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ORIGINAL ARTICLE

Genetics diversity analysis of some Iranian accessions of safflower (*Carthamus tinctorius* L.) by Morphological and Molecular Marker

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ABSTRACT
Safflower (Carthamus tinctorius), is widely cultivated in agricultural systems as a source of high-quality vegetable and industrial oil. 9 RAPD primers and 12 agro-morphological traits were used to assess the genetic diversity of 24 accessions of safflower representing global germplasm variability. For RAPD markers Jacquards' similarity coefficient was used to understanding the genetic relationships among accessions, while for agromorphological traits Euclidian similarity coefficient was used. UPGMA clustering algorithm was used for all markers. RAPD markers grouped accessions into four main clusters also dendrogram of morphology data delineated the accessions into four clusters.

***Keywords**: Carthamus tinctorius L., Genetic diversity, Morphological traits, Molecular Markers*

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INTRODUCTION

Carthamus tinctorius ($2n = 2x = 24$), commonly known as safflower, is a member of the tribe Cynareae, subfamily Tubulifloreae, and family Asteraceae. The eastern part of the Mediterranean region is regarded as the centre of origin of the genus (Ashri and Knowles, 1960).

On the basis of morphological variability existing in C. tinctorius, Knowles (1969b) proposed seven'centers of similarity' [Far East (China, Japan, and Korea), India–Pakistan, Middle East, Egypt, Sudan, Ethiopia, and Europe] with predominant morphotypes in each center. Ashri (1975) extended the number of centers to 10 and named them as 'regional gene pools'. The Middle East center was sub-divided into three gene pools-Iran– Afghanistan, Israel–Jordan–Iraq–Syria, and Turkey with the Kenya gene pool added to the list (Ashri 1975).

Safflower oil is thought to be one of the highest quality vegetable oils, containing oleic acid and linoleic acid (Khan et al., 2008).

Unfortunately, most of the genetic diversity of this plant currently is being lost so the evaluation of genetic diversity will help to provide valuable information on the management and utilization of safflower germplasm (Panahi et al., 2013 b). So far germplasm resources of safflower have been characterized on the basis of agro-morphological traits (Panahi et al., 2013 b), biochemical characters (Zhang, 2001) and molecular markers such as AFLP, SSR and ISSR (Panahi and Ghorbanzadeh, 2013 a). Mohammadi and Parsana (2003) suggested that genetic diversity of safflower is the best estimated if agro-morphological and molecular marker studies are used together.

RAPD markers are markers of choice, because of its simplicity and low-cost nature, rapid, inexpensive and effective system for studying plant genetic relationships (Williams et al., 1990). The RAPD markers could also be used in the study of genetic variability of species or natural populations and in the study of genotype identification (Mahmoudi et al., 2014) Therefore, the aim of this research is the evaluation of

genetic diversity using RAPD and agro-morphological traits as morphological markers in some Iranian and international safflower germplasm.

MATERIAL AND METHODS

Plant materials

The collection of promising genotypes was planted in 2014. Experiment was laid out in a randomized complete block design. In every block, there were three rows and in each row 30 seeds were sown. Each row was 3 m long, and the path between rows was 40 cm. The names of the 24 cultivars investigated are given in Table 1.

Table 1: accessions of safflower used in this study and their Origin

Number	Accession	Origin
1	Saffire	Canada1
2	Lesaf	Canada2
3	Noroeste/84/3/CW	Cimmyt1
4	S-0023	Cimmyt2
5	18-VF	Cimmyt3
6	5150	Cimmyt4
7	CW-88	Cimmyt5
8	PI-250536	Eygpt1
9	PI-250537	Eygpt2
10	Local Ghochan1	Iran1
11	Local Ghochan2	Iran2
12	Local Isfahan1	Iran3
13	Local Isfahan2	Iran4
14	Local Marand	Iran5
15	Local Darab	Iran6
16	IL-111	Iran7
17	Quiriego-88	Mexic1
18	Sahuaripa-88	Mexic2
19	Bacum92	Mexic3
20	Syrian	Syrian
21	Hartman	USA1
22	Finch	USA2
23	Dincer	USA3
24	Mashhad	Wild

DNA extraction

Genomic DNA was extracted from young leaves following the cetyl tri methyl ammonium (CTAB) procedure described by Saghai-Maroof et al. (1984). Extracted DNA concentration was quantified by using the NanoDrop spectrophotometer and qualified using agarose gel electrophoresis.

