Silene scouleri (2)*	*Silene nutans (36)*	*Silene sendtneri (100)*
Sil01	+ (2)	+(1)	-	+(1)	+(1)	+(1)	+(2)	+(1)	+(18)	+(1)
Sil03	+ (2)	+(1)	+(11)	+(1)	+(8)	+(1)	+(4)	+(1)	+(4)	+(15)
Sil16	+ (2)	+(2)	+(2)	+(2)	+(6)	+(4)	+(2)	+(2)	+(12)	+(12)
Sil31	+ (2)	+(1)	+(1)	+(1)	+(2)	+(3)	+(2)	+(2)	+(12)	-
Sil35	+ (2)	-	-	-	+(1)	+(5)	+(1)	+(1)	+(18)	-

Note / Napomena. No amplification (-) and successful amplification (+) with the observed number of alleles; the number of observed individuals for each species is given next to its name / Neuspješna (-) i uspješna amplifikacija (+) sa promatranim brojem alela; broj promatranih jedinki svake vrste dat je iza njenog imena

quite high degrees of polymorphism in *S. nutans* (12 and 18 alleles, respectively). Overall mean allelic diversity for all five loci in *S. nutans* was 12.8. Since Sil01 locus was monomorphic in our study, the expected heterozygosity was 0 and the inbreeding coefficient could not be calculated. Regarding this locus, Godé et al. (2014) noticed high expected heterozygosity (0.784), while the inbreeding coefficient showed that this locus was in outbreeding (-0.008) for *S. nutans*. The highest expected heterozygosity in our study was noticed at Sil03 locus (0.8849), while Sil16 showed heterozygosity lower than expected (0.6724). The inbreeding coefficient for both of these loci was quite high (Sil03 = 0.4395; Sil16 = 0.4093). Overall mean values of H_E and inbreeding for the three observed loci in our study were 0.5191 and 0.4265, respectively. The highest expected heterozygosity for *S. nutans* (Godé et al., 2014) was noticed for Sil35 locus (0.868), while the lowest was obtained for Sil03 locus (0.444). Expected heterozygosity for Sil16 and Sil31 was 0.715 and 0.586, respectively. Overall mean H_E for these five observed loci was 0.6794, and it was higher than the one obtained in our study. Among the remaining four loci, the lowest inbreeding coefficient was for Sil35 (0.067), which is further followed by Sil03 (0.114), Sil16 (0.223), and Sil31 (0.249). Overall mean inbreeding was much lower (0.129) than the one observed in our study (0.4265). Positive values of the inbreeding coefficient indicate the degree of kinship between individuals, which is associated with selfing in plants. The ratio of the effective and detected number of alleles assumes that values close to 1 mean that the higher number of detected alleles contribute to the genetic diversity of the given population. Therefore, such value points to a better distribution of allelic frequencies at a given locus. When we compared three amplified loci in *S. sendtneri*, as an endemic species, and in *S. nutans*, as a typical example of cosmopolitan species, we noticed that Sil03 locus in both species shows no significant deviation of the effective from the detected number of alleles (*S. nutans* – Ra = 0.45, P = 0.059; *S. sendtneri* – Ra = 0.579, P = 0.1139). For Sil16 locus in *S. sendtneri,* we noticed a distinctive deviation of effective (3.05) from the detected number of alleles (12), with a ratio of 0.254 and P = 0.0077. No signifi-

Table 3. Genetic diversity analysis of *S. sendtneri* Boiss. (this study) and *S. nutans* L. (Godé *et al*. (2014)) (N - number of individuals; A_N – allele diversity; A_E – effective number of alleles; Ra – allele ratio; p(Ra) – p-value of Ra; H_E – expected heterozygosity; H_O – observed heterozygosity; f – inbreeding coefficient; PIC - Polymorphic Information Content / **Tabela 3**. Analiza genetičkog diverziteta *S. sendtneri* Boiss. (ovaj rad) i *S. nutans* L. (Godé *et al*. (2014)) (N - broj jedinki; A_N – diverzitet alela; A_E – efektivni broj alela; Ra – odnos alela; p(Ra) – p-vrijednost Ra; H_E – očekivana heterozigotnost; H_O – promatrana heterozigotnost; f – koeficijent inbridinga; PIC - sadržaj polimorfne informacije

