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Genetic diversity analysis of Moroccan saffron (*Crocus sativus* L.) of different origins based on intermicrosatellite markers

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Abstract

Saffron (*Crocus sativus* L.) is an autumnal herbaceous triploid plant; it is the source of saffron spice, recognized as the most expensive spice in the world. In this study, genetic diversity among 14 saffron accessions collected from different ancestral geographic areas in Morocco, Greece and France were assessed using inter-simple sequence repeats (ISSRs) markers system. Ten ISSR primers were amplified into a total of 143 fragments, of which 44.05 % are polymorphic with an average of 6.3 polymorphic fragments per each primer and average of polymorphic information content (PIC) of 0.236. ISSR markers proved to be a powerful tool for assessment of genetic diversity among saffron accessions. Cluster analysis using unweighted pair group method with arithmetic mean (UPGMA), based on Jaccard's similarity coefficient and supported by the principal coordinate analysis (PCoA), divided the studied accessions into three major groups, and showed that genetic distance is independent of geographical distance. In this paper, we report for the first time the level of genetic diversity among Moroccan saffron; this information allows an unequivocal development of a suitable approach for the conservation of *C. sativus* germplasm and reduce its genetic erosion.

Key words Saffron, *Crocus sativus* L., ISSR markers, genetic diversity, conservation.

Introduction

The name of saffron refers to both the spice and the plant (*Crocus sativus* L.) whose dried stigmas of its flowers are used as spice. It is the world's most expensive spice by weight. *C. sativus* L. is a geophyte, perennial and triploid (3 *x* = 24) plant (Karaoğlu *et al.,* 2007). It belongs to Crocus genus (Iridaceae) which includes 100 recognized species (Harpke *et al.,* 2013) vegetatively propagated by corms. Several recent studies have demonstrated that saffron show numerous activities as antioxidant, anti-inflammatory, cardioprotective, neuroprotective, antidepressant and many others (Amin *et al.,* 2016; Ghasemi *et al.,* 2015).

This species is cultivated in different climatic conditions (Gresta *et al.,* 2009). Nowadays, saffron is widely cultivated in Iran, India, Morocco and other countries such as Spain, Greece, Italy, Turkey and France (Babaei *et al.,* 2014). In Morocco, saffron is cultivated for centuries in the area of Taliouine and more recently in Tazenakht area and other regions. Moreover, Morocco ranks third in terms of production with 6.8 t/year. Its saffron is highly reputed nationally and internationally (Lage and Cantrell, 2009).

Because of the commercial and socioeconomic value of this spice, the problem of intense genetic erosion reduced the genetic variation of this crop over the past centuries (Nemati *et al.,* 2014). The evaluation of genetic resources among accessions of this plant have become critically important for conservation, management, and authentication of its germplasm.

Molecular markers are mainly used for the evaluation of genetic diversity of species with high level of reproducibility and polymorphism, possibility of large-scale genotyping, and establishing interchangeable databases. Furthermore, they are unaffected by developmental stages of plants.

In the case of saffron, various molecular markers have been used to assess the genetic diversity such as RAPD, ISSR and SSR (Rubio-Moraga *et al.,* 2009), RAPD and SRAP (Keify and Beiki, 2012 ; Babaei *et al.,* 2014), SSR (Bayat *et al.,* 2016; Fluch *et al.,* 2010; Namayandeh *et al.,* 2012; Nemati *et al.,* 2012; Nemati *et al.,* 2014), SSR and SNP (Javan and Gharari, 2018), AFLP (Siracusa *et al.,* 2013; Torricelli *et al.,* 2019) and IRAP (Alsayied *et al.,* 2015). ISSRs markers amplify DNA sequences between two inverted SSRs. These molecular markers do not require existing knowledge of genomic sequences and are therefore simple, rapid, very effective and are frequently and successfully used for the assessment of genetic diversity of numerous species such as *Argania spinosa* (Mouhaddab *et al.,* 2017), *Acorus calamus* L. (Kasture *et al.,* 2016) and *Vitis vinifera* L (Basheer-Salimia and Mujahed, 2019).

The genetic resources of the Moroccan saffron stay uncharacterized at the molecular level. This is the first attemp on the use of molecular markers for the establishment of the genetic fingerprint of the Moroccan saffron. The data obtained from this study may help in unequivocal development of suitable approaches for the conservation of the Moroccan saffron germplasm.

The aim of the present study is to investigate the spectrum of genetic diversity among 14 saffron accessions of different origins, using ISSRs markers and to identify genetically distinctive accessions within the species. In addition, effectiveness and reliability of ISSRs markers for diversity analysis in *C. sativus* species was evaluated.

