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HIGH LEVELS OF GENETIC VARIATION AS DETECTED BY AFLP IN *Sideritis tmolea* FROM WESTERN TURKEY

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ABSTRACT

The objective of this study was to evaluate the level of genetic diversity among 29 *S. tmolea* genotypes collected from Bozdag, Turkey, using the Amplified Fragment Length Polymorphism (AFLP) technique. A total of 392 AFLP markers were detected using 14 primer combinations. The number of polymorphic bands per AFLP primer combination ranged from 16 to 42, with an average of 28. The Polymorphism Information Content (PIC) values varied from 0.25 (M-CTC/E-AGC) to 0.72 (M-CAC/E-AAG) among 14 selective primers. The genetic dissimilarity that was detected using the NTSYS-PC software ranged from 0.09 to 0.82. According to the dissimilarity results, a high level of genetic diversity existed among the studied genotypes. A model-based structural analysis revealed the presence of 2 populations. The defined population structure was helpful when studying the *S. tmolea* genotypes for diversity and classification.

Keywords: AFLP, genetic diversity, population structure, *Sideritis tmolea*

INTRODUCTION

Sivri çay *(Sideritis tmolea* L.) belongs to the Lamioideae (Stachyoideae) subfamily (Ryding, 1994; Hickey and King, 1997). Although taxa belonging to Lamiaceae Lindl. (Labiatae Juss.) are spread over a large area in habitat ranging from the North Pole to the Himalayas and from South eastern Asia to Hawaii, Australia, Africa and America, they are mainly distributed in the Mediterranean basin (Heywood, 1996). This family, which has 224 types and almost 5600 species, is divided into eight subfamilies according to Cantinoya. *Sideritis* L. is distributed especially in Mediterranean basin, is represented by more than 150 species in a wide area from the Bahamas to China and from Germany to Morocco and is divided into two subgenera. While Lamiaceae in the flora of Turkey is represented by 565 genera and 735 taxa (Guner et al., 2000), *Sideritis* is represented by 3 sections: Hesiodia (Moench) Bentham, Burgsdorfia (Moench) Briquet and Empedoclia (Rafin) Bentham. In Turkey, according to Duman (2013), *Sideritis* is represented by 53 taxa belonging to 45 species. While 40 of these taxa are endemic, the endemism ratio is approximately 75%. In Turkey, there are 31 *Sideritis* taxa, of which 25 are endemic and used in domestic and foreign trade, as well as *S. sipylea*, *S. tmolea* and *S. trojona*, which are endemic and among the first 50 species that are in the danger of extinction due to unconscious collecting (Ozhatay et al., 1998).

There are a number of studies of the pharmaceutical compounds in *Sideritis* species and these studies especially have focused on essential oils, diterpenes and flavonoids (Akcos et al., 1999; Kirimer et al., 2001; Topcu et al., 2002). Various anti-inflammatory, analgesic, diuretical, antiulcer, antidepressant, antimicrobial and insect repellent effects of *Sideritis* species that are grown in Turkey have been studied (Ozturk et al., 1996; Akcos et al., 1999; Bondi et al., 2000). *Sideritis* species have an important place among other plants both as an herbal tea and household remedy. While *Sideritis* species are known under various names in Anatolia, they are usually referred to as "Mountain Tea", "Plateau Tea" and "Sage". *S. tmolea* is known as "Acute Tea".

An analysis of genetic variation between individuals within a species or between different species or populations is useful in identifying the level of genetic variation (Brummer et al., 1995). These diversity studies provide useful information for understanding the genetic bases of various gene pools, for describing and conserving their germplasm and for correctly identifying the varieties in facilitating the selection of sources for new genes for improved yield and quality improvements (Cho et al., 2008). Traditionally, morphological traits, karyotypes and protein and isozyme markers have been widely used to

