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Full Length Research Paper

Evaluation of genetic diversity in *Sulla coronaria* from different geographical populations in Tunisia by inter simple sequence repeat (ISSR)

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Five oligonucleotides generating 116 markers complementary to simple sequence repeats were used in order to characterize wild and cultivated ecotypes of *Sulla coronaria* and assess genetic diversity suitable in breeding programs. While analysing populations, a large genetic variability was revealed and supported by the preferentially allogamous mating system of the species. Moreover, the highest level of intra-population variations (H_{pop}/H_{sp} = 69.9) either of wild or cultivated accessions have been strongly evidenced by a significant adaptation to variety of habitats. In addition, the structure of populations was independent from the bioclimatic stages and was not affected by environmental factors as shown by the non correlation between the geographic and the Nei and Li's genetic distances (r= 0.461 and p=0.068>0.05). The unweighted pair group method with arithmetic mean (UPGMA) genetic relationships showed that some local spontaneous accessions characterised by an orthotropic port (Jebel Zit and Beja) were also molecularly similar to other cultivars.

Key words: *Sulla coronaria*, wild and cultivated forms, intra and inter-populations variability, microsatellites (ISSRs), bioclimatic stages.

INTRODUCTION

Hedysarum and *Sulla* are both taxonomically related Hedysarea genus which represent the most abundant grasses of the Mediterranean basin. These two genera can be diploid or tetraploid, and the Mediterranean species (2n=16) are distinguished by many characteristics related to their morphology, mating system, biological cycle and their native distribution (Pottier-Alapetite, 1979; Trifi-Farah and Marrakchi, 2001). In Tunisia, *Sulla* genus has many abilities such as producing forage, promoting pastoral zones and restoring destroyed pasture land especially in arid and semi-arid areas. Thus, this reliable crop constitutes an important genetic resource since these species are nutritious and highly palatable to sheep (Trifi-Farah et al., 2002). However, these phytogenetic pastoral resources have been currently damaged by severe genetic erosion due to overgrazing and irregular rainfall which are caused by alternations of rainy and drought years.

The *Sulla* genus is composed of six wild annual and perennial species (Choi and Ohashi, 2003): *S. coronaria* (L.) Medik., *S. carnosa* (Desf.) B.H. Choi & H. Ohashi, comb. nov., *S. capitata* (Desf.) B.H. Choi and H. Ohashi, comb. nov., *S. spinosissima* (L.) B.H. Choi and H. Ohashi, comb. nov., *S. flexuosa* (L.) Medik. and *S. pallida* (Desf.) B.H. Choi and H. Ohashi, comb. nov. Among

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Abbreviations: ISSR, Inter simple sequence repeats; **SSR,** simple sequence repeat; **dNTP,** deoxyribonucleotide triphosphate; **EDTA,** ethylenediaminetetraacetic acid; **PPB,** percentage of polymorphic bands; **PCA,** principal component analysis; **PCR,** polymerase chain reaction; **UPGMA,** unweighted pair group method with arithmetic mean.

Table 1. Tunisian *S. coronaria* accessions with bioclimatic area origins.

Accession	Station	Latitude (N)	Longitude (E)	Bioclimatic area
[Be]	Beja	36.7271°	9.1880°	Sub-humid, skin winter
[Bi]	Bizerte	37.2719°	9.8708°	Sub-humid, skin winter
[Eh]	El Haouaria	37.0517°	11.0114°	Sub-humid, warm winter
[Zi]	Jebel Zit	36.4180°	10.3041°	Semi-arid superior, skin winter
[*c*B]	Beja	36.7271°	9.1880°	Sub-humid, skin winter
[Tu]	Tunis	36.8116°	10.1761°	Semi-arid superior, skin winter

Number of individuals by population = 12.

these species, only *S. coronaria* (commonly called Sulla or Spanish sainfoin), is one of the most important forage, thanks to its capacity of silage making and seed production. *S. coronaria* is commonly cultivated in several countries around the Western Mediterranean basin, especially in Spain, Italy and North Africa (Baatout, 1996; Trifi-Farah et al., 2002). *S. coronaria* is very important for the fodder production in Tunisia, which explains the interest particularly developed towards this species. Indeed, the wild populations of this fodder species are well adapted to the local environmental conditions and can be implied in the valuation of the lands. In fact, in the 19th century, the introduction of several cultivars (Grimaldi, Scaravatti, Sparacia, etc.) coming mainly from Italy has failed due to their poor performance on ensilage and their inability to adapt their characteristics to the Tunisian environment except for the North region (Annicchiarico et al., 2008). Their culture concerns the North of Tunisia, where spontaneous populations of *S. coronaria* are located (Trifi-Farah et al., 2002).