Material and methods

Plant material and genomic DNA extraction: In Morocco, the region of Taliouine and Taznakht holds the major part of the production of saffron and represents the cradle of this culture in the country. For these reasons, our accessions originate from this region. In fact, twelve cultivated Moroccan saffron accessions were collected from different ancestral fields in the regions of Taliouine (Taroudant province) and Taznakht (Ouarzazate province). Two cultivated saffron accessions, one from Quercy region in France and one from Kozani (West Macedonia) region in Greece, were used in this molecular study (Table 1).

Table 1. *Crocus sativus* accessions used in this study

No	Code	Origin	Latitude	Longitude	Altitude (m)
1	TB_1	Iznaguen	30° 29,937' N	7° 51,703' W	1498
2	TB_2	Tassousfi	30° 28,468' N	7° 45,948' W	1515
3	TB_3	Sidi Hssaine (Ighri)	30° 28,006' N	7° 42,851' W	1691
4	TM_1	Aglagaln	30° 41,22' N	7° 46,361' W	1761
5	TM_2	Agadir Melloul	30° 45, 229' N	7° 22, 172' W	1775
6	TM_3	Zagmouzen	30° 38,343' N	7° 49, 349' W	1850
7	TH_1	Askaouen 1	30° 44, 434' N	7° 47, 434' W	1939
8	TH_2	Askaouen 2	30° 44,257' N	7° 46, 554' W	1964
9	TH_3	Assif Zimmer	30° 40, 335' N	7° 44,107' W	2213
10	KB	Zaouiyat	30° 29,394' N	7° 19,904' W	1503
11	KM	Tazenakht	30° 30,396' N	7° 19,153' W	1492
12	KH	Taloust	30° 38,147' N	7° 29,530' W	2017
13	G	Plumelec (Greece)	40° 17,202' N	21°48,314' E	-
14	F	Quercy (France)	47°49,481'N	2°37,17' W	--

Total genomic DNA was extracted from fresh young leaves of a single plant of every *C. sativus* accession using modified CTAB method (Doyle and Doyle, 1990). The quality of the extracted DNA was verified by electrophoresis on a 1 % (w/v) agarose gel, and the amount of total genomic DNA obtained was quantified using a Zuzis spectrophotometer 4201/50 (AUXILAB S.L). DNA samples were diluted to 50 µg/mL and stored at -20°C for subsequent ISSR analysis.

ISSR analysis: Ten polymorphic ISSR primers, previously identified by Souto Alves *et al*. (2009), were used to assess the genetic diversity among saffron accessions (Table 2). The PCR reaction was conducted in a reaction volume of 10 µL containing 15 ng of genomic DNA, Taq polymerase buffer (x1), 0.2 mM dNTPs, 0.2 mM of primer (Eurofins), 1 U of Taq polymerase (Bioline Meridian Bioscience Inc, USA) and 1.5 to 2 mM of $MgCl_2$. The concentration of $MgCl_2$ varies according to the nature of the primers. PCR amplification was performed in a thermal cycler (Aeris™ Thermal Cycler, Esco Life Sciences, Thailand), using a program with an initial denaturation cycle at 94 °C for 3 min; 30 cycles of 94 °C for 45 s, 50-60 °C (depending on the type of primers) for 1 min, and 72 °C for 1 min for denaturing, annealing and extension respectively, and a final extension cycle at 72 °C for 7 min. PCR products have been visualized by 2 % w/v agarose gel in TBE buffer at 90 V for 2 h, stained with ethidium bromide (0.5 µg/mL), visualized under UV light, and photographed by a Gel Doc system (Omni DOC Gel Documentation System, Cleaver Scientific Ltd, United Kingdom). A 100bp and 1kb DNA ladder (HyperLadder™, Meridian Bioscience Inc, USA) have been used to identify the size of the fragments.

Table 2. Characteristics of the ISSR primers used for genotyping analysis of 14 *C. sativus* accessions

Code	Motif	Sequence (5′-3′)	[$MgCl_2$] mM	T_H (°C)
ISSR1/8	AG)8C	CAGAGAGAGAGAGAGAGC	2.0	56.0
ISSR3/8	(GA)8YG	YGGAGAGAGAGAGAGAGAGY	1.5	56.8
ISSR4/8	(AC)8YG	YGACACACACACACACACYG	2.0	53.5
ISSR5/8	(GT)8 T	GTGTGTGTGTGTGTGTT	1.5	55.3
ISSR6/8	(AC)8YT	YTACACACACACACACACYT	1.5	56.8
ISSR7/8	(AGC)6	GCAGCAGCAGCAGCAGC	2.0	60.5
ISSR8/8	(CTC)8	CTCCTCCTCCTCCTCCTC	2.0	59.8
ISSR807	(GA)8C	GAGAGAGAGAGAGAGAC	1.5	52.0
ISSR808	(CT)8A	CTCTCTCTCTCTCTCTA	2.0	50.7
ISSR857	(AC)8G	ACACACACACACACACG	2.0	52.0

Data analysis: DNA bands are scored manually as present (1) or absent (0) for each primer for all the samples under study. Only clear and distinguished DNA bands have been considered for data analysis, and the formed binary data have been used for statistical genetic analysis.