Agro-morphological data collection and analysis

For multivariate and other analysis, twenties variables were measured as described below:

Days to flowering (DF) was the date when the first flower bloomed, Blooming time (BT) was the date when 50% of flowers were opened, Plant height (PH) (cm), The firs branch height (BH) (cm), number of secondary branch (NSB), Tributaries angle (TA) (degree), Number of heads (NH), Number of seed per head (NSH), Thousand Seed weight (TSW), Oil percentage (OP), measure with Soxhlet extractor (velp, Italy), Shell percentage (SP), Seed yield (SY) (kg/hec). Agro-morphological data was standardized before using in multivariate analysis by applying in NTSYS-pc software version 2.1. Euclidean distance was used as the similarity coefficient for cluster analysis with the Unweighted Pair Group Arithmetic Means method (UPGMA) using NTSYS-pc software version 2.1 .

RAPD fingerprinting

Twenty random decamer primers were used to amplify RAPD fragments in C. tinctorius accessions. The polymerase chain reaction (PCR) volume of 25 µl in a 0.5 ml thin walled tube contained 10 mM Tris–HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl2, 0.24 mM dNTPs , 0.1% gelatin, 6 µM primer, 20 ng DNA template, and IU Taq polymerase. DNA amplification was performed in a thermal cycler programmed for 45 cycles.

The amplified products were separated on 1.4 % agarose gels and stained with ethidium bromide. Images were photographed, captured by Gel Doc 2000TM (Bio-Rad, USA). Amplified products were scored for the presence (1) or absence (0) of bands and binary matrices were assembled for the ISSR markers. The binary matrices were subjected to statistical analyses using NTSYS-pc software version 2.1. Jacquard's similarity coefficient was employed to compute pairwise genetic similarities. Similarity matrix was used for the cluster analysis and construction of dendrogram using unweighted pair-group method (UPGMA).

Results and Discussion

Nine RAPD primers (Table 2) produced 110 bands (Fig. 1) across the 24 accessions, of which 65 were polymorphic (Table 2). The number of amplified fragments varied from8 (UB91) to 16 (UB67) across the genotypes. The average polymorphic bands per primer were 14.2. The percentage of polymorphism for primers ranged from 66 to 70, with an average polymorphism percent of 65.8 (Table 2).

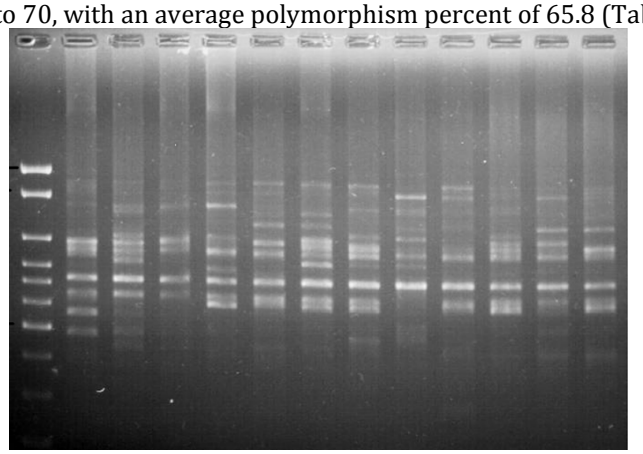

Figure 1: RAPD profile of safflower accession produced by primer UB12.

Primer	Sequence 5'-3'	Total of bands	Polymorphic bands
UB30	CCGGCCTTAG	14	11
UB12	CCTGGGTCCA	12	11
UB89	GGGGGCTTGG	10	4
UB79	GAGCTCGTGT	12	8
UB96	GGCGGCATGG	18	11
UB25	ACAGGGCTC	9	3
UB91	GGGTGGTTGC	8	7
UB18	GGGCCGTTT	12	9
UB67	GAGCACCAGT	16	9

Table 2: primers, sequence and information regarding produced bands.

Pair wise similarities regard to RAPD and morphological markers ranged from 0.53 to 0.75 ,and 21.5 to 135.25, respectively. The clustering pattern obtained with RAPD and morphological data showed distinctive pattern of 24 accessions (Figure 3). The dendrogram of RAPD markers grouped 24 accessions into four main clusters whereas morphological markers grouped the accessions to five accessions (Figure 4).

