UDC 575.630
DOI: 10.2298/GENSR1502711I
Original scientific paper

MOLECULAR AND MORPHOLOGICAL VARIATION IN SOME IRANIAN SAFFRON (*Crocus sativus* L.) ACCESSIONS

F. IZADPANAH [a,*], S. KALANTARI [a], M. E. HASSANI [b], M. R. NAGHAVI [c], M. SHOKRPOUR[a]

[a] Department of Horticulture, Faculty of Agriculture, University of Tehran, Karaj 31587, Iran
[b] Faculty of Agriculture, Food and Natural Resources, University of Sydney, Australia
[c] Department of plant breeding, Faculty of Agriculture, University of Tehran, Karaj 31587, Iran

Izadpanah F., S. Kalantari, M. E. Hassani, M. R. Naghavi , M. Shokrpour (2015): *Molecular and morphological variation in some Iranian saffron (Crocus sativus L.) accessions* - Genetika, Vol 47, No. 2, 711-722

Saffron (*Crocus sativus* L.) is renowned as the most expensive spice in the world. This perennial species belongs to Iridaceae family. Germplasm collection and preservation are one of the main priorities for a better and successful crop improvement. In this study, preliminary evaluation of morphological and molecular diversity among 29 accessions of saffron from Iran was investigated. Several important traits such as length and number of leaves, leaves surface, fresh and dry weight of leaves were measured. RAPD markers using 17 primers were also used to evaluate molecular divergence among the accessions. Simple correlation analysis among morphological traits showed significant positive correlations in most characters, but negative correlations between emergence time and other characters. Cluster analyses based on morphological and molecular data produced dissimilar groups in due to data type. In both dendrograms three distinct groups were resulted and the most of the accessions were placed in the first cluster. Also, the groupings showed no association between diversity patterns and geographical origins. In molecular analysis, out of 17 primers that produced 108 polymorphic bands, 12 primers showed complete polymorphism. The maximum and minimum genetic similarities were 0.98 and 0.42, respectively. This results support abilities of these approaches as economical and quick technique to determination of diversity among saffron accessions.

Key words: Crocus sativus L., Germplasm, Morphological and Molecular variation, saffron

Corresponding author: F. Izadpanah, Department of Horticulture, Faculty of Agriculture, University of Tehran, Karaj 31587, Iran, Tel.: +98 263 2248 721; fax: +98 263 2248 721.
E-mail addresses: izadapanah@ut.ac.ir, izadpanah_f@yahoo.com

INTRODUCTION

Saffron (*Crocus sativus* L.) is a cultivated perennial plant from Iridaceae family which has evolved from *Crocus sativus* L. since ancient time (BRIGTON *et al*., 1980*).* Saffron is a sterile plant propagated vegetatively by corms. It is presented in a field for many years and produce annually valuable stigma as a spice (colorant) and condiment (nutritious food additive). Odor (safranal), tast (picrocrocin) and pigment (crocin) components constituting the spice are localized in the red stigmatic lobes of the flower (HIMENO and SANO, 1987; NEGHBI *et al*., 1989). Recently, some researchers have reported that the extract of *Crocus sp.* has antitumor, antimutagenic and cytotoxic activities (NAIR *et al*., 1991; ABDULLAEV, 2004; LOSCUTOV *et al*., 2000; FATEHI *et al*. 2003). Also, saffron is used in food industry including dairy products, candies, beverage as well as usage in pharmaceutical products (PÉREZ, 1995).

Saffron is cultivated under different climatic conditions, from Mediterranean to Middle East and India, mostly grown in areas with cold winter, low rainfall and hot summer, especially in Iran (Khorasan province) (MOLLAFILABI, 2004; FERNÁNDEZ, 2004). Except Iran as an important and main producer in the world, saffron is being cultivated in other countries such as Spain, Greece, Italy, Switzerland, Morocco, Turkey, India, Pakistan, Mexico, Argentine, New Zealand and recently in Australia (RUBIO-MORAGA *et al*., 2009).

Study of plant genetic diversity is a prerequisite of each breeding program (HADIAN *et al*., 2008). For this purpose, different morphological, molecular, physiological and phytochemical methods have been tested to reveal relationships among different species of plants (ÖZDEMIR *et al*., 2008; OKASAKA *et al*., 2009; IKINCI *et al*., 2010). So far, several researchers have attempted to study morphological, molecular and phytochemical characters of saffron germplasm in different countries (GRILLI CIOLA *et al*., 2001; PARDO *et al*., 2004; ZUBER *et al*., 2004; SOHEILIVAND *et al*., 2007; GRESTA *et al*., 2009; RUBIO-MORAGA *et al*., 2009; BAGHALIAN *et al*., 2010; FERNANDEZ *et al*., 2011).

In recent years, DNA- based markers have been used frequently for the assessment of genetic diversity among populations in many plant species (WILLIAMS, 1990). RAPD makers have been efficiently used to study genetic diversity of different plant species such as *Capsicum annum* (ILBI, 2003), *Allium sativum* L. (BAGHALIAN *et al*., 2005, 2006; ABDOLI *et al*., 2009) and *Prunus* species (SHIRAN, 2007; GOUTA *et al*., 2008; NIKOUMANESH *et al*., 2011).