To measure the level of genetic diversity, numerous components are estimated based on the binary data. The total number of bands per marker (NTB), the number of polymorphic bands (NPB), the percentage of polymorphism (% P), alleles frequency (p and q) and Unbiased Expected Heterozygosity (uHe) were calculated using GenAlEx version 6.5b3 (Peakall and Smouse, 2012). The average number of alleles (Na), average effective number of alleles (Ne), average shannon's Information Index (I) (Lewontin, 1972), average expected heterozygosity or gene diversity (Nei, 1973) for each primer were estimated using POPGENE version 1.32 software package (Yeh *et al.,* 2000).

In order to determine the utility of ISSR markers, various statistical parameters including polymorphic information content (PIC), effective multiplex ratio (EMR), resolving power (RP), discrimination power (DP), and marker index (MI) were calculated using the software IMEC (Amiryousefi *et al.,* 2018).

Two different approaches were used to establish genetic structure of the fourteen saffron accessions. First, a matrix of genetic similarity was compiled using Jaccard's similarity coefficient (JSI) (Jaccard, 1908), and used to generate a dendrogram using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) using the SAHN clustering model in NTSYSpc software version 2.10e (Rohlf *et al.,* 2000). To complement this hierarchical cluster analysis the principal component analysis (PCoA) was carried out by PAST software, ver. 1.34 (Hammer *et al.,* 2001). Secondly, was a Bayesian model-based approach of cluster analysis was conducted by STRUCTURE software version 2.3.4 (Pritchard *et al.,* 2000; Falush *et al.,* 2003) using an admixture model with correlated allele frequencies without any prior knowledge on genotype classification, and with a burn-in length of 50,000 and 500,000 Monte Carlo Markov Chain (MCMC) replicates with ten independent runs for each K ranging from 2 to 14. The most likely number of genetic clusters was estimated by plotting LnP (K) values against Δk values, with the optimal K value selected according to Evanno *et al.* (2005) using the STRUCTURE HARVESTER software (Earl, 2012).

Results

ISSR genetic diversity: A rough analysis of the ISSR amplification shows different profiles depending on the accessions. For example, using the ISSR6 / 8 primer, at least 3 profiles (TB_1, TB_2, TB_3 and TM_1; TM_2 and TM_3; TH_1 and TH_2) stand out clearly. The 10 selected ISSR primer, have yielded 143 bands across all the accessions, ranging from 150 to 2500 pb, with an average of 14.3 loci per primer. The highest number of bands has been observed for the ISSR 4/8 (22 bands), and the lowest one has been detected in the ISSR 1/8 (9 bands). Among the 143 bands, 80 are monomorphic and 63 are polymorphic with about 44.05 % of genetic polymorphism, which indicates a medium level of polymorphism in the genome of *C. sativus.* The maximum percentage of polymorphism 90 % for the ISSR 6/8. This primer is considered as the most powerful primer among the tested ones. It is a potential candidate for the classification of different accessions.

The average number of observed alleles (Na), effective allele number (Ne), Nei's gene diversity index (He). Unbiased Expected Heterozygosity (uHe) and Shannon information index (I) were 1.418,1.279, 0.232 0.158 and 0.164, respectively. The results showed that the 14 saffron accessions had a moderate genetic diversity. Examples of the amplified products of ISSR markers (ISSR6/8, ISSR808, ISSR5/8 and ISSR3/8) are shown in Fig. 1.

ISSR markers utility: PIC value is an indication of population polymorphism and its maximal value for dominant markers is 0.5 (Chesnokov and Artemyeva, 2015). In the present study, half of the used primers exhibited PIC values greater than 0.25. The highest PIC value (0.35) was observed for the primer ISSR6/8 and the lowest one (0.125) was recorded for the primer ISSR8/8 with an average of 0.236. The MI values ranged from 0.010 to 0.023 with an average of 0.018. The RP is an attribute of the markers that represent the discriminatory potential of the primer. For the ISSR markers used in this study, the RP ranged from 0.166 to 10.33 with an average of 3.14, whereas the DP values ranged from 0.139 to 0.56 with an average of 0.42. The primer ISSR 6/8 showed the highest values of all polymorphism indices and it is qualified as the most powerful primer. It is a potential candidate for the classification of different accessions.

