Jordan Journal of Agricultural Sciences, Volume **4**, No.**3**, 2008

Study of Genetic Diversity in *Crocus hyemalis* Boiss. and Blanche Using RAPD Techniques

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ABSTRACT

Genetic relationships among populations of *Crocus hyemalis* Boiss. and Blanche collected from different regions of Jordan were studied using Random Amplified Polymorphic DNA analysis. *C. hyemalis* was also compared to the cultivated species *C. sativus* and *C. vernus*. RAPD and the cluster analysis indicated high degree of inter- and intra-populations variation within the wild *C. hyemalis* populations. High genetic association was found among some wild populations originating from the same collection sites. The close genetic similarity of the *C. hyemalis* collected from Al-Burge with the cultivated *C. vernus* may indicate that this population could be easily brought into cultivation as an ornamental crop due to its showy flowering habit. The possibility of having a new subspecies from *C. hyemalis* population collected from Al-Burge, will be challenged by future rigorous taxonomic analysis and morphological investigation.

Keywords*: Crocus*, RAPD, Genetic diversity, Jordan wild plants.

INTRODUCTION

Crocus hyemalis Boiss. and Blanche (winter saffron) with variation in chromosome number 2n=6 and 6+1-4 (Feinbrurn, 1958) is a perennial stem-less herb of the Iridaceae family. The species is naturally distributed in northern Jordan associated with Oak Park forest and occupies a narrow range of scattered distribution. The distinct characters of this species are the dark purple anthers, yellow perianth throat and honey sent smell flowers with tunic membranous corms. The plant blooms during November to January (Dothan, 1986; Al-Eisawi, 1998).

Crocus sativus L. commonly known as saffron is a male sterile species (2n=3x=24) that is reproduced via corms (Mathew, 1977; Brighton, 1977; Fernandez, 2004). Saffron is not found in natural habitats but has been cultivated in the Mediterranean basins since the late Bronze Age (Zohary and Hopf, 1994; Grilli Caiola *et al*., 2001). The dry stigmas of *C. sativus* L. is currently used as a spice and food colorant (Alonso *et al*., 1990; Escribano *et al*., 1996; Zeng *et al*., 2003). Saffron's therapeutic medicinal benefits are well recognized since

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Received on 25/4/2007 and Accepted for Publication on 28/4/2008.

ancient civilization up to date (Rois *et al*., 1996; Ferrence and Bendersky, 2004). In recent years, the therapeutic value of saffron in certain cancers, cerebrovascular and cardiovascular diseases, has been well documented (Nair *et al*., 1991; Abdullaev and Frenkel, 1992; Abdullaev, 1993; Escribano *et al*., 1996; Rois *et al*., 1996). The known antioxidative activity of *Crocus* is attributed to crocins, long chain highly unsaturated and conjugated tetraterpenes, which give the stigmas their color, the picrocrocin which gives the bitterness and the safranal which gives its odor and used in flavoring (Zarghami and Heinz, 1971; Visvanath *et al*., 1990; Zeng *et al*., 2003; Auria *et al*., 2006). Recently and using LC-MS analysis, Al-Balas (2004) isolated and identified four compounds from the Jordanian *C. hyemalis*: crocin-5, crocin-3, crocin-2 and crocin-1. All compounds showed medium cytotoxicity when they were tested using the brine shrimp lethality test. These compounds were reported for the first time from this species. In a recent ethnopharmacological survey of medicinal herbs of Ajloun Heights in Jordan, *C. hyemalis* was the plant of the highest use value (Aburjai *et al*., 2007).

The cultivation of *C. sativus* L. has decreased steadily and it is about to disappear in some traditional producing countries (Visvanth *et al*., 1990; Molaina *et al*., 2005; Chen *et al*., 2004). Despite the value and the significance of this species, studies related to identifying the genetic resources and genetic diversity are lacking. An understanding of genetic diversity is essential to properly maintain and exploit germplasm resources and to develop a global strategy for better management and more effective use of variation in collected germplsam (Brown, 1989a; 1989b).

However, usage of morphological characters alone for genetic diversity studies can not be considered a successful tool because their genetic control is largely unknown and their expression depends on environmental and geographical factors (Boulli *et al*., 2001; Degani *et al*., 1998); while DNA fingerprinting techniques overcome the previous limitation and exhibit a great potential as a tool for a wide range of areas in plants including genotype identification, population genetics and others (Weising *et al.*, 1995).