Marker	N	A_N	A_E	Ra	p(Ra)	H_E	H_O	f	PIC
S. sendtneri									
Sil01	100	1	1	1	/	0	0	/	0
Sil03	100	15	8.69	0.579	0.1139	0.8849	0.5000	0.4395	0.8738
Sil16	100	12	3.05	0.254	0.0077	0.6724	0.4000	0.4093	0.6422
Sil31	100	/	/	/	/	/	/	/	/
Sil35	100	/	/	/	/	/	/	/	/
Mean	100	9.3333	/	/	/	0.5191	0.3000	0.4265	0.5054
S. nutans (Godé et al. (2014))									
Sil01	36	18	4.63	0.257	0.0081	0.784	0.819	-0.008	
Sil03	36	4	1.8	0.45	0.059	0.444	0.375	0.114	
Sil16	36	12	3.51	0.292	0.0138	0.715	0.474	0.223	
Sil31	36	12	/	/	/	0.586	0.495	0.249	
Sil35	36	18	/	/	/	0.868	0.778	0.067	
Mean	36	12.8	/	/	/	0.679	0.588	0.129	

cant deviation of the effective from the detected number of alleles was observed (Ra = 0.292, P = 0.0138) for this same locus in *S. nutans*. Sil01 locus was monomorphic in the case of *S. sendtneri*, however, for *S. nutans* we noticed a statistically significant deviation of the effective (4.63) from the detected number of alleles (18). The allele ratio for this locus was 0.257 and P = 0.0081. In the case of an average number of alleles for these three loci in both species, a significantly lower number of the effective from the detected number of alleles was observed (*S. nutans* – Ra = 0.25, P = 0.0072; *S. sendtneri* – Ra = 0.223, P = 0.0041).

The proportion test, in regards to amplification success, showed that *S. sendtneri* has a statistically significant difference only to *S. nutans* (Tab. 4). Cross-utilization of markers in different species of one genus can be affected by the presence of substitutions or indels within the flanking regions (Moccia et al., 2009), as well as by the phylogenetic relatedness of analyzed species (e.g. *S. sendtneri* belongs to the section Graminiformes, while *S. nutans* is a part of the section Holopetalae). For polymorphic ratio, a statistically significant difference was observed only in regards to *S. nutans* (Tab. 4) as well. Considering the average values of amplified and polymorphic ratio for nine other *Silene* species and *S. sendtneri,* a statistical difference was noticed in terms that *S. sendtneri* had a lower number of successfully amplified loci ($\chi^2 = 23.755$, P< 0.0001) as well as a lower number of polymorphic loci ($\chi^2 = 6.076$, P = 0.0137).

The fact that only 60% of the investigated markers (i.e. Sil01, Sil03, Sil16, Sil31, and Sil35) designed for *S. nutans* successfully amplified in *S. sendtneri,* and 40% of them were polymorphic, raises a question concerning the applicable value of these loci in assessing the genetic diversity of this endemic species. Other observed species (*S. acaulis, S. italica, S. latifolia, S. noctiflora, S. otites, S. paradoxa, S. vulgaris,* and *S. scouleri*), in most cases, displayed the successful amplification at the same level as in *S. nutans* (Tab. 4). However, only *S. acaulis* had the same number of polymorphic loci as *S. nutans,* while *S. noctiflora* even showed a lower level of polymorphic rate than the one observed in *S. sendtneri*. Polymorphism of loci can be related directly to different genetic processes, such as selfing and inbreeding. Nonetheless, a monomorphic locus should not be automatically discarded as unusable since the absence of variation can be one of the indicators of different processes occurring within a population. Regarding the genus *Silene*, the amplification ratio can be a clear parameter of the applicability of microsatellite markers in assessing genetic diversity.