Genetic relationships and clustering analysis: The data matrix analyzed included 2002 entries, 1630 of which were for present loci (1) and 372 were for absent loci (0). Accordingly, the Jaccard coefficient was calculated and presented in Table 3. The genetic distance matrix showed a distance ranging from 0.061 to 0.296 with a mean of 0.177 (Table 4). The maximum genetic distance value of 0.296 (70.4 % of similarity) was exhibited between Iznaguen (TB_1) and Zagmouzen (TM_3) accessions, whereas the lowest genetic distance of 0.061 (93.9 % of similarity) was shown between Tassousfi (TB_2) and Sidi Hssaine (TB_3) accessions.

UPGMA dendrogram clustered the *C. sativus* accessions under study into three major clusters (Fig. 2). The first cluster consisted of eight accessions: Askaouen 1 (TH_1), Askaouen 2 (TH_2), Assif Zimmer (TH_3), Aglagaln (TM_1) Zaouiyat (KB), Taloust (KH), Greece (G) and France (F). The second contained Iznaguen (TB_1), Tassousfi (TB_2) and SidiHssaine(TB_3). The third cluster contained only two accessions: Agadir Melloul (TM_2) and Zagmouzen (TM_3). The TB_2 and TB_3 accessions were found to be very close. They both come from the medium altitude and belong to 2 regions very close geographically. The TH_1, TH_2 and TH_3 accessions were very close in the dendogram. They belong to the same high-altitude region of Askaouen. The KH_1 accession stands out on its own as representative of the high altitude of the Sirwa mountains. In addition, ISSR UPGMA analysis indicated that the saffron of France and Greece are very close genetically, and the closest Moroccan accession to these two accessions was that of Taloust (KH).