Random Amplified Polymorphic DNA (RAPD) is one of the most extensively used molecular techniques for assessing species genetic diversity and is established as a preferable technique over morphological characterization (Degani *et al*., 1998; Ferguson *et al*., 1998; Lowe *et al*., 1996; Syouf *et al*., 2006; Migdadi *et al*., 2004; Al-Nashash *et al*., 2007; Khasawneh *et al.* , 2007).

Furthermore, Grilli Caiola *et al*. (2004) used RAPD technique to test *Crocus sativus* relatedness to *C. cartwrightianus* and *C. thomasii*.

In this research, we report, for the first time, on the genetic diversity present in the wild *C. hyemalis* populations collected from Jordan in comparison to the cultivated species *C. sativus* and *C. vernus* using RAPD techniques.

MATERIALS AND METHODS

Plant Material and Their Collection Sites

During November to January of 2004-2005, 42 populations of *C. hyem*alis were collected from different regions in Jordan. The cultivated *C. sativus* was brought from Morocco and the ornamental C. *vernus* was purchased from the local market. Collection sites, population's codes and their major ecogeographical parameters are presented in Table 1.

DNA Isolation

DNAs were extracted from (0.5 g) leave tissue using a Promega Wizard genomic DNA purification plant kit according to instructions provided by the manufacturer (htt:\\www.promega.com).

The DNA pellet was rehydrated using 100 µL of DNA rehydration solution and stored at −20 °C until use. The isolated DNA was checked for purity and quantity by spectrophotometer method as described by Sambrook *et al.* (1989).

Table (1): *Crocus hyemalis* populations, their collection sites, codes, elevation, latitude, longitude and collection dates during the year 2004-2005.

	Population Name/ Collection Site	*Population Code*	*Elevation (m)*	*Latitude East*	*Longitude North*	*Collection Date*
1.	*Crocus hyemalis*/ Souf.	1-4	1052.5	35 49 17.7	32 20 07.5	21/12/2004
2.	*C. hyemalis/* Bergish	5-9	843.1	35 45 24.1	32 25 10.9	25/11/2004
3.	*C. hyemalis*/ Junaid	10-14	1052.8	35 47 20.5	32 21 27.0	25/11/2004
4.	*C. hyemalis*/ Samta	15-19	1055.0	35 49 06.2	32 23 21.2	21/12/2004
5.	*C. hyemalis/* Rehaba 1	20-24	1015.0	35 49 15.5	32 42 15.6	21/12/2004
6.	*C. hyemalis*/ Rehaba 2	25-29	943.0	35 48 53.6	32 24 30.1	28/12/2004
7.	*C. hyemalis*/ Usaim	30-35	913.0	35 48 26.2	32 24 09.6	28/12/2004
8.	*C. hyemalis*/ Rasoon	36-40	852.0	35 46 43.2	32 23 58.6	28/12/2004
9.	*C. hyemalis*/ Al-Burge	41-42	1026.4	35 51 20.8	32 19 23.7	5/01/2005
10.	*C. vernus* (Local market)	43				
11.	*C. sativus* (Morocco)	44				

RAPD Analysis

The 42 DNA wild *C. hyemalis* samples and the two cultivated *Crocus* species were subjected to DNA fingerprinting. In order to determine the typeability, reproducibility and discrimination of each primer, separate amplification of each primer was conducted (three trials for each primer). The output of each experiment was compared to the previous one.

The standard RAPD amplification protocol recommended by Williams *et al.* (1990) was followed with some modifications. Amplifications were carried out in 25 µl reaction mixture containing 1X PCR buffer mixed with $MgCl_2$ [50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5mM $MgCl_2$ and 0.1% triton X-100)] (Promega, catalog # M2661), 100 µM of each dNTP (Promega, USA), 1.5µl 10-base primers at five picomoles/µl, 0.5 µl (5u/ µl) Taq DNA polymerase (Promega, USA) and 10 ng template DNA. The final volume of the reaction was brought to 25 µl nuclease free water and placed in a PCR tube.