In order to elaborate suitable improvement, many studies have been developed to evaluate and to preserve this important forage crop. These studies applied morphological, iso-enzymatic and particularly molecular analyses which are basically organellar DNAs and nuclear rDNA (Baatout et al., 1985, 1990; Trifi-Farah and Marrakchi, 2001, 2002). Previous study concerning the selection of reference genes in *Hedysarum coronarium* under various stresses and stages of development have also been realised (Cordoba et al., 2011). As regard the use of molecular techniques, microsatellites have proven to be effective in the examination of genetic diversity and the characterization of phytogenetic resources. In this scope, the “inter simple sequence repeats” based on polymerase chain reaction (ISSRs-PCR) procedure has been successfully applied in many crop species (Zietkiewicz et al., 1994; Moreno et al., 1998; Pasakinskiene et al., 2000; Ghariani et al., 2003; Chennaoui et al., 2007, 2008).

As part of our work in improving *S. coronaria* plants, we investigated the microsatellites (ISSR) in a wide range of spontaneous Tunisian *S. coronaria* populations. These accessions were selected from different Tunisian bioclimatic stages in comparison with one sample of cultivar forms in order to characterize wild ecotypes and assess genetic diversity suitable in breeding programs.

MATERIALS AND METHODS

This study was carried out on five spontaneous *S. coronaria* accessions and one cultivar [*c*B] sampled from different distribution areas in the North and the middle of Tunisia covering the sub-humid and the semi arid stage (Table 1). Collected seeds from each accession were germinated. The resultant seedlings where first transferred to Petri dishes then incubated in a greenhouse in optimal temperature condition (25°C) until cotyledons emerged. 12 seeds that constitute a sample of each population were used to assess intra- and inter-populations variation levels.

DNA extraction

The individual isolation of total cellular DNA from fresh seedlings was done according to the protocol of Dellaporta et al. (1983) with minor modifications adapted to mini extraction. The resultant DNA was quantified using Gene-Quant spectrophotometer (Pharmacia) and its quality was determined through analysing minigel electrophoresis according to Sambrook et al. (1989).

Primers screening and PCR-ISSR assays

Seven oligonucleotides were tested in order to explore the genetic diversity of *S. coronaria* at the inter-populations level. The resultant analysis of the intra-populations polymorphism level of spontaneous and cultivated forms was performed using five of them. The ISSR-primers were used to amplify the sequence of DNA between two microsatellites using two types of primers which are the unanchored primers which consist of a simple microsatellite sequence, and the anchored primers were added with a single extra nucleotide in the 3’- end of a microsatellite sequence. The primers’ sequences used in this study and their properties are summarized in Table 2.

DNA amplification was achieved in 25 µL of final volume reaction mixture containing 2.5 µL of enzyme buffer (10X), 50 mM of dNTPs, 60 pg of primers and 1.5 U of *Taq* DNA polymerase (QBIOgene, France). The amount of DNA extracted to generate a reproducible fingerprinting for each sample was from 20 to 25 ng. Amplifications were performed in a Crocodile III thermocycler (QBIOgene, France) according to the following conditions: an initiation step of 5 min at 94°C; then 35 cycles each; one includes 30 s at 94°C for denaturation, 90 s at primer-specific appropriate melting temperature (Tm, °C) for hybridization and 90 s at 72°C for elongation, and finally a 5 min step at 72°C was programmed as a final elongation. Each reaction vial was overlaid with a mineral oil

Table 2. Percentage of polymorphic bands and resolving power of ISSR primers used in this study.

Primers sequence	Tm (°C)		ISSR amplified band		Resolving power (Rp)	Average band informativeness (AvIb)
	Theoretical	Optimal	Total	Polymorphic (%)		
$(AG)_{10}T$	62	57	19	100	11.101	0.584
$(AG)_{10}G$	64	60	32	100	18.821	0.588
$(TC)_{10}C$	64	60	23	100	12.240	0.532
$(GACAC)_4$	64	55	28	100	16.211	0.579
$(AGG)_6$	60	55	14	100	7.216	0.515
Total			116	100	Sum=65.589	Average=0.559

drop. A negative control was added in each run to test for contamination. The final PCR-ISSR products were separated by electrophoresis on a 1.5% agarose gel in 0.5X tris borate EDTA (pH 8.3) buffer for 3 h at 80 V. The obtained fragments were visualized by an ethidium bromide staining method under ultraviolet (UV) light (Sambrook et al., 1989). Polymerase chain reaction (PCR) was carried out 3 times, only the repeatable bands were considered for diversity assessment. Each ISSR band was considered as an ISSR locus scored according to molecular weight marker (1Kb Ladder, Q-Biogene).