Preserving Saffron germplasm by establishing collections of Iranian local accessions is appeared necessary. The first step for achieving this goal is to collect all saffron accessions from several natural growing parts in Iran. In this research, after establishment of collection of accessions from Khorasan province, their phenotypic and genotypic diversity were evaluated. Also, possible correlations between morphological traits in different accessions were determined and finally, molecular markers (RAPDs) was used to assess genetic divergence among the accessions.

MATERIALS AND METHODS

Plant material

The 29 saffron accessions obtained by collecting the corms from different fields in north east of Iran (Khorasan Province) during June 2008. The sampling was performed with cooperation of local Agricultural Extension Offices and producers. Geographical origins of the 29 accessions are listed in Table 1.

Morphological studies

In order to study the accessions for morphological attributes, a two-year field experiment was conducted during two growing seasons of 2008-2010 at the experimental station of horticulture department, University of Tehran, Karaj, Iran. The geographical location of the station was, 35º 48´ N, 51º E, 1320 m above m. s. The collected corms were sown in separate lines, at 20 cm depth, 15 cm apart between rows and 25 cm within row. Irrigation was done ten days after planting in September. Weed control and irrigation have been performed in succession. The soil texture was Clay – Loamy and average annual precipitation was 254.5 mm.

Table 1. Geographical origins of the 29 Iranian saffron accessions

Accessions	origins	Latitude (N)	Longitude (E)	Altitude (m)
1	Ghandeshtan	35º18.4´	59 º 11.7´	1412
2	Ghandeshtan	35º18.4´	59 º 11.7´	1412
3	Abrood	35 º 19´	59 º 17.7´	1413
4	Abrood	35 º 19´	59 º 17.7´	1413
5	Kajderakht	35 º 12´	59 º 05´	1213
6	Kajderakht	35 º 12´	59 º 05´	1213
7	Kajderakht	35 º 11´	59 º 06´	1231
8	Kajderakht	35 º 11´	59 º 06´	1231
9	Kajderakht	35 º 11´	59 º 07´	1212
10	Kajderakht	35 º 11´	59 º 07´	1212
11	Dasht- e- nook	34 º 31´	58 º 10´	1250
12	Dasht- e- nook	34 º 31´	58 º 10´	1250
13	Chahrbagh	34 º 51´	58 º 16´	1242
14	Chahrbagh	34 º 51´	58 º 16´	1242
15	Chahrbagh	34 º 51´	58 º 16´	1242
16	Baghestan	34 º 38´	58 º 70´	1252
17	Research Station, Gonabad	34 º 38´	58 º 70´	1253
18	Research Station, Gonabad	34 º 38´	58 º 70´	1253
19	Research Station, Gonabad	34 º 38´	58 º 70´	1253
20	Noghab(Ghasabe)	34 º 21´	58 º 43´	1083
21	Noghab(pe -ye- deh)	34 º 20´	58 º 42´	1098
22	Biland	34 º 24´	58 º 42´	1055
23	Dooghabad	35 º 02´	58 º 49´	990
24	Dooghabad	35 º 06´	58 º 53 ´	1075
25	Dooghabad	35 º 05´	58 º 50´	1025
26	Shadmehr	35 º 10´	59 º 03´	1207
27	Afghoo	34 º 01´	58 º 19´	1236
28	Ghaien	33 º 57´	59 º 16´	1428
29	Research Station, Gonabad	34 º 38´	58 º 70´	1253

At the end of each growing season (May 2010), aerial parts (leaves) of the plants were harvested from all the accessions. Afterwards, they were taken to laboratory and number of leaf, leaf length, leaf area and fresh weight were measured, and then to measure dry weight they were placed in oven at 70°c for 24 h. The length of leaves in each plant was measured from the end of leaf spath to the tip of leaf, and the number of leaf was the total leaves of each plant then the average of these data was calculated. Calculating of emergence time was based on the period after the first

watering up to emergence of leaves. Corms were weighted firstly, before planting and secondly at the end of second growing season.

Molecular studies

Total genomic DNA was isolated from 120 mg of fresh young leaves using the procedure described by Dellaporta et al., 1983. The genomic DNA quality and quantity were determined by comparing with a standard λ DNA set (SM0313 Fermentase, TM Life Scinces) on 1.2% agarose gel. For RAPD analysis, PCR reactions were carried out in a 15 reaction volume containing 1x PCR buffer, 1.75 Mm $mgcl_2$, 200 m $dNTP_s$, 0.4 M of each RAPD primer (OPERON & TIB-MOLBOIL Co., Germany), 1U Taq DNA polymerase (CinnaGene, Iran) and 10 ng of genomic DNA. Amplification was performed in a 96 well-block thermocycler (iCycler, Bio Rad Co., USA) programmed for 45 consecutive cycles, initial step of 4 min at 94 ° C, followed by 35 cycles of 92 ° C for 1 min, 37º C for 1 min, 72 ° C for 2 min and a final extension at 72ºC for 5 min. Amplification products were resolved by electrophoresis through a 1.2 % (w/v) agarose (Roche Co., Germany) gel in 1x TBE buffer for 120 min under 120V. PCR products were visualized by ethidium bromide ($5\mu gml^{-1}$) staining and photographed under UV- transiluminator, by a Gel Doc system (UVP, Bio Doc Co., USA).