assess genetic diversity (Mudibu et al., 2011). Therefore, the measurement of genetic variation based on morphological characteristics is time-consuming and incoherent and requires extensive field trials and evaluation (Astarini et al., 2004). Different DNA techniques (RFLP, RAPD, AFLP, SSR, CAPS, ISSR and SNP) that are not affected by the environment (Maciel et al., 2003) are necessary for genetic screening among categories of closely related species in germplasm banks (Lapitan et al., 2007). The major applications of DNA markers for plant genetics include the assessment of phylogeny in selecting suitable parents for the generation of heterosis (Biton et al., 2012). Thus, several researchers have developed a wide range of molecular markers that have been used in several genetic diversity studies (Shimomura and Hirashima, 2006; Martins-Lopes et al., 2008; Cui et al., 2010). Among the different molecular marker approaches, AFLP is one of the most widely used in various plant species with different degrees of relatedness (Witkowicz et al., 2003). AFLP is suitable for studying diversity. The AFLP technique is based on the amplification of short restriction endonuclease-digested genomic DNA fragments onto which adaptors have been ligated at both ends using Polymerase Chain reaction (PCR) (Saiki et al., 1988). The presence or absence of these selective nucleotides in the genomic fragments being amplified provides the polymorphism. Alternately, the AFLP technique relies on a system of dominant markers that allow for the simultaneous analysis of a large number of markers in the genome. This method is highly reproducible and can be used to survey the overall genetic differences in the nuclear genome in a single assay without the need for primary sequence knowledge to design primers (Vuylsteke et al., 1999).

The objective of this research was to investigate the level of molecular diversity and phylogenetic relationships at the AFLP marker level among epidemic *Sideritis* that were collected from Bozdag.

MATERIALS AND METHODS

The collected S. tmolea species

The material of the current study is *S. tmolea* P.H. Davis by Lamiaceae. *S. tmolea* is distributed on the West side of Bozdag and on the slopes of the North and Northwest sides of Kangal Mount, which is a part of Bozdağlar at an altitude of 1450-2100 m. DNA samples were collected from the Bozdag Mountains; a population of individuals from different points was sampled in at least 10 - m intervals and GPS data for all of the samples was recorded (Table 1).

Table 1. Geographic distribution of *S. tmolea* landraces sampled in this study.

Genotype number	Altitude (m*)	GPS*	Genotype number	Altitude (m*)	GPS*
1	1654	38º16' 06" N* 028º04'52" E*	**16**	1564	38º201' 14" N 028º07'22" E
2	1695	38º19' 12" N 028º04'58" E	**17**	1615	38º20' 12" N 028º07'21" E
3	1747	38º19' 16" N 028º04'58" E	**18**	1566	38º20' 47" N 028º08'27" E
4	1651	38º19' 44" N 028º06'53" E	**19**	1566	38º20' 47" N 028º08'27" E
5	1703	38º19' 37" N 028º06'49" E	**20**	1555	38º20' 49" N 028º08'27" E
6	1857	38º19' 29" N 028º06'42" E	**21**	1980	38º19' 27" N 028º05'39" E
7	1967	38º19' 26" N 028º06'23" E	**22**	1960	38º01' 24" N 028º05'36" E
8	1967	38º19' 26" N 028º06'23" E	**23**	1960	38º01' 24" N 028º05'36" E
9	1709	38º19' 48" N 028º06'30" E	**24**	1920	38º19' 23" N 028º05'29" E
10	1669	38º19' 40" N 028º06'32" E	**25**	1920	38º19' 23" N 028º05'29" E
11	1597	38º20' 34" N 028º06'34" E	**26**	1786	38º19' 46" N 028º07'20" E
12	1597	38º20' 34" N 028º06'34" E	**27**	1751	38º19' 48" N 028º07'17" E
13	1641	38º20' 59" N 028º05'47" E	**28**	1664	38º19' 49" N 028º07'12" E
14	1534	38º21' 19" N 028º05'39" E	**29**	1588	38º19' 52" N 028º07'60" E
15	1534	38º21' 19" N 028º05'39" E			

*N: North, E: East, m: meter, GPS: Global Positioning System

DNA isolation

The young leaves from *Sideritis* were ground to a fine powder with liquid nitrogen in a TissueLyser (Technogen Co. Izmir Turkey). The total genomic DNA was isolated according to the protocol of Doyle and Doyle (1987) with some modifications. The purified DNA was resuspended in 100 µL of 10 mM Tris-HCl (pH 8.0) buffer containing 1 mM EDTA for use in the analysis. The DNA concentration of the sample was determined spectrophotometrically by measuring the absorbance at 260 and 280 nm using a NanoDrop ND-1000 (Thermo Sci. Co) spectrophotometer and the quality was checked on 1% agarose gels. The stock DNA was diluted to a working solution of 200 ng/µL and the samples were stored at -20 °C for the PCR reactions.