Data analysis

Polymorphic amplified products were scored as present (1) or absent (0) to form a binary data matrix. The ability of the most informative primers to differentiate between accessions was assessed by calculating the percentage of polymorphic bands (PPB) and their resolving power (Rp) (Prevost and Wilkinson, 1999) according to the Gilbert et al. (1999) formula:

$$Rp = \sum Ib$$

Where, $Ib = 1- (2 \times |0.5 - p|)$, p is the proportion of species containing the I band. Ib is a measure of closeness of a band to be present in 50% of the accessions and Rp is the sum of Ib values of all the bands generated by primers.

First, the binary matrix established from ISSR-markers was computed with the statistical analysis system software version 6.07 (SAS, 1990) to perform a principal component analysis (PCA) carried out in the Centre Inter Regional d'Informatique et d'Automatisme El Khawarizmi (CIRIA-El Khawarizmi). Secondly, Shannon's index was calculated for each ISSR oligonucleotide among all individuals and populations to perform intra- and inter-populations diversity according to the formula (Lewontin, 1972; Lynn and Schaal, 1989; Bussel, 1999):

$$H = - \sum_{i=1}^{k} p_i \, Log_n \, p_i$$

Where, k is the band number, p_i is the frequency of the band i in a population, and H is the genetic diversity. A diversity index average (H_0=intra-population diversity) was calculated for each population. The obtained averages of index reflect the intra-population diversity average (H_{pop}). The total diversity H_{sp} is calculated considering all the individuals and without taking into consideration the populations. The ratio of the index of average genetic diversity and the total diversity $[(H_{pop} / H_{sp}) \times 100]$ corresponds to the percentage of intra- population diversity. The following formula: $G_{st} = (H_{sp} - H_{pop}) / H_{sp} \times 100$ reflects the percentage's estimation of the inter-population diversity.

Molecular variance (AMOVA) analysis was also carried out on ISSR dataset using GenAlEx 6.4 (Peakall and Smouse, 2006). The AMOVA components were used as estimation of molecular diversity at the hierarchical level among and within populations. In addition, the binary matrix was also computed with the Gendist program (PHYLIP 3.5) (Felsenstein, 1995) using the Nei and Li's (1979) formula to generate the genetic distance matrix exploited by the Neighbour program in order to define relationships between the studied populations. The obtained tree files were then submitted to the TreeView (Win32. 1.5.2) software to map phylogenetic diagrams based on the unweighted pair group method with the arithmetic averaging (UPGMA) (Page, 1996). Branch support was estimated by bootstrapping (500 replicates) with the program WINBOOT (Yap and Nelson 1995). Correlations between Nei and Li's genetic distances and geographic distances were evaluated by the test of Mantel using XLStat Version 2008 (Addinosoft SARL, Paris, France) (Mantel, 1967).

RESULTS

ISSR primers

Five spontaneous populations and one cultivar of *S. coronaria* were subject to our experiments. Out of the seven tested oligonucleotides complementary to SSR, five produced clear and reproducible polymorphic amplifications (Table 2). In fact, even with some adjustments, $(CT)_{10}T$ primers still revealed a smear and $(TG)_{10}$ indicates the absence of amplification. A total of 116 polymorphic DNA bands ranging from 100 to 3054 bp were successfully generated and considered as molecular markers suitable for assessing the genetic diversity among the *S. coronaria* populations.

Figure 1 shows typical examples of ISSR amplification patterns using anchored (Figure 1a) or unanchored primers (Figure 1b) (respectively $(AG)_{10}G$ and $(AGG)_6$). As expected, the ISSR banding patterns varied depending on the primers sequence. Indeed, 14 to 32 bands were recorded from different primers with a mean of 23.2 bands/primer (Table 2). As well, the number of bands generated was generally greater when using the anchored primers than the simple ones; 32 polymorphic bands for $(AG)_{10}G$, whereas 14 markers for $(AGG)_6$). Variation in average informativeness band (AvIb) and resolving power (Rp) are reported in Table 2. The data