Data analysis

In order to evaluate relationships between morphological parameters, Pearson's correlation coefficients were calculated by SPSS software v.19. UPGMA clustering was conducted based on mean values of 2 years of the measured characters using Jaccard distance matrix.

Amplified products were scored as present (1) or absent (0) to form a binary matrix. Nei & Li (1979) index was used to estimate the genetic similarities between the accessions. PIC coefficient ($1-\Sigma p_i^2-\Sigma\Sigma 2p_i^2\ p_j^{2)}$ and Marker Index (PIC × no. of polymorphic bands) was assayed to determine the primer efficiency in clarifying the accessions relationships. Molecular grouping was obtained by UPGMA method using NTSYS package v. 2.02 (ROHLF, 1998).

RESULTS

Morphological analysis

In morphological evaluations, significant and positive correlations were observed between length and number of leaves, leaves surface, fresh and dry weight of leaves variables. For example, when the number of leaves increase the fresh and dry weight of leaves increase too. Among quantitative traits, emergence time had negative relation with other studied attributes in the accessions (Table 2). Cluster analysis based on morphological traits resulted in grouping the 29 accessions into three main groups (Fig. 1). Out of the 29 accessions, twenty five were included in the first cluster, while accessions of Dooghabad and Shadmehr were placed in second cluster and the accessions of Noghab and Afghoo were grouped in third cluster. There was not good correspondence among the morphological grouping and their geographical origins.

Table 2. Pearson correlation coefficients of morphological traits in 29 accessions of saffron (C. sativus L).

Traits ET	LN	LA (cm^2)	LL (cm)	FW (g)	DW(g)	CW(g)	
LN	1						
LA	0.66**	1					
LL	0.93**	0.9**	1				
FW	0.87**	0.97**	0.97**	1			
DW	0.88**	0.94**	0.99**	0.99**	1		
CW	0.88**	0.94**	0.99**	0.99**	1.00**	1	
ET	-0.35	-0.73**	-0.57**	-0.67**	-0.63**	-0.63**	1

**P<1%,LN (leaf number), LA (leaf area), LL (leaf length), FW (fresh weight), DW (dry weight), CW (corm weight), ET (emergence time).

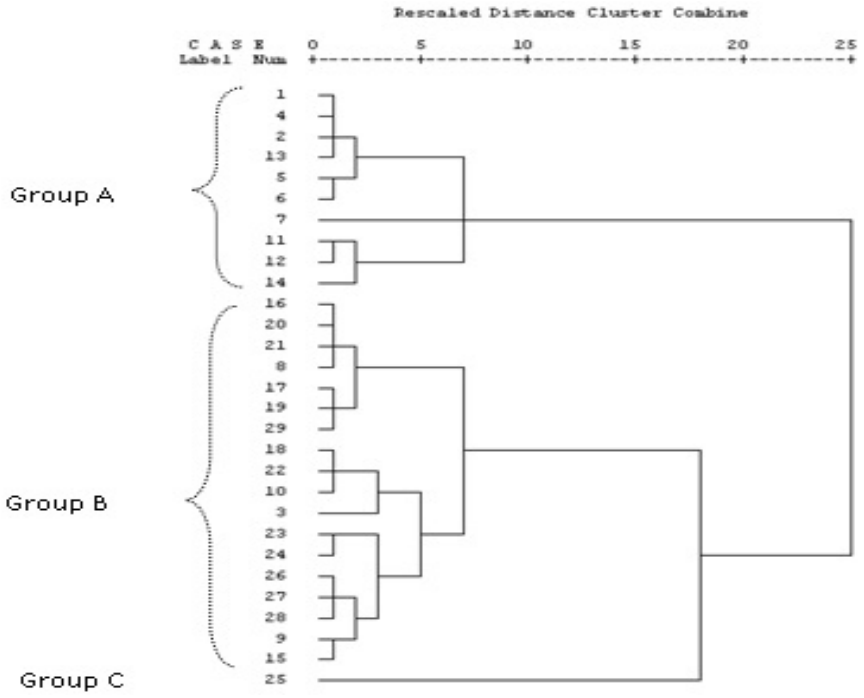


Fig.1. UPGMA clustering of morphological traits in the saffron accessions (see table 1 for the name of the accessions)

Molecular analysis

Table 3. Sequences of primers employed and the number of total and polymorphic bands produced on RAPD analysis