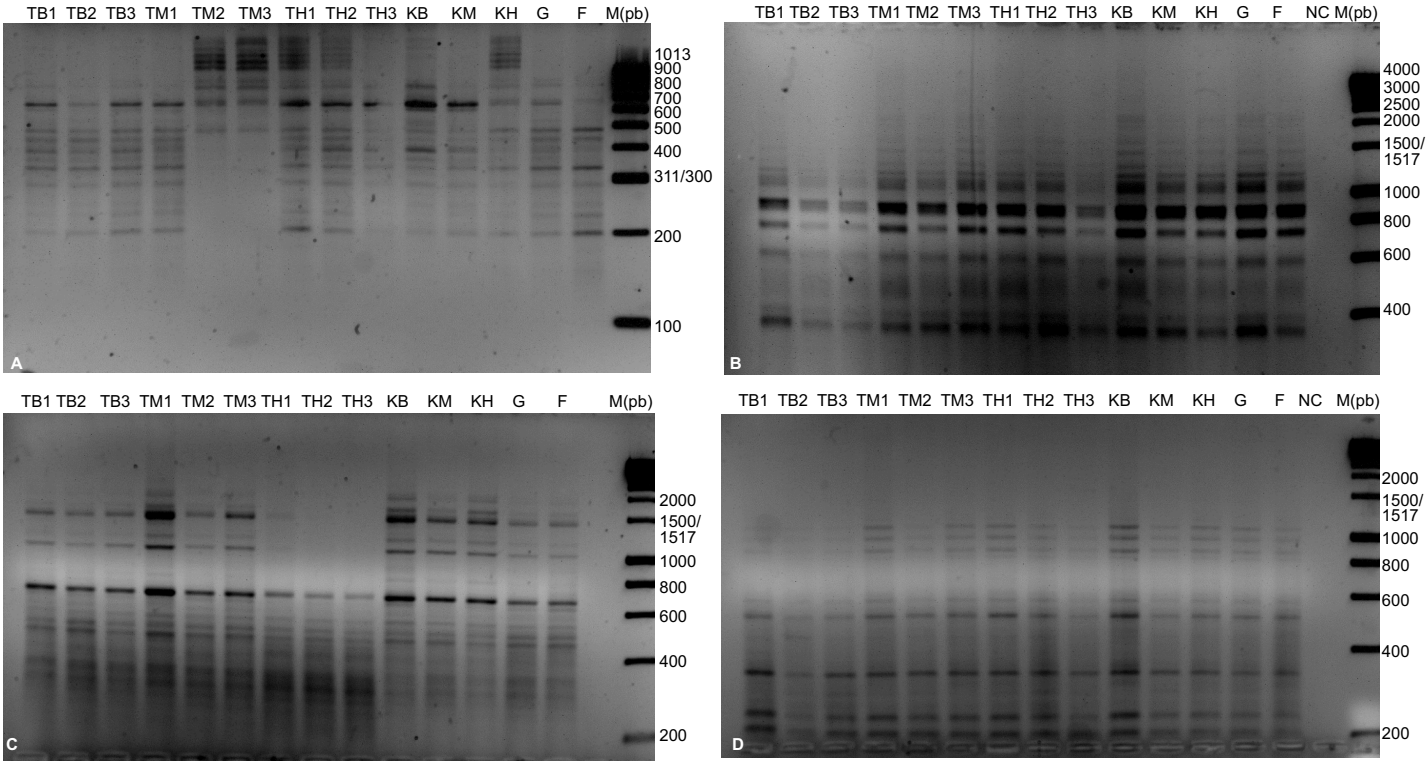

Fig. 1. PCR-ISSR banding patterns generated from 14 saffron accessions using primer ISSR6/8 (A), ISSR808 (B), ISSR5/8 (C) and ISSR3/8 (D) on 2% w/v agarose gel; Lane 1-14 represent different *C. sativus* accessions as listed in Table 1; Lane 15 is a negative control (NC); Lane 16: M (molecular weight marker 1kb or 100pb

Table 3. Genetic diversity revealed by the 10 ISSR markers used in the study

Code of primer	p	q	TNB	Na	Ne	NPB	% P	I	He	uHe	PIC	MI	EMR	RP	DP	Size in (bp)
ISSR1/8	0.850	0.150	9	1.333	1.300	3	33.33	0.222	0.158	0.164	0.14	0.011	8.25	1.5	0.16	240-1013
ISSR3/8	0.904	0.096	10	1.100	1.008	1	10.00	0.016	0.007	0.007	0.15	0.012	9.08	0.17	0.17	200-1100
ISSR4/8	0.722	0.278	22	1.546	1.367	12	54.54	0.300	0.205	0.213	0.24	0.020	18.25	3.5	0.31	200-2500
ISSR5/8	0.668	0.332	13	1.571	1.405	7	53.84	0.331	0.226	0.235	0.31	0.023	9.75	3.83	0.44	300-2000
ISSR6/8	0.455	0.545	20	1.950	1.670	18	90.00	0.559	0.382	0.397	0.35	0.025	13.16	10.33	0.56	200-1500
ISSR7/8	0.750	0.250	12	1.333	1.197	4	33.33	0.179	0.119	0.123	0.26	0.021	9.66	3.33	0.35	400-2500
ISSR8/8	0.883	0.117	15	1.267	1.191	4	26.66	0.160	0.110	0.114	0.12	0.010	13.91	1.5	0.14	200-1000
ISSR807	0.814	0.186	19	1.211	1.055	4	21.05	0.072	0.042	0.043	0.23	0.019	16.00	2.0	0.29	150-700
ISSR808	0.696	0.304	11	1.455	1.275	5	45.45	0.230	0.154	0.160	0.30	0.023	8.25	2.16	0.44	200-2000
ISSR857	0.732	0.268	12	1.417	1.325	5	41.66	0.256	0.178	0.185	0.26	0.021	9.75	3.16	0.34	300-2500
Total	7.474	2.526	143	14.183	12.793	63	-	2.325	1.581	1.641	2.36	0.180	116.06	31.476	3.2	-
Average	0.747	0.252	14.3	1.418	1.279	6.3	44.05	0.232	0.158	0.164	0.24	0.018	11.6	3.14	0.32	-

p and q: allele frequency; TNB: Total number of bands; NPB: number of polymorphic bands; % P: percentage of polymorphism; I : Shannon's Information Index He: Expected heterozygosity or gene diversity; uHe : Unbiased Expected Heterozygosity; PIC: polymorphism information content; MI: Marker index; EMR: Effective multiplex ratio; RP: resolving power; DP: discrimination power; Na, Observed number of alleles; Ne, Effective number of alleles