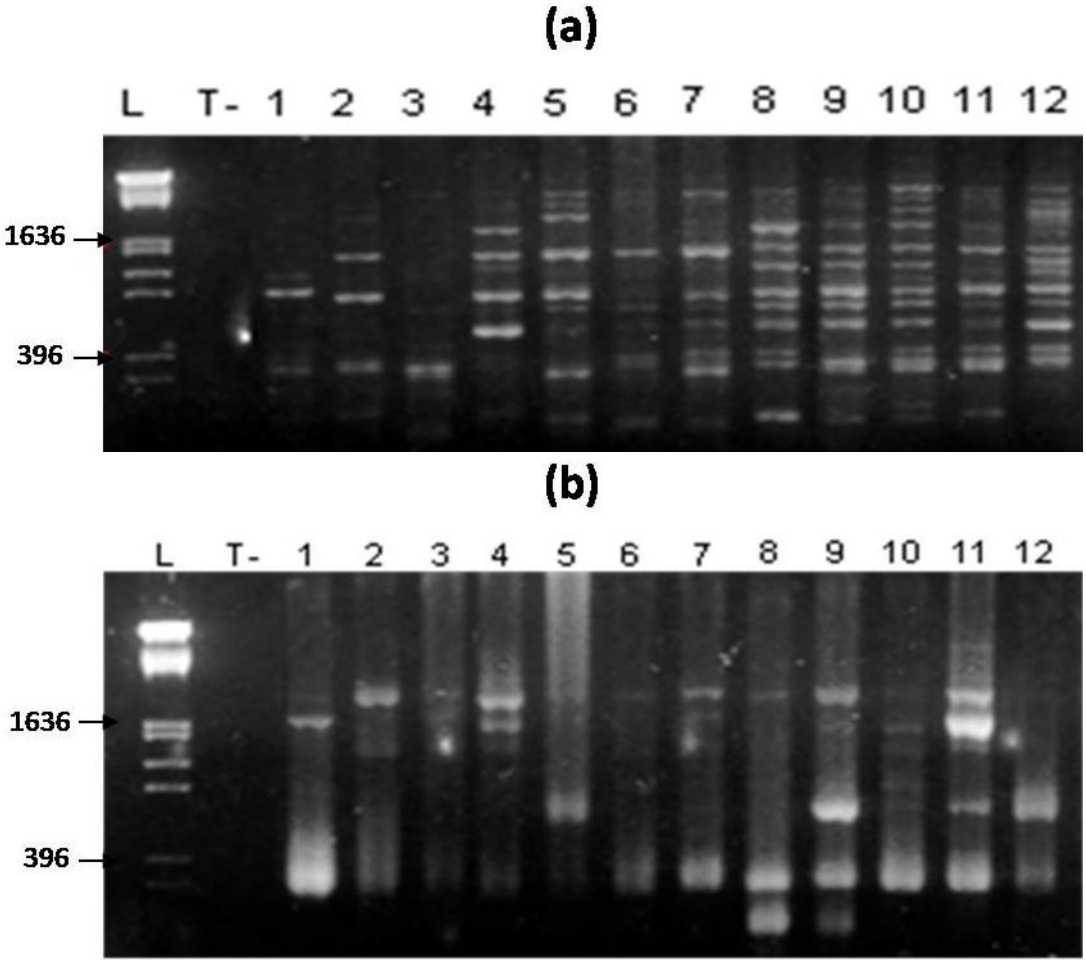

Figure 1. ISSR amplification products of 12 individuals of Tunis wild accessions *Sulla coronaria* using 3'anchored primers $(AG)_{10}G$ (a) and unanchored oligonucleotide $(AGG)_6$ (b). L, Standard size marker (1Kb ladder; Q-biogene, France); T-, negative control; lanes 1 to 12, DNA templates.

analysis showed that AvIb ranged from 0.515 to 0.588 with a mean value of 0.559 and the Rp values ranged from 7.216 to 18.821 with a sum Rp value of 65.589. The primers $(AG)_{10}G$ showed the highest value of Rp (18.821) and of AvIb (0.588). Hence, we assumed that oligonucleotides, based on repeated AG, contributed effectively towards the examination of genetic diversity in this crop.

Intra-population variability

Throughout all the studied accessions of *S. coronaria*, 116 polymorphic ISSR bands were detected. However, considering each population, some bands were revealed as monomorphic, varying from three in Tunis [Tu] population to 17 in Beja cultivated population. Furthermore, the number of markers ranged from 76 within the Beja cultivar to 113 within the Tunis population (Table 3). The five wild populations of *S. coronaria* showed significant difference at the genetic distances level ranging from 0.000 to 0.402 (Beja [Be]) with an average distance of 0.223 and from 0.234 to 0.967 (Tunis [Tu]) with a mean of 0.600 (Table 3). It is worthy to note that in the area of Beja, the averages of genetic distances between individuals from wild and cultivated forms are similar (respectively, 0.223 and 0.232) (Table 3). The relatively great genetic distance scored (up to 0.967) proves that the analyzed populations are characterized by a high intra-population variation level. This is strongly supported by the relatively high values scored for the genetic diversity within populations as reported in Table 4 (H_0 varied from 4.39 to 7.60 respectively for Beja and Tunis accessions) or by the percentage of diversity within populations (H_{sp} is of 8.99; H_{pop}/H_{sp} is of 69.9).