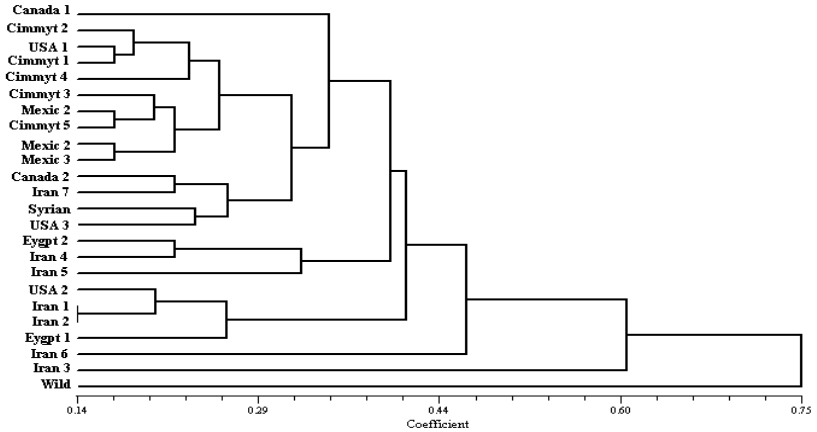

Figure 3: Dendrogram of different accessions of Safflower accession based on RAPD Markers.

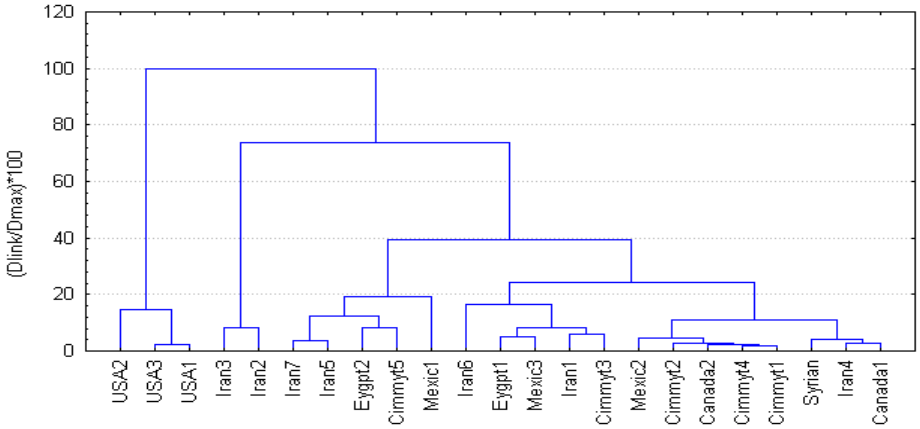

Figure 4: Dendrogram of different accessions of Safflower accession based on agro morphological traits.

The cluster analysis based on Molecular and agromorphological data showed that there was a considerable agreement between geographic origin and their genomic similarities. Similar results were obtained in the study by Arzani and Rezaei (2011). Similarities in genotypes grouped in the same cluster could also appear because of participating a common lineage, convergent evolution and selection of superior genotypes.

There was no association between agromorphological diversity and molecular diversity. Similar disparity between morphological traits and RAPDs was reported in different studies (Panahi et al, 2013 b). There could be many reasons for the lack of correlation between RAPDs, AFLP and morphological distances. One reason could be that RAPDs detect polymorphisms in coding as well as non-coding regions of the genome (Mahmoudi et al., 2014), of which only a small portion is coding, therefore, it is very likely that the polymorphism found is in a non-coding region. The relationship between molecular markers and phenotypic traits could be significant if the markers were linked to selected loci. Also, plants that are morphologically similar are not necessarily genetically (Souframanien et al, 2004). Discordance between various DNA marker systems is not uncommon and is reported in many plant taxa (Powell et al., 1996). The incongruence between the marker systems also suggests that RAPD and morphological marker systems have different mutation rates under similar selective forces in safflowers.

According to Powell et al. (36), the relationships may be rather dependent on genome coverage and/or the type of sequence variation recognized by each marker system. More detailed studies are needed for safflowers before any conclusions can be made with regard to genome coverage of markers.

Therefore, this result demonstrates proper distribution of RAPD markers through entire genome and confirms the results of cluster analysis. Also the result showed these genotypes have high genetic diversity, thus, for success in safflower breeding programs use to recommended Iranian safflower local.

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