AFLP analysis

An amplified fragment length polymorphism analysis was performed using the Li-Cor AFLP Kit (catalog number: 830-06195 AFLP2-DYE Selective Amplification Kit, Lincoln, NE, USA). followed the manufacturer's instructions. According to the kit, genomic DNA (200 ng/µL) was digested with the *Eco*RI and *Mse*I restriction enzymes at 37 °C for 2 h followed by 15 min at 70 °C. The digested DNA fragments were then ligated with specific enzyme adaptors in the presence of T4 DNA ligase. A ligation reaction was performed at 37 °C for 3 h. Pre-amplification PCR was performed after diluting the ligated DNA ten-fold with double deionized water and then pre-amplifying using *Eco*RI and *Mse*I non-selective primers with one additional selective nucleotide at the 3´ end of the *Mse*I primer (*Mse*I + C) and the *Eco*RI primer (*Eco*RI + A). The primer combinations were named according to the restriction enzyme initials, such as M-CAC/E-GGA: "M" stands for *Mse*I, "CAC" stands for the nucleotide extensions and "E" stands for the *Eco*RI enzyme. After the pre-selective PCR, the reaction mixture was diluted 1:40 and used as a template for selective amplification. Selective amplifications were performed with the selective primer combinations of *Eco*RI (labeled with two different fluorescent dyes (IRD 700 or 800) at the 5´ ends) and *Mse*I (unlabeled) with three selective nucleotides. Fourteen primer combinations were used to screen for polymorphisms among the samples (Table 2). The AFLP products were separated electrophoretically in denaturing 8% polyacrylamide gels (19:1 ratio acrylamide:bisacrylamide, 7,5 M urea and 1X TBE buffer) at 45 W constant power for approximately 4 h 30 min and run on a Li-Cor 4300s DNA Analyzer. The size of the products was determined using the Li-Cor® IRDye® as an internal size standard (50 to 700 bp).The imaging of the AFLP fragments was performed using the SAGA software.

Table 2. Number of polymorphic bands and PIC for each AFLP primer pair that was used in the analysis of the 29 *S. tmolea* genotypes

Primer Number	Primer Pairs	Number of polymorphic bands	PIC
1	M-CAA / E-AAG	19	0.54
2	M-CAA / E-ACT	20	0.56
3	M-CAC / E-AAG	19	0.72
4	M-CAC / E-ACT	23	0.57
5	M-CTT / E-AAC	31	0.41
6	M-CTT / E-AGG	29	0.34
7	M-CTC / E-ACA	23	0.42
8	M-CTC / E-AGC	16	0.25
9	M-CTC / E-AAG	27	0.43
10	M-CTC / E-ACT	22	0.39
11	M-CTG / E-ACA	40	0.56
12	M-CTG / E-AGC	41	0.52
13	M-CTG / E-AAG	42	0.64
14	M-CTG / E-ACT	40	0.48
	TOTAL	**392**	
	AVERAGE	**28**	**0.49**

Data analysis

The AFLP profiles were assessed individually for each primer pair combination. The polymorphic AFLP bands were manually scored as a binary data matrix with presence as "1" and absence as "0" across all of the 29 genotypes. Only the clear and strong bands were recorded and used for the analysis. Genetic dissimilarity estimates were calculated between the genotypes using Jaccard's coefficient of dissimilarity. The calculations and analyses were generated with the help of NTSYS-PC software version 2.02 (Rohlf, 1998). The PIC of each marker was calculated using PIC = 1 - Σ pi^2, where P_i is the band frequency of the i^{th} allele (Smith et al., 1997).

Assessment of population structure with AFLP

All of the AFLP markers that were developed in this study were used to infer the population structure in the set of 29 genotypes. For the analysis of population structure, we used the STRUCTURE version 2.3.4 software, which implements a Bayesian, model-based clustering algorithm (Pritchard et al., 2000). The individuals in the sample are

assigned to populations (clusters) or jointly to additional populations if their genotypes indicate that the populations are admixed. The optimal number of populations (K) was determined under a burn-in period of 100,000 with 100,000 Markov Chain Monte Carlo iterations. The membership of each genotype was run for the range of genetic clusters from value of K = 1 to 10 with the admixture model and for each K, the run was replicated 10 times. For each K value, the runs showing the highest posterior probability of data were considered. The true value of K and the number of populations were detected by an ad hoc quantity based on the second order rate of change of the likelihood function with respect to K (ΔK) (Evanno et al., 2005).