A principal component analysis (PCA) was applied to the data matrix. The resultant distribution of *S. coronaria* genotypes were mainly based on the first three principal components explaining 21.242% of the total variation. This percentage is considered consequent for such

Table 3. Intra-population variability in *S. coronaria* based on ISSR markers.

Accession	ISSR amplified band			Genetic distance (Nei and Li, 1979)		
	Total	Polymorphic	PPB (%)	Minimum	Maximum	Average
Beja [Be]	93	76	81.7	0.000	0.402	0.223
Bizerte (Bi]	98	84	85.7	0.25	0.776	0.475
El Haouaria [Eh]	102	92	90.2	0.298	0.724	0.507
Jebel Zit [Zi]	105	99	94.3	0.358	0.724	0.522
Tunis [Tu]	116	113	97.4	0.234	0.967	0.600
cv. Beja [*c*B]	102	85	83.3	0.000	0.393	0.232

Table 4. Genetic diversity within and among *S. coronaria* populations (Shannon's index) based on ISSR data.

Primer	*c*B	Be	Bi	Tu	Eh	Zi	H_{pop}	H_{sp}	H_{pop}/H_{sp}	G_{st}
H: $(AG)_{10}T$	3.97	3.79	5.70	6.27	6.15	6.02	5.32	7.31	72.8	27.2
H: $(AG)_{10}G$	5.87	6.18	8.70	10.68	9.65	10.25	8.55	12.01	71.2	28.8
H: $(TC)_{10}C$	4.68	4.04	6.73	7.57	6.66	7.51	6.20	9.72	63.8	36.2
H: $(GACAC)_4$	5.46	5.40	8.89	9.16	9.02	9.08	7.83	9.52	82.2	17.8
H: $(AGG)_6$	2.43	2.55	3.12	4.34	4.45	4.18	3.51	6.39	54.9	45.1
H_o	4.48	4.39	6.63	7.60	7.18	7.41	6.28	8.99	69.9	30.1

*c*B, cultivar Beja; Be, Beja; Tu, Tunis; Eh, El Haouaria; Zi, Jebel Zit; H, intra-population diversity for each primer; H_o, intra-population diversity; H_{pop}, intra-population diversity mean; H_{sp}, total diversity; $H_{pop}/H_{sp}\times100$, percentage of diversity within population against the total diversity; $G_{st} = (H_{sp}-H_{pop})/H_{sp}\times100$, percentage of inter-population diversity against the total diversity.

molecular markers. The first component explicates 11.013% of inertia and it is basically defined by ISSR markers generated by $(AG)_{10}G$ and $(GACAC)_4$ primers. At the second axis, it absorbs 5.167% of total inertia and it is defined by ISSRs corresponding to $(AG)_{10}T$ and $(TC)_{10}C$ primers. The third dimension explicates 5.062% of inertia deals with $(AGG)_6$ primers. The graphical representation of populations dispersion in the plan generated by the two axes (1 - 2) showed the existence of an important diversity within the analysed populations (Figure 2). Tunis population displayed the greatest diversity. The accession from Beja formed the tightest cluster, while the other spontaneous populations displayed a wide range of variation.

Inter-populations variability

In order to estimate variation in the inter-population's level, the genetic distance matrix based on ISSR markers using Nei and Li's (1979) formula was established among the studied populations. Results exhibited a large average distance ranging from 0.226 to 0.797 with a mean of 0.565 reflecting the high level of genetic variability offered by ISSR markers within the species *S. coronaria* (Table 5). The smallest distance (0.226) value was observed between populations displaying the same geographical area: Beja's cultivar [*c*B] and Beja's spontaneous population [Be] suggesting their high degree of genetic similarities. On the other hand, the largest distance value (0.797) reflecting a high divergence was recorded between the littoral population of El Haouaria [Eh] and the continental one of Jebel Zit [Zi]. All the remaining ones presented intermediate distances.

The ISSR UPGMA dendrogram shows two main clusters separated with bootstrap values of 100% (Figure 3). The first one (I) contained wild populations from Tunis and El Haouaria. All the remaining accessions were ranged in the second cluster (II) that exhibited two secondary ramifications. The first labeled (II-1) consisted of both spontaneous and cultivated Beja's accessions (86%), while the second ramification (II-2) was composed of populations from Bizerte and Jebel Zit (92%). We also noted that clustering was independent from bioclimatic stages. This independence was confirmed by the evaluation of the correlation between molecular variability and geographical distances using the Mantel test. Results revealed that the Nei and Li's genetic distances were not correlated with geographical distances ($r= 0.461$ and $p=0.068>0.05$ after 100 permutations).