Name of primer	Sequence (5´- 3´)	Total no. of bands	No. of polymorphic bands	percent of polymorphism	PIC	Marker Index
BA08	CCACAGCCGA	11	11	100	0.69	7.59
BA10	GGACGTTGAG	12	12	100	0.70	8.4
BA11	CCACCTTCAG	8	8	100	0.65	5.2
BA12	TGTTGGGCAC	3	2	66.66	0.24	0.48
BA15	GAAGACCTGG	5	5	100	0.53	2.65
BA17	TGTACCCCTG	9	9	100	0.70	6.3
BA18	CTCGGATGTC	10	10	100	0.60	6
BB20	CCAGGTGTAG	4	4	100	0.46	1.84
BC10	AACGTCGAGG	8	8	100	0.63	5.04
BC14	GGTCCGACGA	6	6	100	0.60	3.6
BC16	CTGGTGCTCA	6	4	66.66	0.60	2.4
BD13	CCTGGAACGG	7	1	14.28	0.54	0.54
BE02	ACGCCTGTAG	3	3	100	0.37	1.11
OPC5	GATGACCGCC	4	4	100	0.46	1.84
OPC7	GTCCCGACGA	7	4	57.14	0.64	2.56
OPC9	CTCACCGTCC	7	5	71.42	0.64	3.2
OPAB4	GGCACGCGTT	5	5	100	0.47	2.35
Total		118	101			
Average		6.5	5.6	92.85		

Out of 100 RAPD primers, 17 primers with good and repeatable bands were selected for molecular description of the accessions (Table 3. A total of 101 polymorphic bands were obtained by 17 primers. The size of amplified fragments ranged between 250 and 3500 bp for all primers. The maximum similarity value (Dice coefficient) was 0.98 between Kajderakht accessions (No.9 and No.5) and the minimum was 0.42 between Afghoo and Dooghabad accessions (Table 4). The

primer of TIBMBA-10 had high PIC (0.7) value and Marker Index that implied its efficiency in clarifying molecular variation among the accessions. The minimum PIC and marker index were found in primer of TIBMBA-12.

The cluster analysis based on molecular data formed three distinct groups (Fig. 2). First group consisted of 27 accessions, but in both groups of B and C, just one accession, Noghab and Afghoo, were grouped, respectively.

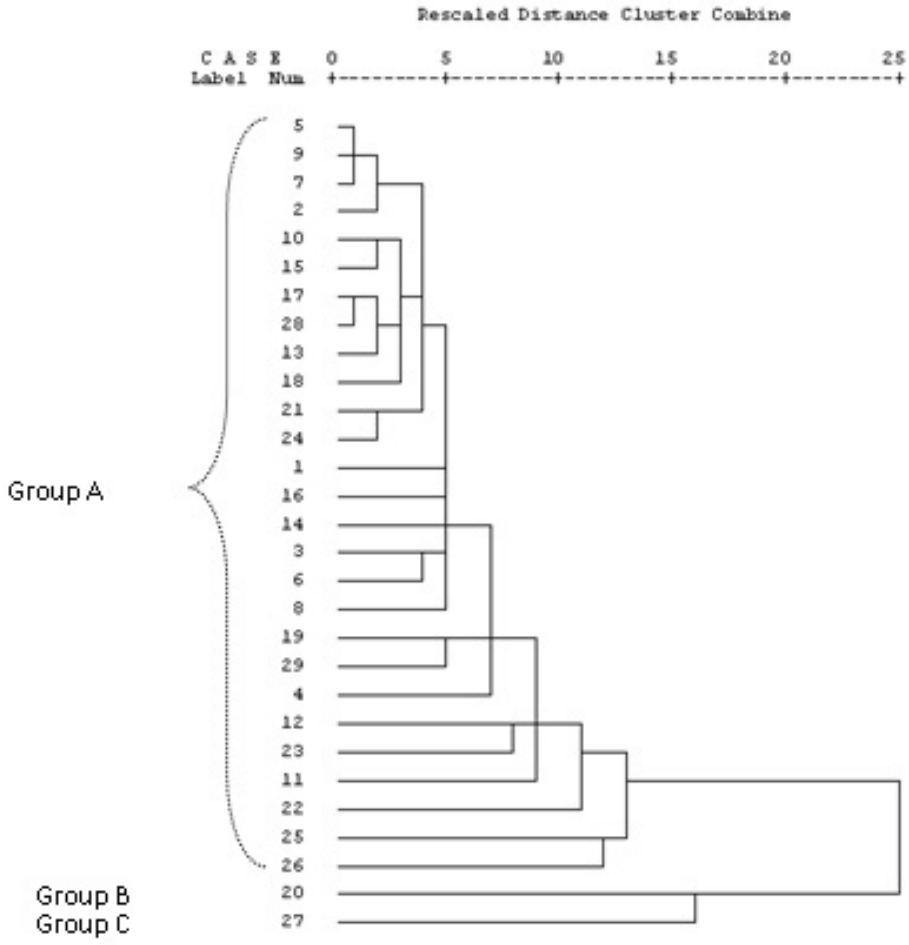
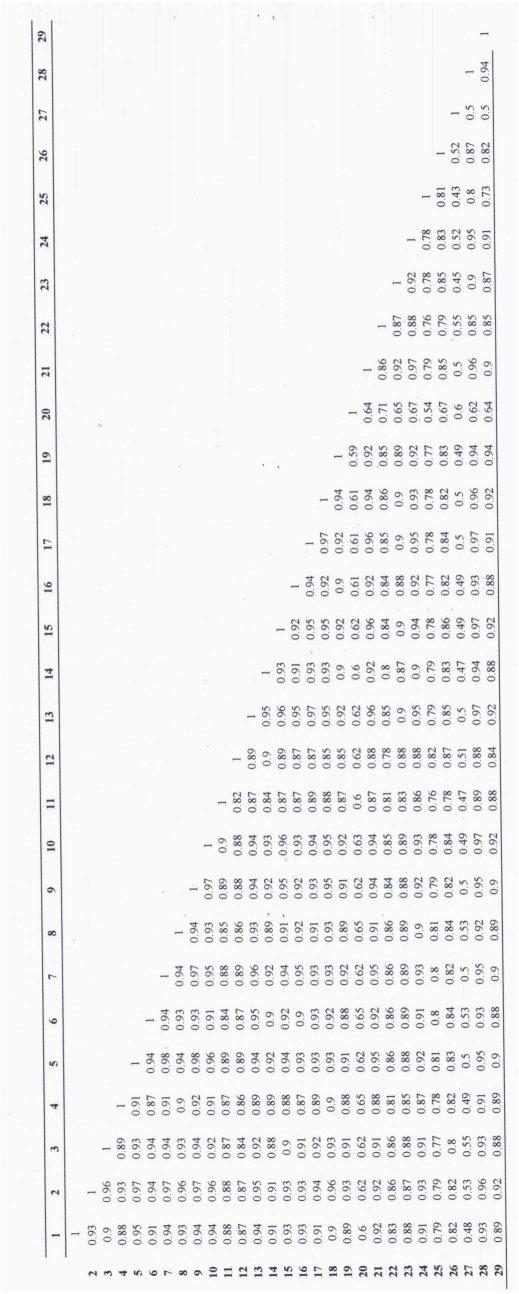