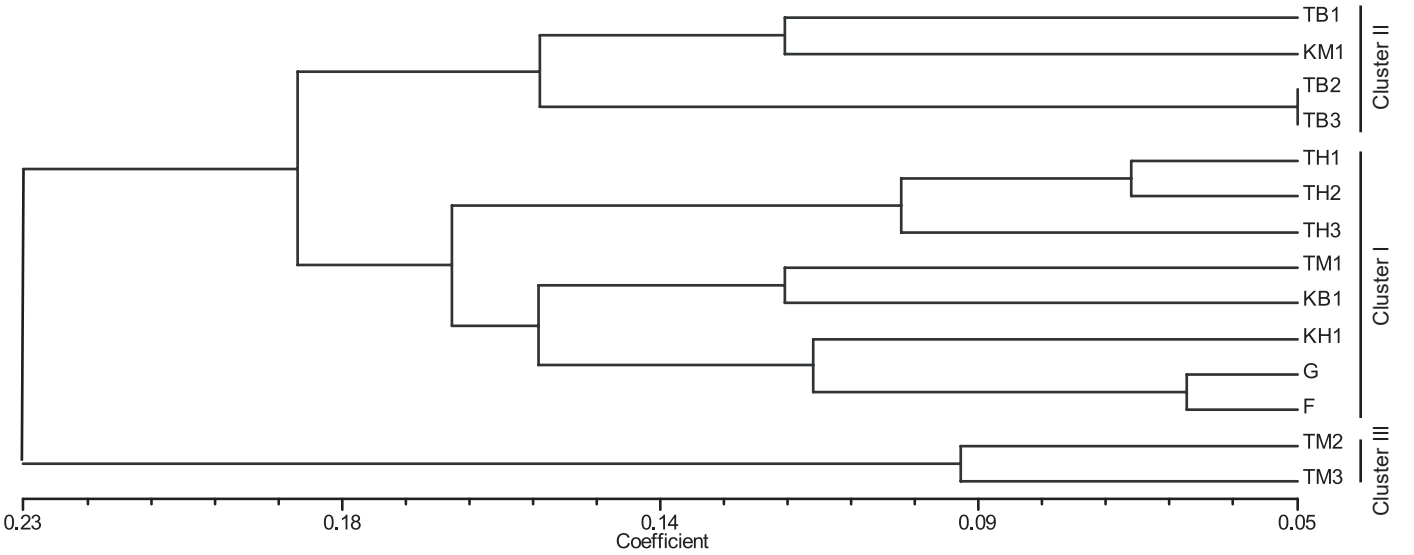

Fig. 2. UPGMA dendrogram illustrating relationship among the 14 accessions of *C. sativus*, based on the genetic Jaccard's similarity matrix data obtained using 10 ISSR markers

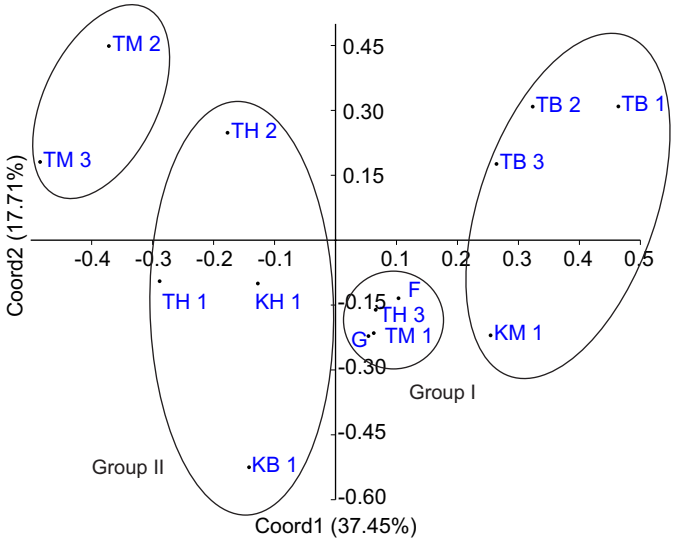

Fig. 3. Principal coordinates analysis (PCoA) of 14 accessions based on ISSR banding patterns

The principal coordinates analysis (PCoA) based on Jaccard distances of the 14 accessions explain 55.16 % of the total variation by the first two axes (Fig. 3). The first principal coordinate (37.45 % of a total variation) divided the cluster I indicated by ISSR UPGMA analysis into two groups: The first group contained G, F, TH_3 and TM_1 accessions and the second group consisted of TH_1, TH_2, KH and KB accessions. PCoA analysis supports mostly and corresponds largely to cluster analysis. It should be noted that the group formed by TB_1, TB_2, TB_3 and KM_1 contained accessions all belonging to the medium altitude while group 2 formed by TH_1, TH_2, KH and KB included accessions from high altitude except by KB. Bayesian model-based structure analysis showed the maximum DK value of K=3 (Fig. 4A), which illustrating the 14 accessions of saffron were divided into three groups (Fig. 4E). This result is in agreement with the results obtained by UPGMA dendrogram. The STRUCTURE clustering analysis is also consistent with UPGMA analysis and PCoA, except TH_1 and TH_2 accessions who were clustered with TM_2 and TM_3 accessions.