Furthermore, the PCA allowed us to examine the relationships between the six analyzed populations. Graphical representation (1-2) of the dispersion of accessions (Figure 2) concurred with the existence of an important diversity and permit the identification of two main clusters: The first one integrated Jebel Zit, wild population and cultivar originating from Beja's site; while

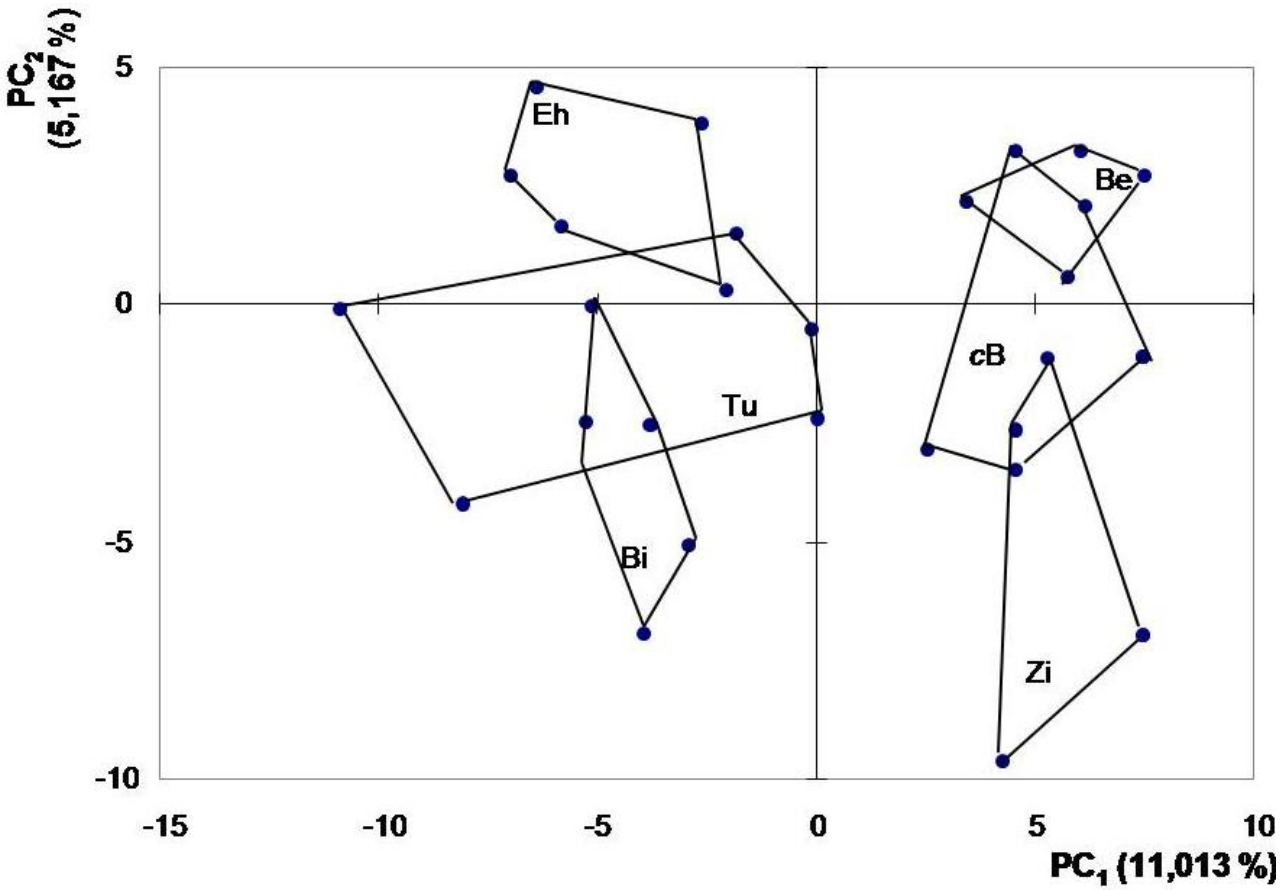

Figure 2. Principal component analysis of *S. coronaria* accessions based on ISSR markers. See Table 3 for population labels.

Table 5. Genetic distances matrix among analysed accessions of *S. coronaria* using Nei and Li's (1979) formula based on ISSR markers.