RESULTS AND DISCUSSION

In the current study, we detected the genetic diversity and phylogenetic relationships of *S. tmolea* at the AFLP marker level. The size range between the smallest and largest alleles that were observed for a given AFLP varied from 50 to 500 bp, which was able to discriminate between the 29 *Sideritis* genotypes. Similar results were observed by Murtaza (2006) and Strikic et al. (2010), who found band sizes ranging from 50 to 500 bp using cotton and olive genotypes, respectively. AFLP marker systems were highly effective in discriminating the 29 *S. tmolea* genotypes that were analyzed. The total number of assays included 14 primer combinations for AFLP, as listed in Table 2. In total, the 14 selective primer combinations amplified 392 polymorphic products for the 29 analyzed genotypes (Table 2). Previously, AFLP marker systems were used to characterize various genotypes (Maras et al., 2008; Strikic et al., 2010; Nemli, 2013; Panahi et al., 2013). For example, Grati-Kamoun et al. (2006) found 172 polymorphic bands from 9 primer combinations in olive genotypes. Khalighi et al. (2008) obtained 387 bands from 14 primer combinations. Colomba and Gregorini (2011) studied durum wheat using 8 AFLP primer pairs that generated 137 amplification products and 17 polymorphic bands per primer combination. Farah Fazwa et al. (2013) detected a total of 170 AFLP fragments among 62 accessions for two varieties of *L. pumila* (*L. pumila* var. *pumila* and *L. pumila* var. *alata*). From the studies that are mentioned above, the authors concluded that AFLP was a very sensitive technique for detecting markers for genetic studies. Based on the results of our study, 14 AFLP primer pairs amplified in the 29 *S. tmolea* genotypes from Turkey, the number of polymorphic bands was higher than that of previous studies (Colomba and Gregorini, 2011; Farah Fazwa et al., 2013). These findings indicate a high level of genetic variation among the studied genotypes. The number of polymorphic bands per AFLP primer combination ranged from 16 (M-CTC / E-AGC) to 42 (M-CTG / E-AAG). On average, 28 polymorphic bands were amplified by each primer combination (Table 2). These results are similar to those of this study in terms of the average number of scored polymorphic bands. Khalighi et al. (2008) found the average number of 27 in *Triticum* spp. and *Aegilops* spp. and Zhang et al. (2012) reported 31 polymorphic bands per primer combination from their AFLP study in *Brachypodium* species.

The PIC is defined as the probability that two alleles that are taken at random from a population can be distinguished using the marker in question and is a measure of allele diversity at a locus. The success of AFLP markers in a variety of genotypes that were corrected with the PIC value indicates the genetic variation among the genotypes (Muse et al., 2005). The PIC was calculated to identify the most informative AFLP primers. The PIC value ranged from 0,25 (M-CTC / E-AGC) to 0,72 (M-CAC / E-AAG) among the 14 selective primers. The average PIC was calculated as 0,49, demonstrating the good discriminatory power of the identified markers (Table 2). Furthermore, except for M-CTC/E-AGC (PIC value: 0,25), all of the AFLP combinations were highly informative, with an average PIC of 0.6 (Table 2). However, higher mean PIC values were obtained by AFLP markers compared to those of other studies, such as Yuan et al. (2011) (0,33) and Zhang et al. (2012). Pecina-Quintero et al. (2012) reported a mean PIC value of 0,294 with 10 primer combinations using 41 genotypes of the sweet sorghum germplasm. The same AFLP markers were used by Pecina-Quintero et al. (2012) to produce a lower PIC value (0,27) for the M-CAC/E-AAG and M-CTG/E-ACA primer combinations. However, in this study, the PIC values were 0,72 and 0,56 for the M-CAC/E-AAG and M-CTG/E-ACA primer combinations, respectively. Therefore, the AFLP combinations that were used in this study have a high discriminatory power and are recommended for use in genetic diversity analyses of *S. tmolea*.

Understanding the genetic similarity/dissimilarity among genotypes is crucial in germplasm collection and also facilitates the thorough utility of these genetic resources in crop improvement programs (Farah Fazwa et al., 2013). The genetic distance of the improved *Sideritis* genotypes is shown in Table 3. The 0/1 matrix was used to calculate the distance among the 29 *Sideritis* genotypes according to Jaccard (1908). The matrix of genetic dissimilarity ranged from 0,09 to 0,82. Among these values, the highest degree of dissimilarity (0,82) was found between genotypes 13 and 23, indicating that these genotypes were the most distinct from each other. The lowest degree of dissimilarity (0,09) that was revealed by the AFLP analysis was found between genotypes 27 and 28. In this study, a higher AFLP polymorphism was observed among the genotypes than in several previous reports using AFLP markers. The genetic distance values demonstrated a large genetic variation among the studied genotypes in a narrow geographic region. A high level of genetic variation was found among the genotypes that may be attributed to habitat fragmentation due to anthropopression, geographic isolation and genetic drift (Qian et al., 2013). Similarly, Cinar et al. (2009) used 8 *Sideritis* species for genotyping and reported that the genetic distance varied from 0,284 (between *Sideritis arguta* 1370 and *Sideritis arguta* 778) to 0,903 (between