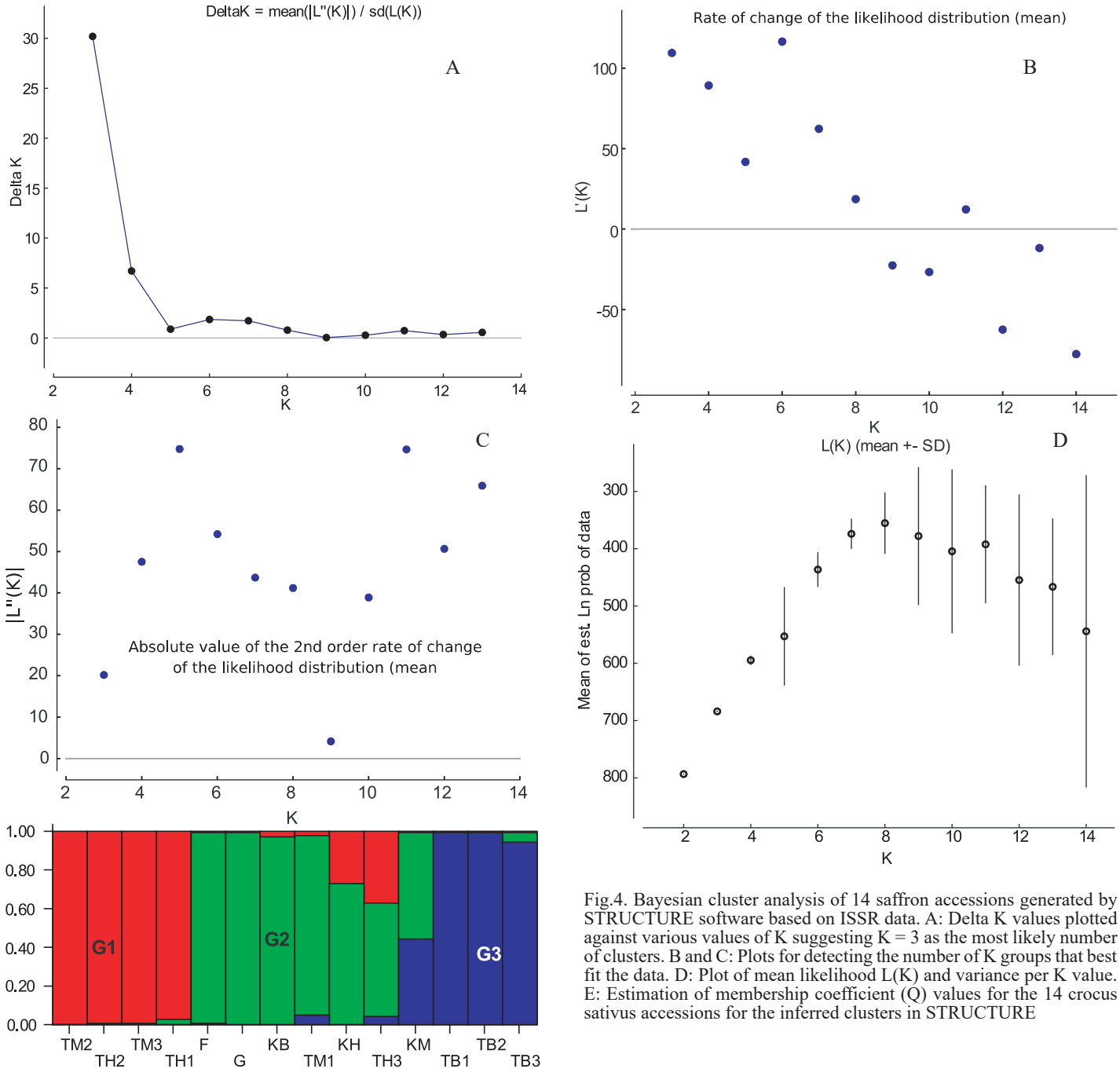

Fig.4. Bayesian cluster analysis of 14 saffron accessions generated by STRUCTURE software based on ISSR data. A: Delta K values plotted against various values of K suggesting K = 3 as the most likely number of clusters. B and C: Plots for detecting the number of K groups that best fit the data. D: Plot of mean likelihood L(K) and variance per K value. E: Estimation of membership coefficient (Q) values for the 14 crocus sativus accessions for the inferred clusters in STRUCTURE

Table 4. Jaccard's genetic distance index generated for the 14 accessions of *C. sativus* based on ISSR data

	TB_1	TB_2	TB_3	TM_1	TM_2	TM_3	TH_1	TH_2	TH_3	KB	KM	KH	G	F
TB_1	0													
TB_2	0.154	0												
TB_3	0.161	0.061	0											
TM_1	0.190	0.170	0.134	0										
TM_2	0.265	0.229	0.207	0.219	0									
TM_3	0.296	0.261	0.266	0.197	0.109	0								
TH_1	0.266	0.232	0.198	0.157	0.172	0.177	0							
TH_2	0.219	0.198	0.161	0.175	0.147	0.182	0.081	0						
TH_3	0.221	0.170	0.132	0.134	0.221	0.239	0.127	0.100	0					
KB	0.268	0.235	0.201	0.120	0.229	0.207	0.154	0.200	0.174	0				
KM	0.138	0.178	0.170	0.127	0.243	0.247	0.206	0.212	0.140	0.182	0			
KH	0.232	0.198	0.163	0.149	0.135	0.196	0.143	0.147	0.163	0.133	0.156	0		
G	0.207	0.173	0.165	0.165	0.209	0.200	0.186	0.192	0.136	0.149	0.129	0.137	0	
F	0.188	0.168	0.130	0.131	0.190	0.222	0.182	0.187	0.115	0.171	0.138	0.103	0.074	0