Fig.2. UPGMA clustering based on RAPD data in the saffron accessions (see table 1 for the name of the accessions)



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29
1	1																												
2	0.93	1																											
3	0.9	0.96	1																										
4	0.88	0.93	0.89	1																									
5	0.95	0.97	0.93	0.91	1																								
6	0.91	0.94	0.94	0.87	0.94	1																							
7	0.94	0.97	0.94	0.91	0.98	0.94	1																						
8	0.93	0.96	0.93	0.9	0.94	0.93	0.94	1																					
9	0.94	0.97	0.94	0.92	0.98	0.93	0.97	0.94	1																				
10	0.94	0.96	0.92	0.91	0.96	0.91	0.95	0.93	0.97	1																			
11	0.88	0.88	0.87	0.87	0.89	0.84	0.88	0.85	0.89	0.9	1																		
12	0.87	0.87	0.84	0.86	0.89	0.87	0.89	0.86	0.88	0.88	0.82	1																	
13	0.94	0.95	0.92	0.89	0.94	0.95	0.96	0.93	0.94	0.94	0.87	0.89	1																
14	0.91	0.91	0.88	0.89	0.92	0.9	0.92	0.89	0.92	0.93	0.84	0.9	0.95	1															
15	0.93	0.93	0.9	0.88	0.94	0.92	0.94	0.91	0.95	0.96	0.87	0.89	0.96	0.93	1														
16	0.93	0.93	0.91	0.87	0.93	0.9	0.95	0.92	0.92	0.93	0.87	0.87	0.95	0.91	0.92	1													
17	0.91	0.94	0.92	0.89	0.93	0.93	0.93	0.91	0.93	0.94	0.89	0.87	0.97	0.93	0.95	0.94	1												
18	0.9	0.96	0.93	0.9	0.93	0.92	0.93	0.93	0.95	0.95	0.88	0.85	0.95	0.93	0.95	0.92	0.97	1											
19	0.89	0.93	0.91	0.88	0.91	0.88	0.92	0.89	0.91	0.92	0.87	0.85	0.92	0.9	0.92	0.9	0.92	0.94	1										
20	0.6	0.62	0.62	0.65	0.62	0.65	0.62	0.65	0.62	0.63	0.6	0.62	0.62	0.6	0.62	0.61	0.61	0.61	0.59	1									
21	0.92	0.92	0.91	0.88	0.95	0.92	0.95	0.91	0.94	0.94	0.87	0.88	0.96	0.92	0.96	0.92	0.96	0.94	0.92	0.64	1								
22	0.83	0.86	0.86	0.81	0.86	0.86	0.86	0.86	0.84	0.85	0.81	0.78	0.85	0.8	0.84	0.84	0.85	0.86	0.85	0.71	0.86	1							
23	0.88	0.87	0.88	0.85	0.88	0.89	0.89	0.89	0.88	0.89	0.83	0.88	0.9	0.87	0.9	0.88	0.9	0.9	0.89	0.65	0.92	0.87	1						
24	0.91	0.93	0.91	0.87	0.92	0.91	0.93	0.9	0.92	0.93	0.86	0.88	0.95	0.9	0.94	0.92	0.95	0.93	0.92	0.67	0.97	0.88	0.92	1					
25	0.79	0.79	0.77	0.78	0.81	0.8	0.8	0.81	0.79	0.78	0.78	0.87	0.85	0.83	0.86	0.82	0.84	0.82	0.83	0.67	0.85	0.79	0.85	0.83	1				
26	0.82	0.82	0.8	0.82	0.83	0.84	0.82	0.84	0.82	0.84	0.78	0.87	0.85	0.83	0.86	0.82	0.84	0.82	0.83	0.67	0.85	0.79	0.85	0.83	0.81	1			
27	0.48	0.53	0.55	0.49	0.5	0.53	0.5	0.53	0.5	0.49	0.47	0.51	0.5	0.47	0.49	0.49	0.5	0.5	0.49	0.6	0.5	0.55	0.45	0.52	0.43	0.52	1		
28	0.93	0.96	0.93	0.91	0.95	0.93	0.95	0.92	0.95	0.97	0.89	0.88	0.97	0.94	0.97	0.93	0.97	0.96	0.94	0.62	0.96	0.85	0.9	0.95	0.8	0.87	0.5	1	
29	0.89	0.92	0.88	0.89	0.9	0.88	0.9	0.89	0.9	0.92	0.88	0.84	0.92	0.88	0.92	0.88	0.91	0.92	0.94	0.64	0.9	0.85	0.87	0.91	0.73	0.82	0.5	0.94	1