Table 3. Genetic dissimilarity coefficient matrices of the 29 genotypes of *S. tmolea* using AFLP markers (Jaccard's coefficient)

Genotype number	01	02	03	04	05.	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
01	0.00																												
02	0.29	0.00																											
03	0.40	0.23	0.00																										
04	0.48	0.59	0.56	0.00																									
05	0.33	0.49	0.49	0.36	0.00																								
06	0.33	0.24	0.26	0.46	0.45	0.00																							
07	0.31	0.24	0.30	0.49	0.44	0.23	0.00																						
08	0.55	0.50	0.45	0.52	0.42	0.43	0.45	0.00																					
09	0.40	0.35	0.39	0.48	0.36	0.36	0.40	0.41	0.00																				
10	0.43	0.47	0.56	0.46	0.45	0.47	0.44	0.56	0.36	0.00																			
11	0.52	0.33	0.39	0.71	0.62	0.41	0.43	0.51	0.34	0.44	0.00																		
12	0.45	0.30	0.35	0.67	0.55	0.35	0.37	0.51	0.32	0.37	0.15	0.00																	
13	0.56	0.37	0.39	0.71	0.62	0.41	0.44	0.56	0.39	0.45	0.11	0.18	0.00																
14	0.56	0.40	0.41	0.71	0.63	0.43	0.45	0.56	0.39	0.47	0.14	0.21	0.11	0.00															
15	0.41	0.45	0.47	0.60	0.50	0.44	0.44	0.51	0.35	0.33	0.31	0.29	0.34	0.34	0.00														
16	0.51	0.34	0.40	0.73	0.64	0.44	0.40	0.44	0.44	0.45	0.16	0.21	0.18	0.22	0.32	0.00													
17	0.47	0.37	0.43	0.69	0.58	0.43	0.44	0.53	0.41	0.45	0.16	0.20	0.18	0.18	0.31	0.13	0.00												
18	0.40	0.49	0.52	0.47	0.49	0.47	0.47	0.52	0.41	0.34	0.47	0.42	0.50	0.50	0.37	0.50	0.47	0.00											
19	0.40	0.51	0.52	0.48	0.45	0.52	0.49	0.49	0.39	0.41	0.46	0.38	0.47	0.48	0.35	0.47	0.47	0.32	0.00										
20	0.50	0.58	0.63	0.56	0.58	0.61	0.52	0.65	0.52	0.44	0.53	0.46	0.55	0.52	0.42	0.57	0.52	0.38	0.37	0.00									
21	0.49	0.68	0.72	0.45	0.44	0.64	0.57	0.58	0.60	0.49	0.79	0.66	0.81	0.80	0.54	0.77	0.72	0.49	0.38	0.44	0.00								
22	0.41	0.65	0.68	0.44	0.42	0.65	0.60	0.56	0.48	0.45	0.73	0.65	0.79	0.80	0.52	0.76	0.68	0.43	0.38	0.42	0.32	0.00							
23	0.61	0.82	0.79	0.49	0.57	0.81	0.73	0.66	0.60	0.52	0.80	0.68	0.83	0.79	0.54	0.83	0.80	0.48	0.43	0.43	0.38	0.33	0.00						
24	0.45	0.47	0.47	0.56	0.51	0.49	0.45	0.62	0.43	0.42	0.39	0.38	0.42	0.44	0.40	0.45	0.41	0.43	0.44	0.42	0.50	0.45	0.56	0.00					
25	0.49	0.42	0.49	0.65	0.56	0.44	0.41	0.55	0.42	0.41	0.31	0.32	0.33	0.35	0.33	0.28	0.28	0.41	0.45	0.51	0.63	0.60	0.71	0.29	0.00				
26	0.52	0.73	0.71	0.40	0.46	0.67	0.60	0.45	0.52	0.43	0.72	0.63	0.77	0.76	0.47	0.72	0.71	0.45	0.36	0.43	0.37	0.35	0.33	0.49	0.60	0.00			
27	0.51	0.42	0.43	0.75	0.62	0.45	0.49	0.58	0.45	0.47	0.24	0.28	0.21	0.22	0.38	0.26	0.23	0.45	0.49	0.55	0.73	0.71	0.79	0.40	0.28	0.74	0.00		
28	0.52	0.38	0.43	0.75	0.61	0.46	0.49	0.52	0.47	0.48	0.25	0.30	0.25	0.26	0.41	0.28	0.26	0.48	0.49	0.57	0.73	0.72	0.81	0.46	0.33	0.70	0.10	0.00	
29	0.48	0.42	0.49	0.52	0.52	0.45	0.44	0.57	0.40	0.37	0.34	0.32	0.37	0.35	0.34	0.41	0.33	0.38	0.41	0.42	0.59	0.55	0.61	0.38	0.38	0.53	0.31	0.33	0.00