Amplification reactions were performed in a thermal cycler (MJ-Research, Model PTC 200 programmed as follows: initial denaturizing step at 95 °C for 2 minutes, followed by 35 cycles of denaturizing at 95 °C for 40 seconds each, annealing at 34 °C for 40 seconds and extension at 72 °C for 2 minutes. DNA sampls were subjected to final extension cycle at 72°C for 5 minutes. Finally, the samples were either held at 4°C for direct

use or stored at −20°C until needed.

Amplified products were electrophoresed in 1.5 % agrose gels (Bio-RAD PAC 300, USA) at 100 Volt for 120 minutes and the banding patterns were visualized using ethidum bromide staining at 0.5 µg/mL (Sigma, USA). The size of the PCR products was estimated using 100 bp DNA ladder (Promega, USA). Gels were viewed using the gel documentation system (Vilber Lourmat, France).

Data Analysis of RAPD Profiles

The banding pattern of each primer was coded by determining the total number of unique bands observed in all of the samples examined to generate a binary matrix for each prime and coded by 0 and 1 for the absent and present bands, respectively.

Data were analyzed using NTSYSpc version 2.0 (1997) software packages. Similarity analysis was run based on Dice coefficient: 2a/ (2a+b+c), where; a: the number of bands commonly present in individual a and b; b: the numbers of bands present in individual a but not in b and c: the numbers of bands present in individual b but not in a.

The dendrogram was constructed using the Unweighted Pair Group Method (UPGMA) (average linkage) (Sneath and Sokal, 1973).

RESULTS

Amplification of the DNA from different *Crocus* populations was obtained at 5-10 ng/µL template concentrations. The DNA quality at the ratios of UV absorbency at 260/280 fell within the range 1.8-1.9. The primers; OPB-12 **(**5'-GGAGGGTGTT-3'**)**, OPM-01 (5'-GTTGGTGGCT-3'**),** OPM-02 (5'-ACAACGCCTC-3'), OPM-03 (5'-GGGGGATGAG-3) and OPM-05 (5'-GGGAACGTGT-3') showed clear amplification patterns. The number of polymorphic markers across the populations ranged from 1 to 6.

The maximum number of markers across the population with primer OPB12, OPM01, OPM02, OPM03 and OPM05 was 6, 4, 4, 3 and 3, respectively. The size of the amplified DNA fragment ranged from 0.28 to 1.5 Kb, part of banding patterns for primers OPM03 and OPM05 are shown in Figures 1 and 2, respectively. RAPD markers produced by the five primers and their distributions across the populations are presented in Table 2. The primers generated a total of 26 markers, where 24 markers were polymorphic which accounted 92% and the remaining 8 % were monomorphic (Table 2). Theses primers also produced a total of 615 differently sized fragments (bands) across populations.

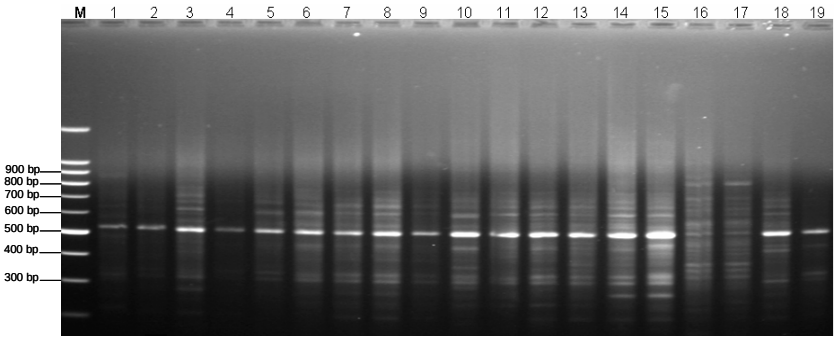

Figure (1): Amplification with primer OPM03 lane 1-19, *C. hyemalis* collected from Souf, Bergish, Junaid and Samta; M; 1000bp DNA ladder (Promega, 2005).