Table 4. Similarity coefficients among the saffron accessions based on RAPD data

DISCUSSION

One of the important reasons for studying the saffron accessions in this research was lack of enough information around variation of the different saffron clones grown in a broad range of areas in Iran. Saffron is a sterile plant which vegetative propagation may also reduce and influence its genetic diversity and spatial genetic structure of local accessions that often consist of the patches of clonal individuals. Despite of this propagation method in saffron and relatively restricted geographical range covered by the investigation, the accessions studied exhibited a pronounced genetic divergence.

The correlation coefficients displayed strong relations among all the attributes. Positive correlation between corm weight and other growing traits is an important relationship for improving quantitative yield. The effect of choosing larger corms at planting saffron has been considered by several researchers (NEGBI *et al*., 1989; SADEGHI, 1993; LATIFI and MASHAYEKHI, 1996; and SOHEILIVAND *et al*., 2006). They have shown that by increasing corm size, the highest yield were obtained during several cropping years. Since, increasing the morphological traits e.g. leaf number can stimulate the photosynthesis and other metabolic pathways in plant. So, it is not unexpected that elevation of morphological traits such as leaf number and area may cause to increase other traits e.g. stigma, flower no. and also phytochemical compounds such as safranal and picrocrocin (BAGHALIAN *et al*., 2010).

Combining morphological evaluation and molecular markers leads to more reliable results in the assessment of genetic variation (HADIAN *et al*., 2008; HEND *et al*., 2009; NIKOUMANESH *et al*., 2011).

According to similarity matrix and dendrogram based on RAPD data, some of accessions were placed in the same groups, representing a close genetic similarity. Natural selection process in each population leads to accumulation of adaptive genes. RAPD is dispersed through the genome and their association with agronomic traits is influenced by the breeder only in the region under selection pressure (FERNÁNDEZ *et al*., 2002). There was not clear relationship between genetic divergence and geographical origins in the clusters. In morphological cluster, the accessions from similar geographical places (No.1 and No.3) grouped in different clusters. Moreover, the same results were obtained in molecular clustering. This result may be found because of frequent translocation of saffron corms among different villages and cities. Therefore, these exchanges of corms throughout the country could explain the overlapping grouping of some accessions from different regions of Iran.

In recent years, several studies were focused on classification of different genus of Crocus (GRILLI CIOLA *et al*., 2004; ÖZDEMIR *et al*., 2008; BAGHALIAN *et al*., 2010), but few of them included the differences between various accessions of cultivated saffron. BAGHALIAN *et al* (2010) have confirmed necessary of research on genetic variation and heritability of agro-morphological and phytochemical traits of this crop. They remarked genetic variation of vegetative and qualitative traits of this species and independence of similarity among populations and their origin.

In conclusion, morphological and molecular analyses showed a good genetic diversity between different accessions. These achievements are important for germplasm management and also will help breeders in selection programs to obtain a desirable cultivar. So, it is expected that this collection could provide a sufficient genetic variation and good sample set for choosing highly polymorphic markers; however, the result of this research is not the large and more database needed to calculate meaningful parameters of population genetic structure e.g. allel frequencies.

ACKNOWLEDGMENT

We gratefully acknowledge the excellent cooperation of Jahad Keshavarzi, Khorasan Razavi in Mashhad through this research, especially Mr. Masood Vejdani.

Received February 02h, 2015
Accepted May30th, 2015

REFERENCES

ABDOLI, M., B.HABIBI-KHANIANI, K. BAGHALIAN, S. SHAHNAZI, H RASSOULI, H.NAGHDI BADI (2009): Classification of Iranian Garlic (*Allium sativum* L.) Ecotypes Using RAPD Marker. - J. Medicinal Plant, *8*: 45-51.

ABDULLAEV, F.I. (2004): Antitumor effect of saffron (*Crocus sativus* L.). Overview and perspectives. - Acta Hort., *650*: 491-499.