Population	*c*B	Bi	Be	Tu	Eh	Zi
*c*B	0.000					
Bi	0.535	0.000				
Be	0.226	0.537	0.000			
Tu	0.621	0.674	0.611	0.000		
Eh	0.532	0.608	0.528	0.582	0.000	
Zi	0.526	0.599	0.519	0.580	0.797	0.000

the second included the remaining accessions of *S. coronaria* species. Thus, the spontaneous populations of Beja and Jebel Zit seem to be similar to the cultivar in opposition to El Haouaria, Tunis and Bizerte according to the first principal component.

Partitioning of genetic variation

AMOVA analysis allowed the partitioning of the genetic variation among and within populations using ISSR data (Table 6). The AMOVA result indicates more variation within (72.43%) than between populations (27.57%). This was not surprising for *S. coronaria* local populations since they are cross-pollinated. Thus, AMOVA also supported the results of Shannon's information measure that there was a relatively high level of genetic differentiation within populations.

DISCUSSION

In this study, the ISSR technology was applied to generate markers as tools to assess the genetic diversity

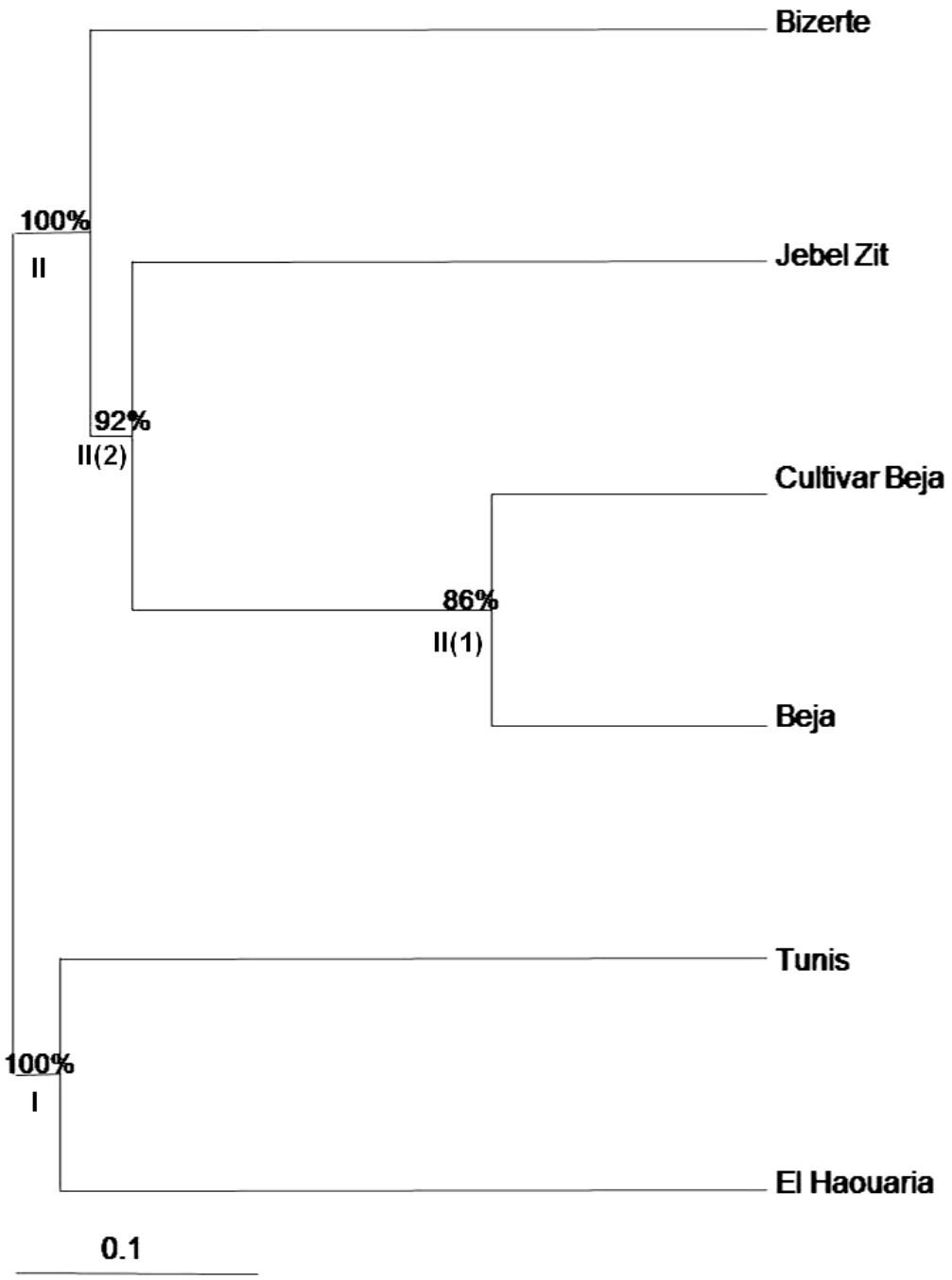

Figure 3. UPGMA phenogram of the genetic relationships among *Sulla coronaria* accessions produced from the bootstrap analysis. The numbers on the branches represent the bootstrap value of 500 bootstrap replicas. Each arm of the tree corresponds to the scaled genetic distance. See Table 3 for population labels.