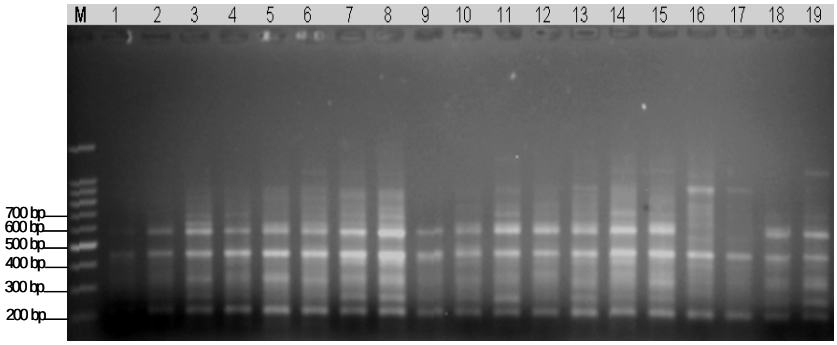

Figure (2): Amplification with primer OPM05 lane 1-19, *C. hyemalis* collected from Souf, Bergish, Junaid and Samta; M; 1000bp DNA ladder (Promega, 2005).

Table (2): RAPD primer, total number of markers, polymorphic markers, total number of bands and polymorphic percentage produced across all samples.

Primer	**Total no. of markers per primer**	**Polymorphic markers**	**Total number of bands across populations**	**Polymorphic %**
OPB12	6	5	152	83
OPM05	5	5	95	100
OPM01	7	6	128	86
OPM03	3	3	102	100
OPM02	5	5	138	100
Total	**26**	**24**	**615**	**92**

Based on Dice (1945) mathematical model, a similarity matrix was constructed to assess the genetic identity among the *C. hyemalis* populations (Table 3). Different levels of variation were detected among different *C. hyemalis* populations. The overall similarity was 0.74, which implies that 74% of the RAPD fragments were shared between the *C. hyemalis* populations. Wild *C. hyemalis* populations had the highest similarity percentage (1.0), while the cultivated *Crocus* showed the lowest percentage (0.35). The lowest similarity was between the cultivated *C. vernus* and the wild *C. hyemalis* population collected from Useem (0.35). While the highest similarity was for the *C. hyemalis* populations collected from Rehaba sites (1.0), other *wild C. hyemalis* populations collected from Samta, Souf and Junaid locations had a high similarity with one population of *C. hyemalis* collected from Bergish (1.0).

Tested *Crocus* species were grouped into four main clusters (Figure 3): one cluster containing all wild *C. hyemalis* populations collected from Rasoon and Junaid locations, one *C. hyemalis* population collected from Samta, two *C. hyemalis* populations collected from Bergish, seven *C. hyemalis* populations collected from Rehaba and two populations collected from Usaim; the second cluster containing three *C. hyemalis* populations collected from each Souf, Bergish, Rehaba and Samta , two populations collected from Useem*;* the third cluster containing two *C. hyemalis* populations collected from Al- Burge and one population collected from Usaim; and the last cluster grouping the cultivated species *C. sativus*

and *C. vernus*.

High inter- and intra-polymorphisms were found for wild *Crocus* populations collected from the eight different locations. RAPD analysis identified location specific differences and separated the cultivated *C. sativus* and *C. vernus* from the wild *C. hyemalis* populations. Populations of *C. hyemalis* which originated from diverse habitats in Jordan have been found to vary genetically as revealed by RAPD techniques. High genetic association between some wild populations was found within the same population of Rehaba. Interestingly, the population of Al-Burge was distinctly separated in one group and it was the closest to the cultivated *C. vernus* and C. *sativus* species. Other distinctly separated populations are those that were collected from Useem (Figure 3).

Table (3): Genetic similarity index based on Dice coefficient for *Crocus* species collected during 2005-2006 (1-44, see Table 1).