Discussion

This is the first report on identification and evaluation of genetic diversity among Moroccan saffron by molecular markers. The PCR-ISSR technique has been used to identify and assess the genetic diversity of Moroccan saffron and establish its relationship with French and Greek saffron. The primers used in this study have been successfully used to study numerous species including *Murraya koenigii* (Verma and Rana, 2011), *Pterosperma oblate* (Su *et al.*, 2008) and *Argania spinosa* L. *Skeels* (Mouhaddab *et al.*, 2017). These primers are highly reproducible due to their primer length and high renaturation stringency and are found to provide highly polymorphic fingerprints with few primers detecting relatively high numbers of loci.

Despite the vegetative propagation of saffron, the ISSR markers used in this study revealed considerable level of genetic diversity within the studied accessions (44.05 %), which prove that the ISSR markers are useful, informative, and simple tools for the characterization of saffron germplasm and fingerprinting purposes. Our results are broadly consistent with the results of previous studies based on diversity of the SRAP markers and reported by Babaie *et al.* (2014) and Keify and Beiki (2012) among the Iranian saffron. The detected level of polymorphism in these studies were 43.88 and 43 %, respectively. Otherwise, the level of polymorphism detected by the ten ISSR primers used in this study is significantly higher than that revealed by the AFLP markers used by Torricelli *et al.* (2019), which did not exceed 4.13 %.

RAPD, ISSR, SSR (Rubio-Moraga *et al.,* 2009), chloroplast and microsatellites markers (Fluch *et al.,* 2010) were used in order to assess the genetic diversity of saffron from different geographical areas; the data from these studies suggest that saffron is a monomorphic species. However, based on ISSR data of this study, we can firmly report that saffron is not a monomorphic plant, and we can identify accessions for breeding purposes. Studies of Nemati *et al.* (2012) and Bayat *et al.* (2016) using SSR markers for saffron support this finding.

The cultivation of saffron in Morocco dates back more than 4 centuries in the Taliouine region where the village of Souktana is the cradle of this culture. Its propagation in neighboring regions which differ in their climates, altitudes and soil composition are factors which have their importance in the genetic diversification of current saffron accessions in these regions where the major Moroccan saffron production is located.

According to Botstein *et al.* (1980), the PIC value varies from 0 to 0.5 for dominant markers. The average of PIC value (0.236) indicates that the ISSR markers could develop medium loci polymorphism among saffron accessions, which is higher than the PIC value (0.150) detected by SRAP markers (Babaei *et al.,* 2014), and confirms the usefulness of this technique for the evaluation of genetic variation among saffron accessions.

The low genetic distance values can be assigned to the vegetative propagation of C. *sativus* species as a triploid and sterile plant (Fernández and Pandalai, 2004; Negbi, 1999) and to the artificial selection of the best traits (Agayev *et al.*, 2009). It indicates that the studied accessions are close, which confirms that saffron accessions may have originated from a common ancestry (Bayat *et al.,* 2016).

The dendrogram, PCoA and STRUCTURE analysis obtained in the present study shows that Moroccan, French and Greek accessions are closely linked; therefore, the separation of species is independent of geographical origin. This result is consistent with the findings of earlier genetic studies based on RAPD and ISSR data (Rubio-Moraga *et al.,* 2009), SSR data (Nemati *et al.,* 2014; Bayat *et al.,* 2016), and SRAP data (Babaei *et al.,* 2014).

Does the correlation found between access groups and the altitude level of their plantation, particularly at the level of high and medium altitude accessions reflect an effect of altitude on the genetic diversity of these accessions? This question deserves careful study.

Despite the asexual reproduction of *C. sativus*, a consistent level of genetic diversity was revealed in the present study using molecular analysis. The inter-microsatellite markers used are suitable for the molecular characterization and examination of genetic diversity among *C. sativus* accessions as well as elucidating genetic relationships among this species. The data of this study can potentially be used for forming a core collection of Moroccan *C. sativus* germplasms and to conserve this valuable crop in Morocco and in the world.

Although the number of accessions used in this work was limited, we obtained important results concerning the diversity of this plant. Therefore, it would be interesting to expand sampling and to apply more informative markers such as SSRs.

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