Table 6. Analysis of molecular variance among six populations of *S. coronaria* using ISSR markers.

Source of variation	*df*	Sum of square	Percentage of variation (%)
Among populations	5	67.243	27.57[a]
Within populations	66	199.785	72.43[a]
Total	71	267.028	

[a]Significant at alpha 0.001 (after 1000 random permutations); *df*, degree of freedom.

within *S. coronaria* and to define the relationships in between the five wild accessions and the cultivated form. Therefore, a selection of primers was required to fingerprint the genome of these species. The percentage of polymorphic bands (PPB=100%) obtained for the six analyzed *S. coronaria* accessions suggest that ISSR procedure constitutes a suitable alternative approach to examining genetic diversity among populations The developed data provide evidence of a large genetic variability among all analyzed populations. This provided evidence suggests an interesting genetic potential for selection and also indicates that ISSR technology is a powerful and an efficient approach, which can supply sufficient information in diversity analysis either at the intra- or the inter-populations level. This is an agreement with studies using ISSR technique to assess genetic diversity in several crops (Belaïd et al., 2006; Chennaoui et al., 2008; Ghariani et al., 2003; Marzougui et al., 2009; Pasakinskiene et al., 2000; Salhi-Hannachi et al., 2004; Xia et al., 2007).

In addition, the oligonucleotides based on repeated AG, contributed effectively to the examination of genetic diversity in this crop (Rp=18.821). Similar results are in agreement with the fact that repeated AGs are very abundant in plants, and reveal the highest polymorphism (Tsumura et al., 1996; Sarla et al., 2003; Salhi-Hannachi et al., 2004; Chennaoui et al., 2008). Shannon's index and AMOVA analysis revealed that the vast majority of genetic variation occurred within populations with relatively low genetic diversity among populations. Our results support the high variation within groups in other cross-pollinating species such as Buffalo grass (Huff et al., 1993), *Lolium multiflorum* (Vieira et al., 2004), *Bromus innermis* (Diaby and Casler, 2005), *Bromus riparius* Rehm (Ferdinandez et al., 2001), *Bromus auleticus* (Rivas, 2001), *Chloris gayana* K. (Ubi et al., 2003), *Pascopryum smithii* (Larson et al., 2003) and *Eucalyptus globulus* (Nesbitt et al., 1995). The preferentially allogamous mating system of the studied species (Yagoubi and Chriki, 2000) with a high dispersal of seeds does not prevent isolation of populations and strongly supports the non correlation between the geographic distances and the Nei and Li's genetic distances. In fact, the genotypic structure of populations was independent from the bioclimatic stage and was not affected by environmental factors, thus supporting an equal adaptation to variety of habitats. *S. coronaria* may be effectively considered as a well adapted plant which is grown under a wide range of soil and climatic conditions.

Moreover, ISSR banding patterns are genetically informative about the genetic diversity at the DNA level and they are allowed to provide molecular markers correlated with plant traits. In fact, results illustrated a significant divergence especially between the plagiotropic El Haouaria population and the orthotropic accessions of Jebel Zit or cv. Beja. These spontaneous populations were characterised by an opposite geotropism on the bases of morphological and molecular analysis (Marghali et al., 2006; Trifi-Farah and Marrakchi, 2000). Similar quantitative characters exhibiting local adaptation were reported in populations of *Triticum dicoccoides*, *Hordeum spontaneum* and *Pinus edulis* (Ivandic et al., 2003; Peng et al., 2003; Travis et al., 1998). In addition, it is noticeable that the cultivated and the wild populations coexisting in the same locality of Beja exhibited a closely cluster. This suggests that both wild and cultivated forms may exchange gene flow taking into account the preferentially allogamous mating system of the species (Ureta et al., 2008).

Conclusion

This work constitutes an alternative to improving the insight of the genetic diversity within Mediterranean *Sulla* accessions and to detect molecular markers that allow discrimination between genotypes with contrasting traits of interest (plagiotropic and orthotropic tendency). Research is currently in progress by the SSR markers in order to shed light on the genetic organization of *Sulla* species and for *Sulla* breeding program.

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