1
2	0.79
3	0.77	0.92
4	0.71	0.93	0.92
5	0.76	0.90	0.89	0.97
6	0.80	0.87	0.86	0.93	0.97
7	0.73	0.87	0.86	0.93	0.90	0.94
8	0.97	0.76	0.74	0.69	0.73	0.77	0.77
9	0.90	0.77	0.69	0.77	0.81	0.85	0.85	0.94
10	0.93	0.86	0.77	0.79	0.83	0.80	0.73	0.90	0.90
11	0.90	0.83	0.74	0.83	0.87	0.84	0.77	0.87	0.94	0.97
12	0.88	0.75	0.67	0.75	0.79	0.82	0.82	0.91	0.97	0.88	0.91
13	0.84	0.77	0.69	0.77	0.81	0.79	0.79	0.88	0.94	0.90	0.94	0.97
14	0.90	0.77	0.69	0.77	0.81	0.85	0.85	0.94	1.00	0.90	0.94	0.97	0.94
15	0.90	0.83	0.74	0.83	0.87	0.84	0.77	0.87	0.94	0.97	1.00	0.91	0.94	0.94
16	0.97	0.76	0.74	0.76	0.80	0.84	0.77	0.93	0.94	0.90	0.93	0.91	0.88	0.94	0.93
17	0.71	0.93	0.92	1.00	0.97	0.93	0.93	0.69	0.77	0.79	0.83	0.75	0.77	0.77	0.83	0.76
18	0.64	0.86	0.85	0.93	0.90	0.87	0.87	0.62	0.71	0.71	0.76	0.69	0.71	0.71	0.76	0.69	0.93
19	0.62	0.83	0.81	0.90	0.87	0.84	0.84	0.60	0.69	0.69	0.73	0.73	0.75	0.69	0.73	0.67	0.90	0.97
20	0.71	0.86	0.85	0.86	0.90	0.87	0.80	0.69	0.71	0.79	0.76	0.69	0.71	0.71	0.76	0.69	0.86	0.93	0.90
21	0.79	0.71	0.69	0.79	0.83	0.80	0.73	0.76	0.84	0.86	0.90	0.81	0.84	0.84	0.90	0.83	0.79	0.86	0.83	0.86
22	0.79	0.71	0.69	0.79	0.83	0.80	0.73	0.76	0.84	0.86	0.90	0.81	0.84	0.84	0.90	0.83	0.79	0.86	0.83	0.86	1.00
23	0.81	0.74	0.72	0.74	0.79	0.76	0.69	0.79	0.80	0.89	0.86	0.77	0.80	0.80	0.86	0.79	0.74	0.81	0.79	0.89	0.96	0.96
24	0.64	0.80	0.87	0.80	0.77	0.74	0.74	0.62	0.57	0.64	0.62	0.55	0.57	0.57	0.62	0.62	0.80	0.88	0.85	0.88	0.72	0.72	0.75
25	0.81	0.67	0.64	0.67	0.71	0.69	0.62	0.79	0.80	0.81	0.86	0.77	0.80	0.80	0.86	0.86	0.67	0.74	0.71	0.74	0.89	0.89	0.85	0.75
26	0.64	0.86	0.85	0.93	0.90	0.87	0.87	0.62	0.71	0.71	0.76	0.69	0.71	0.71	0.76	0.69	0.93	1.00	0.97	0.93	0.86	0.86	0.81	0.88	0.74
27	0.79	0.71	0.69	0.79	0.83	0.80	0.73	0.76	0.84	0.86	0.90	0.81	0.84	0.84	0.90	0.83	0.79	0.86	0.83	0.86	1.00	1.00	0.96	0.72	0.89	0.86
28	0.81	0.74	0.72	0.74	0.79	0.76	0.69	0.79	0.80	0.89	0.86	0.77	0.80	0.80	0.86	0.79	0.74	0.74	0.71	0.81	0.89	0.89	0.92	0.67	0.77	0.74	0.89
29	0.86	0.79	0.69	0.71	0.76	0.73	0.67	0.83	0.84	0.93	0.90	0.81	0.84	0.84	0.90	0.83	0.71	0.71	0.69	0.79	0.86	0.86	0.89	0.64	0.81	0.71	0.86	0.96
30	0.71	0.79	0.85	0.86	0.83	0.87	0.87	0.69	0.71	0.64	0.69	0.69	0.65	0.71	0.69	0.76	0.86	0.79	0.76	0.71	0.64	0.64	0.59	0.72	0.59	0.79	0.64	0.59	0.57
31	0.67	0.81	0.80	0.89	0.86	0.90	0.90	0.64	0.73	0.67	0.71	0.71	0.67	0.73	0.71	0.71	0.89	0.89	0.86	0.81	0.74	0.74	0.69	0.75	0.62	0.89	0.74	0.62	0.59	0.89