Sideritis perfoliata 20 and *Sideritis perfoliata* 47). These results indicate a high degree of genetic diversity among different species. We calculated a high degree of genetic diversity within *S. tmolea* samples that were collected in a narrow area of existing endemic species. Furthermore, the reproductive system directly influences the degree and distribution of genetic variation and can lead to reproductive isolation and eventually to speciation (Gonza´lez-Pe´rez et al., 2008). The level of total heterozygosity in outcrossing plants was higher than that from comparable analyses of self-pollinating plants (Nybom and Bartish, 2000). The high genetic diversity may be an indication that the number of individuals of this species was larger than previously studied (Prohens et al., 2007). The species in the genus *Sideritis* are distributed throughout the world and the distribution of these species could have once been much wider than it is currently, which could explain the high levels of genetic variation that were detected in this endemic species.

Twenty-nine *Sideritis* genotypes were analyzed in order to determine the population structure using the STRUCTURE. After running the K value, the curve peaked at ΔK = 2 for the final analysis (Figure 1), indicating that the 2 population, namely Population 1 (red) and Population 2 (green), were the most capable of explaining the 29 *Sideritis* genotypes. Population 1 consists of 16 accessions (genotypes 1, 4, 5, 6, 7, 8, 9, 10, 18, 19, 20, 21, 22, 23, 24 and 26) showed admixture levels at 1555, 1703, 1857 and 1980 m. The remaining 13 accessions from seemingly similar levels, ranging from 1500-1700 (m) except for genotype 25 (1920 m), were classified as Population 2 by the STRUCTURE version 2.3.4 software (Figure 1). The genotypes collected from same location did not form into one cluster. The differences among the genotypes may be explained by the assessment of certain morphological characteristics.

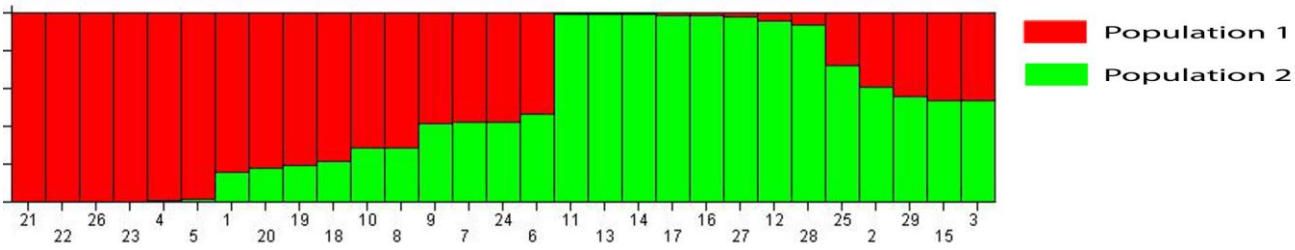

Figure 1. Bar plot showing the genetic diversity structure for the 29 *S. tmolea* genotypes using the program STRUCTURE version 2.3.4. Each population is represented by a different color as listed: red (POP1) and green (POP2).

In conclusion, the genus *Sideritis* provides a wide range of research possibilities due to its richness in compounds. This study is the first report of the use of the AFLP technique to evaluate the genetic relationships among *S. tmolea* genotypes and provides information on the population structure. A wide degree of genetic diversity is important for the characterization and development of improved varieties. Some *Sideritis* species are very difficult to distinguish morphologically due to lack of ecotype variation; therefore, this study demonstrates that the AFLP technique could play an important role in the identification of closely related taxa in *Sideritis* for future germplasm collection efforts in a cost-effective way. These preliminary results suggest that AFLP can be used in marker-assisted parental selection for future genetic diversity in genotypes while introgressing the desirable characters. The high levels of genetic variation that were observed between genotypes in *S. tmolea* indicate that management should aim to conserve as many of the small populations as possible in this study.

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