AKASAKA, M., J. USHIKI, H. IWATA, R, ISHIKAWA, T. ISHII (2009): Genetic relationships and diversity of weedy rice (*Oryza sativa* L.) and cultivated rice varieties in Okayama Prefecture, Japan. - Breeding Science, *59*: 402-409.

BAGHALIAN, K., ZAIAI, S.A., NAGHDI BADI, H.A. (2006): Post-planting evaluation of morphological characters and allicincontent in Iranian garlic (*Allium sativum* L.) ecotypes.- Sci. Hortic.,*107*: 405-410.

BAGHALIAN, K., M. SHABANI SHESHTAMAND, A.H. JAMSHIDI (2010): Genetic variation and heritability of agro-morphological and phytochemical traits in Iranian saffron (*Crocus sativus* L.) population. - Ind. Crops Prod., *31*: 401-406.

DELLAPORTA, S.L., J. WOOD, J.B. HICKS (1983): A plant DNA miniprepration version Π. - Plant Molecular Biology Reporter, *1*: 19-21.

FERNÁNDEZ, M.E., A.M. FIGUEIRAS, C. BENITO (2002): The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity among barely cultivars with known origion.- Theor. Appl. Genet., *104*: 845-851.

FERNÁNDEZ, J.A., O.SANTANA, J.L. GUARDIOLA, R.V, MOLINA, P.HESLOP-HARISON, G. BORBELY, F.,BRANCA, S. ARGENYTO, E. MALOUPA, T. TALOU, J.M.THIERCELIN, K, GASIMOV, H. VURDU, M., ROLDÁN, M. SANTAELLA, E. SANCHÍ, A, GARCÍA-LUIS, G, SURANYI, A, MOLNÁR, G, SRAMKO, G. GULYAS, L. BALAZ, O. HORVAT, M., RUDRÍGUEZ, R. SNCHEZ-VIOQUE, M.Á,ESCOLANO, N.KRIGAS, T. PASTOR, B.RENAU-MORATA, C. RAYNAUD, O.,IBADLI, M. POLISSIOU, Z. M. TSIMIDOU, A, TSAFTARIS, M. SHARAF-ELDIN, J. MEDINA, T. CONSTANTINIDIS, T.KARAMPLIANIS, M.DE-LOS-MOZOS-PASCULA (2011): The World Saffron and Crocus collection: strategies for establishment, management, characterisation and utilisation. - Genet Resour Crop Evol., *58*: 125-137.

GOUPTA, H., E.KSIA, , N.ZOGHLAMI, , M.ZARROUK, , A. MLIKI, (2008): Genetic diversity and phylogenetic relationships among Tunisian almond cultivars revealed by RAPD markers. - J. Hortic. Sci. Biotechnology, *83* (6): 707-712.

GRILLI CIOLA, M., P. CAPUTO, R. ZANIER (2004): RAPD analysis in *Crocus sativus* L. accessions and related *Crocus* species. - Biol Planty, *48*: 375-380.

GRESTA, F., G. AVOLA, G. M. LOMBARDO, L. SIRACUSA, G. RUBERTO (2009): Analysis of flowering, stigmas yield and qualitative traits of saffron (*Crocus sativus* L.) as affected by environmental conditions. - Scientia Horticulturae, *119*: 320–324.

HADIAN, J., S.M.F.TABATABAEI, M.R. NAGHAVI, Z. JAMZAD, T.RAMAK-MASOUMI, (2008): Genetic diversity of Iranian accessions of *Satureja hortensis* L. based on horticultural traits and RAPD markers. - Scientia Horticulturae, *115*: 196-202.

HEND., B.T., B. GHADA, B.M. SANA, M. MOHAMED, T. MOKHTAR, S.H. AMEL (2009): Genetic relatedness among Tunisian plum cultivars by random amplified polymorphic DNA analysis and evaluation of phenotypic charecters. - Sci. Hortic., *121*: 440-446.

HIMENO, H., K. SANO (1987): Synthesis of crocin, picrocrocin and safaranal by saffron stigma- like structures prolifrated in vitro. - Agric. Biol. Chem., *51*: 2395-2400.

ILBI, H. (2003): RAPD markers assisted varietal identification and genetic purity test in Pepper, *Capsicum annum.* - Sci. Hortic., *97*: 211-218.

IKINCI, N., C.OBERPRIELER (2010): Genetic relationships among NE Turkish *Lilium* L. (Liliaceae) species based on a random amplified polymorphic DNA analysis. - Plant Syst. Evol., *284*: 41-48.

LAGE, M., C.L. CANTRELL (2009): Quantification of saffron (*Crocus sativus* L.) metabolites crocins, picrocrocin and safranal for quality determination of the spice grown under different environmental Moroccan conditions. - Sci. Hortic., *121*: 366-373.

LATIFI, N., K.MASHAYEKHI, (1996): Effect of corm weight on saffron flowering. - In: Proceeding of 4th Congress on Cultivation and Plant Breeding of Iran. Technology University of Esfahan, Iran.

MOLLAFILABI, A. (2004): Evaluations of saffron (*Crocus sativus* L.) yield components. - Acta Hort., *650*: 211-214.

NEGBI, M., B. DAGAN, A. DROR, D.BASKER (1989): Growth, flowering, vegetative reproduction and dormancy in the saffron (*Crocus sativus* L.). - Israel J. Bot., *38*: 95-113.