32	0.75	0.67	0.64	0.67	0.72	0.69	0.62	0.72	0.74	0.83	0.80	0.71	0.74	0.74	0.80	0.72	0.67	0.67	0.64	0.75	0.83	0.83	0.87	0.57	0.70	0.67	0.83	0.87	0.83	0.50	0.61
33	0.86	0.79	0.69	0.71	0.76	0.73	0.67	0.83	0.84	0.93	0.90	0.81	0.84	0.84	0.90	0.83	0.71	0.71	0.69	0.79	0.86	0.86	0.89	0.64	0.81	0.71	0.86	0.81	0.86	0.57	0.67	0.83
34	0.58	0.58	0.55	0.67	0.64	0.62	0.62	0.56	0.67	0.67	0.72	0.64	0.67	0.67	0.72	0.64	0.67	0.67	0.64	0.58	0.75	0.75	0.70	0.48	0.61	0.67	0.75	0.61	0.58	0.50	0.61	0.60	0.75
35	0.74	0.74	0.72	0.81	0.79	0.76	0.76	0.71	0.80	0.81	0.86	0.77	0.80	0.80	0.86	0.79	0.81	0.81	0.79	0.74	0.89	0.89	0.85	0.67	0.77	0.81	0.89	0.77	0.74	0.67	0.77	0.78	0.89	0.87
36	0.81	0.81	0.72	0.74	0.71	0.69	0.69	0.79	0.80	0.89	0.86	0.77	0.80	0.80	0.86	0.79	0.74	0.74	0.71	0.74	0.81	0.81	0.85	0.67	0.77	0.74	0.81	0.77	0.81	0.59	0.69	0.78	0.96	0.78	0.92
37	0.74	0.74	0.72	0.81	0.79	0.76	0.76	0.71	0.80	0.81	0.86	0.77	0.80	0.80	0.86	0.79	0.81	0.74	0.71	0.67	0.81	0.81	0.77	0.67	0.77	0.74	0.81	0.77	0.74	0.67	0.69	0.70	0.81	0.78	0.92	0.85
38	0.74	0.81	0.80	0.89	0.86	0.83	0.83	0.71	0.80	0.81	0.86	0.77	0.80	0.80	0.86	0.79	0.89	0.81	0.79	0.74	0.81	0.81	0.77	0.67	0.69	0.81	0.81	0.77	0.74	0.74	0.77	0.70	0.81	0.78	0.92	0.85	0.92
39	0.77	0.85	0.83	0.85	0.81	0.79	0.79	0.74	0.76	0.85	0.81	0.73	0.76	0.76	0.81	0.74	0.85	0.77	0.74	0.77	0.77	0.77	0.80	0.70	0.64	0.77	0.77	0.80	0.77	0.69	0.72	0.73	0.85	0.73	0.88	0.88	0.88	0.96
40	0.76	0.83	0.74	0.83	0.80	0.77	0.77	0.73	0.81	0.83	0.87	0.79	0.81	0.81	0.87	0.80	0.83	0.76	0.73	0.69	0.76	0.76	0.71	0.62	0.71	0.76	0.76	0.71	0.76	0.69	0.71	0.64	0.83	0.72	0.86	0.86	0.86	0.93	0.89
41	0.76	0.69	0.67	0.69	0.67	0.71	0.77	0.80	0.81	0.76	0.73	0.79	0.75	0.81	0.73	0.73	0.69	0.62	0.60	0.62	0.69	0.69	0.71	0.54	0.57	0.62	0.69	0.71	0.69	0.62	0.64	0.64	0.76	0.72	0.79	0.79	0.79	0.79	0.81	0.80
42	0.67	0.59	0.56	0.59	0.57	0.62	0.69	0.71	0.73	0.67	0.64	0.71	0.67	0.73	0.64	0.64	0.59	0.52	0.50	0.52	0.59	0.59	0.62	0.42	0.46	0.52	0.59	0.62	0.59	0.52	0.54	0.52	0.67	0.78	0.69	0.69	0.69	0.69	0.72	0.71	0.93
43	0.52	0.52	0.56	0.52	0.50	0.55	0.62	0.57	0.53	0.44	0.43	0.58	0.53	0.53	0.43	0.50	0.52	0.52	0.57	0.52	0.44	0.44	0.46	0.50	0.38	0.52	0.44	0.38	0.37	0.52	0.54	0.35	0.44	0.52	0.46	0.46	0.38	0.46	0.48	0.50	0.64	0.69
44	0.59	0.59	0.64	0.67	0.64	0.69	0.76	0.64	0.67	0.52	0.57	0.65	0.60	0.67	0.57	0.64	0.67	0.67	0.64	0.59	0.59	0.59	0.54	0.67	0.62	0.67	0.59	0.46	0.44	0.67	0.69	0.43	0.52	0.52	0.62	0.54	0.62	0.62	0.56	0.64	0.64	0.62	0.77