Table 4. Comparison of the ratio of amplified and the ratio of polymorphic loci between *S. sendtneri* and other 9 species from *Silene* genus / **Tabela 4**. Poređenje omjera amplificiranih i polimorfnih lokusa između *S. sendtneri* i ostalih 9 vrsta roda *Silene*

Species	A.R. (%)	χ^2	*p value*	P.R. (%)	χ^2	*p value*
S. sendtneri	60	-	-	40	-	-
S. acaulis	100	2.575	0.1086	100	5.618	0.0178
S. italica	80	2.340	0.1261	20	2.340	0.1261
S. latifolia	60	0.000	1.0000	40	0.000	1.0000
S. noctiflora	80	0.164	0.6858	20	0.164	0.6858
S. otites	100	3.818	0.0507	60	0.926	0.3358
S. paradoxa	100	5.035	0.0248	60	1.209	0.2715
S. vulgaris	100	5.035	0.0248	80	4.792	0.0286
S. scouleri	100	1.303	0.2536	40	0.000	1.0000
S. nutans	100	20.250	<0.0001	100	38.368	<0.0001

Note / Napomena. A.R. (%) – the ratio of amplified loci (%); P.R. (%) – the ratio of polymorphic loci (%); χ2 – Chi squared test / A.R. (%) – omjer amplificiraih lokusa (%); P.R. (%) – omjer polimorfnih lokusa (%); χ2 – Hi kvadratni test

In summary, in 100 analyzed individuals of *S. sendtneri*, two loci did not amplify, Sil01 was monomorphic and only two loci showed a high level of allelic diversity. Monomorphism of Sil01 locus was not surprising since it displayed the same pattern in *S. italica*, *S. noctiflora*, *S. otites*, *S. paradoxa*, and *S. scouleri*. This suggests that this locus can be considered as an indicator of genetic processes within a given population. However, the impossibility of amplification of two loci, with Sil31 locus being amplified within all other species, calls into question the validity of this microsatellite set concerning *S. sendtneri*, endemic species of the Dinaric region. Even though a high number of detected alleles at two loci for *S. nutans* and *S. sendtneri* was noticed, the effective number of alleles in the case of Sil01 for *S. nutans* and Sil16 for *S. sendtneri* showed a statistically significant lower number of alleles that contribute to genetic diversity. Only Sil03 locus for *S. sendtneri* had a high number of alleles contributing to the genetic diversity, in which case this locus could be considered adequate for diversity assessment.

These conclusions lead to the main question: What are the main indicators of adequacy, an amplification ratio (ratio of amplified loci in regards to the observed) or a polymorphic ratio (ratio of polymorphic loci in regards to the observed) for the given set of microsatellite loci? The absence of amplification of microsatellite loci is the most obvious hampering factor in the context of predicting genetic diversity. Studies on the transferability of plant microsatellites in the same genus reported an average success rate of about 70% between species (overview in Hernández-Espinosa et al., 2020). However, an increase in the number of observed microsatellite loci does not automatically guarantee a successful assessment (Guichoux, 2011; Rossetto, 2001). Monomorphic loci cannot be used as the valid input parameters for calculating different measures of heterogeneity (heterozygosity, number of effective alleles, etc). Nonetheless, monomorphic loci can still be informative since the fixation of alleles in a given population provides insight into the past or current genetic processes that might have caused the absence of more alleles (Nazareno & dos Reis, 2011).