NIE, M., W.H. LI (1979): Mathematical model for studying genetic variation in terms of restriction endonucleases. - Proc. Natl. Acad. Sci. U. S.A. *76*: 5269- 5273.

NIKOUMANESH, K., A. EBADI, M. ZEINALABEDINI, Y. GOGORCENA (2011): Morphological and molecular variability in some Iranian almond genotypes and related *Prunus* species and their potential for rootstock breeding. - Sci. Hortic., *129*: 108-118.

NEGBI, M. (1999): Saffron cultivation: past, present and future prospects. - In: Negbi, M. (Ed.): Saffron *Crocus sativus* L. Pp.19-30. Harwood Academic Publ., Amsterdam.

ÖZDEMIR, C., M. KILINÇ (2008): Morphology and anatomy of three subsp. Of *Crocus Speciosus* Bieb. - Bangladesh J. Bot., *37*(2): 97-103.

PARDO, J., J.A. FERNANDEZ, L.GÓMEZ-GÓMEZ (2004): Development of molecular markers for origin determination in Saffron. - Acta Hort., *650*: 95-97.

PÉREZ, M. (1995): El azafrán, historia, cultivo, comerico, gastronomía, Ediciones, Agrotécnicas, Madrid, Spain.

ROHOLF, F.J. (1998): NTSYS-pc Numerical Taxonomy and Multivariate Analysis System.Version 2.02. - Exeter Software, Setauket, Newyork.

RUBIO-MORAGA, A., R.CASTILLO–LÓPEZ, , L.GÓMEZ-GÓMEZ, O. AHRAZEM (2009): Saffron is a monomorphic species as revealed by RAPD, ISSR, microsatellite analyses. - BMC Research Notes, *2*:189.

SADEGHI., B. (1993): Effect of corm weight on saffron flowering. - I.R.O.CS.T. Mashhad Center, Mashhad, Iran.

SHIRAN, B., N. AMIRBAKHTIAR, S.KIIANI, SH MOHAMMADI, B.E. SAYED-TABATABAEI, H. MORADI,(2007): Molecular charecterization and genetic relationship among almond cultivars assessed by RAPD and SSR markers. - Sci. Hortic., *111*: 280-292.

SOHEILIVAND, S., Y. AGAYEV, A.MSHAKIB, M.FATHI (2007): Comparision of diversity in flowering rate of two Saffron (*crocus sativus* L.) popultion of Iran. - Acta Hort. 650: 317-320.

WILLIAMS, J.G.K., A.E.,KUBELIK, K.J. LIVAK, J.A. RAFALSKI, S.C. TINGEY (1990): DNA polymorphism amplified by arbitrary primer are useful as genetic markers. - Nucl Acids Res., *18*: 6531-6536.

ZUBOR, A.A., G. SURANYI, Z. GYORII, G. BORBELY, J.PORKISH (2004): Molecular Bilogical Approach of the systematics of *crocus sativus* L.and Allies. - Acta Hort., *650*: 85-93.

MOLEKULARNO I MORFOLOŠKO VARIRANJE NEKIH GENOTIPOVA IRANSKOG ŠAFRANA (*Crocus sativus* L.)

F. Izadpanah [1,*], S. Kalantari [1], M. E. Hassani [2], M. R. Naghavi [3], M. Shokrpour[1]

[1] Odelenje za hortikulturu, Poljoprivredni fakultet, Univerzitet u Teheranu, Karaj 31587, Iran

[2] Poljoprivredni fakultet, hrana i prirodni resursi, Univerzitet u Sidneju, Australija

[3] Odelenje za oplemenjivanje biljaka, Poljoprivredni fakultet, Univerzitet u Teheranu, Iran

Izvod

Šafran (*Crocus sativus* L.) je najskuplji začin u svetu. Perenialne vrste pripadaju porodici *Iridaceae*. Vršena je preliminarna evaluacija morfološke i molekularne divergentnosti između 29 genotipova šafrana iz Irana u dužini i broju listova, površine lista, sveža i suva masa lista . Korišćeni su RAPD markera sa 17 prajmera za ocenu molekularne divergentnosti između genotipova. Analizom jednostavne korelacione analize utvrđene su signifikantne pozitivne korelacije među morfološkim osobinama ali negativne korelacije između vremena nicanja i drugih karakteristika. Analiza dendograma zasnovanih na morfološkim i molekularnim podacima daju različite grupe zbog tipa podataka. Kod oba dendograma tri različite grupe su dobijene kod većine genotipova i smeštene su u prvom klasteru. Grupisanja su pokazala da nema asocijacije između geografskog porekla i divergentnosti. Od 17 prajmera koji su proizveli 108 polimorfnih traka 12 je pokazalo kompletan polimorfizam. Utvrđene su maksimalne (0.98) i minimalne (0.42) genetičke sličnosti. Rezultati pokazuju da se ove tehnike mogu koristiti kao ekonomske i brze u determinaciji diverziteta između genotipova šafrana.

Primljeno 03. II. 2015.
Odobreno 30.V. 2015.