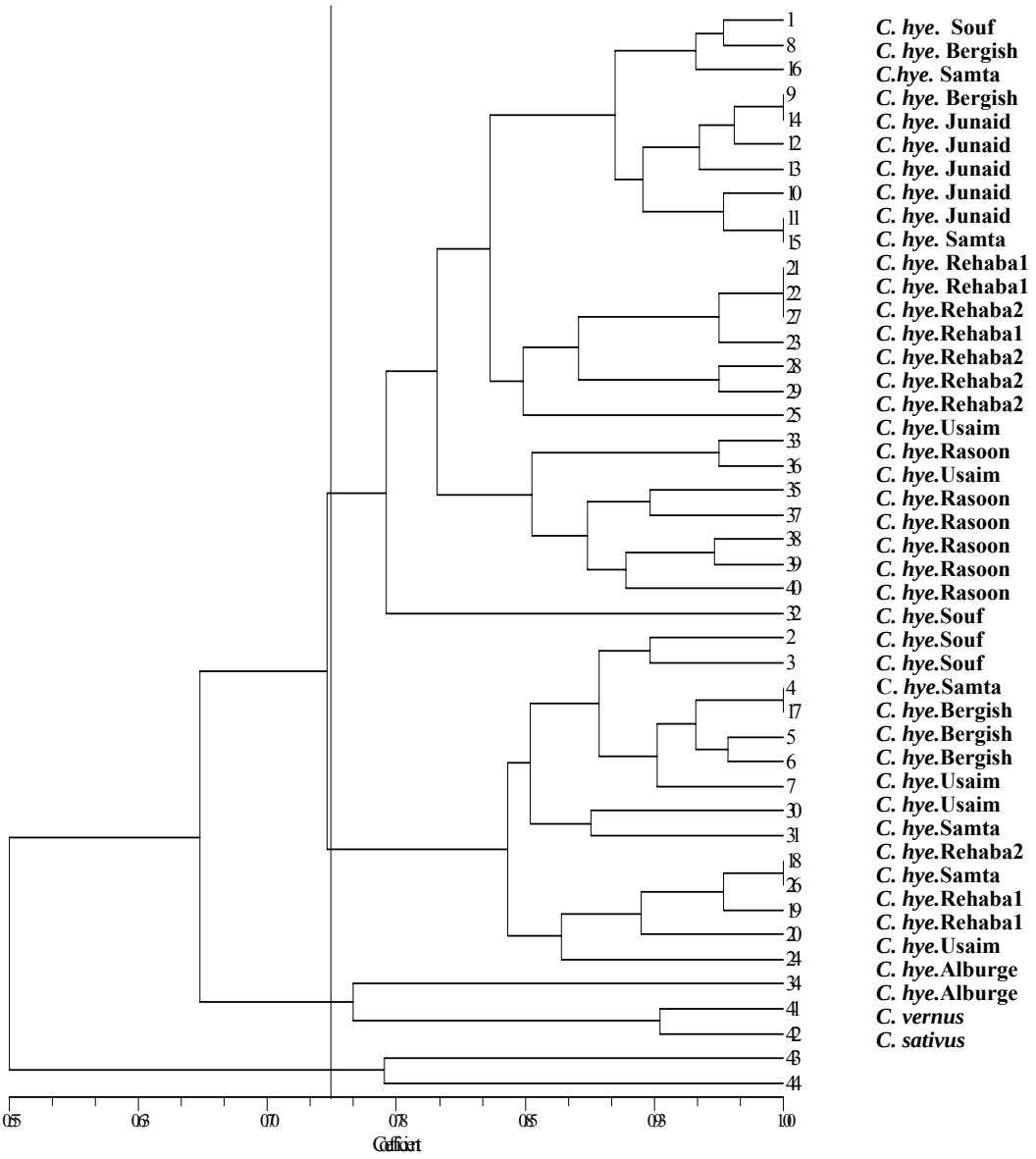

Figure (3): Hierarchical cluster of the wild *Crocus hyemalis* populations and the cultivated *Crocus* species with identification code number and collection sites performed on the basis of genetics characters using Dice coefficient based on the Un-weighted Pair Group Method (UPGMA).