The number of applicable loci, especially in plants, is one of the most commonly cited factors influencing the population genetic diversity assessment (Wang et al., 2021). Although some studies investigate the question of the lowest number of microsatellite loci required for the diversity assessment (Pfeiffer et al., 2011; Arthofer et al., 2018), that requirement cannot be met very often. This further raises the question of the validity of results. Assessing the Hardy-Weinberg equilibrium is not a sufficient indicator by itself (Roux, 1974). However, estimating the ratio of the effective and the detected number of alleles proposed by Pojskic & Kalamujic (2015) and Pojskić (2019), together with the number of alleles and expected heterozygosity, could be an indicator of the validity of the given microsatellite loci set. If the number of effective alleles is close to a detected number (ideal situation, Ra =1), then that microsatellite locus surely possesses a potential for assessing the genetic diversity of plant species population.

4. CONCLUSION / ZAKLJUČAK

To conclude, in analyses of plant endemic species such as *S. sendtneri*, caution should be exercised when selecting microsatellite markers designed for cosmopolitan species of the same genus, since different genetic factors affect the amplification success and polymorphism of the given loci. When determining the applicability for endemic species, attention should be given to the number of detected and effective alleles and their ratio, the success of locus amplification for the complete set of markers used, and the ratio of polymorphs to the total number of observed loci. In particular, selfing should be taken into account as one of the processes that increase inbreeding, and thus have the effect of reducing variation in the population. These analyses should be carried out before assessing the heterogeneity and interpopulation relationships of a given species.

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Sažetak

Silene sendtneri Boiss. (Caryophyllaceae) je dinarska endemska vrsta s bijelim, ukrasnim, mirisnim cvjetovima čiji *locus classicus* je opisan u Bosni (Šilić, 1990). Prethodne studije zasnivale su se na istraživanju morfološke varijabilnosti i efektima klijanja sjemena nakon tretiranja salicilnom kiselinom (Slavnić, 1969; Kukuljac et al., 2016). Do sada, ova vrsta nije bila istraživana sa molekularno-genetičkog aspekta.

Mikrosateliti su vrlo korisan molekularni marker u procjeni genetičkog diverziteta, dva do 20 puta informativniji od SNP-ova (Arthofer i sur., 2018). Prema istraživanju koje su sproveli Godé i sur. (2014), razvijena su 24 mikrosatelitna markera za vrstu *Silene nutans* koji su testirani u unakrsnoj amplifikaciji kod dodatno osam vrsta. Cilj naše studije bio je procijeniti primjenjivu vrijednost mikrosatelitskih lokusa stvorenih za kosmopolitske vrste na endemskim vrstama kao što je *S. sendtneri,* za koju prethodno ne postoje molekularni podaci.

Biljni materijal je prikupljen na 18 lokaliteta. Iako je najčešći ograničavajući faktor ekstrakcija visokokvalitetne DNK uslijed prisustva brojnih sekundarnih metabolita, izmjena standardnog CTAB protokola dodavanjem vitamina C u pufer za lizu rezultirala je uspješnom ekstrakcijom 100 analiziranih uzoraka. Testirana je amplifikacija pet mikrosatelitnih lokusa, odabranih pod pretpostavkom da će biti amplificirani i polimorfni. Iako je amplifikacija bila uspješna u tri SSR lokusa, za lokus Sil03 otkriveno je 15 alela dok je prema studiji Godé i sur. (2014) prijavljeno četiri. U obje studije, lokus Sil16 je pokazao po 12 alela. Lokus Sil01, u našoj studiji je pokazao monomorfnost, dok je kod vrste *S. nutans* bio visoko polimorfan (18). Dva testirana lokusa, Sil31 i Sil35, koji se nisu amplificirali, pokazivali su izuzetan polimorfizam kod vrste *S. nutans* sa 12, odnosno, 18 alela. Stoga, ovaj rad predstavlja preliminarne rezultate upotrebljivosti mikrosatelitskih lokusa stvorenih za kozmopolitske vrste u procjeni genetičke raznolikosti endemskih biljnih vrsta.

Ključne riječi: diverzitet, endemične vrste, kosmopolitske vrste, mikrosateliti, *Silene sendtneri*