DISCUSSION

The results obtained demonstrated that RAPD markers can be applied to discriminate among wild *Crocus* populations. It has been cited that RAPD markers can be of great value in measurement of inter-specific variation as was revealed in studying *C. sativus* (Grilli Caiola, 2004). High polymorphism was detected among inter- and intra- populations of the nine wild *C. hyemalis* populations collected from their natural habitats in Jordan (Figure 3 and Table 3). Genetic diversity, inter- and intra- *C. hyemalis* populations, could be attributed to ecogeographically structured and adaptive parameters. Cluster analysis also revealed that all populations belonging to the wild species were not clustered in the same group, suggesting a greater degree of inter- and intra-specific variation within the wild *Crocus* species. This variability may be attributed to new combination of alleles or due to increased mutation rate, fixation of alleles from the parents or due to environmental factors. This genetic diversity reflects the range of ecological environment under which the species evolved over millennia. The low degree of intra-specific variation in the cultivated *Crocus* may suggest that the cultivated species passed through a genetic bottle neck during domestication. This conclusion is supported by previous results of RAPD markers of Grilli Caiola *et al*. (2004).

Close genetic relations between some populations collected from the same localities could be attributed to the vegetative multiplication character of this species especially for those populations collected from Rehaba locations (Dothan, 1986). The closer similarity of population in cluster 3 (population of *C. hyemalis*- Al-Burge and *C. hyemalis* –Useem) to the cultivated species appears to be interesting and needs further investigation. The population of Al-Burge in particular was characterized by a distinct honey smell and relatively larger stigmas and petals. The close genetic similarity of the *C. hyemalis* collected from Al-Burge with the cultivated *C. vernus* may indicate that this species could be easily brought into cultivation as an ornamental crop due to its showy sweet scent flowering habit. Pending on future rigorous taxonomic analysis and morphological investigation, *C. hyemalis* collected from Al-Burge may be reconsidered taxonomically.

These findings also proved that Jordan's northern and central heights are important centers of *Crocus* diversity; thus strategies for *in situ* conservation should be given high priorities to conserve these gene pools which can provide valuable genetic resources in the future.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the Higher Council for Science and Technology for funding this activity and the National Center for Agricultural Research and Extension (NCARE) for making this research possible.

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دراسة التنوع الوراثي في الزعفران البري *Crocus hyemalis* Boiss. and Blanche باستخدام تقنية RAPD

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ملخـص

تناول هذا البحث دراسة العلاقة الوراثية بين أنواع الزعفران البري في الأردن *Crocus hyemalis* Boiss. and Blanche باستخدام تقنية المكاثرة العشوائية لقطع DNA المتباينة (RAPD). تم جمع عينات الزعفران البري *C. hyemalis* من مواقع بيئية مختلفة في الأردن خلال شهري كانون الأول وكانون الثاني 2005/2004، ثم تمت مقارنته مع أنواع الزعفران المزروع *C. vernus* and *C.sativus*. اظهرت نتائج التحليل باستخدام تقنية RAPD اختلاف الزعفران البري الناشئ في بيئات مختلفة من الأردن وراثياً. كما دل التحليل التجميعي على وجود درجة عالية من التنوع بين الزعفران البري *C. hyemalis*. كما دلت الدراسة على وجود علاقة وراثية قوية بين بعض العينات ضمن نفس مواقع الجمع. دل التشابه الوراثي الكبير بين الزعفران البري *C. hyemalis* الذي تم جمعه من منطقة البرج مع الزعفران المزروع *C. vernus* على امكانية زراعة هذا النوع بسهولة وذلك لخاصية إزهاره. وتدل النتائج على ضرورة دراسة C. *hyemalis* الذي جمع من منطقة البرج دراسة متقدمة.

الكلمات الدالة: الزعفران، تقنية RAPD، التنوع الجيني، نباتات الأردن الطبية.

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تاريخ استلام البحث 2007/4/25 وتاريخ قبوله 2008/4/28.