Phylogenetic Relationships and Genetic Diversity in Collected Resources of *Carthamus tinctorius* by Random Amplified Polymorphic DNA Markers

Jung Sook Sung, Gyu Taek Cho, Gi An Lee, Hyung-Jin Baek and Man Kyu Huh[1]*

National Academy of Agricultural Science, RDA, Suwon 441-100, Korea
[1]*Department of Molecular Biology, Dongeui University, Busan 614-714, Korea*

Carthamus tinctorius L. (Compositae) is an herb primarily distributed throughout in the world. The species is regarded as ecologically important in the world. Safflower was used for medicines, as well as making red (carthamin) and yellow dyes. We have used the RAPD technique to investigate the phylogenetic relationships and genetic diversity of *C. tinctorius*. We obtained 123 bands from all the 26 cultivars. The average number of bands was 9.5 per primer. The genetic diversity of safflower is found among cultivars and there is a high among-cultivar differentiation. The OPC18-01 band is the specific marker for Syria cultivar, whereas no products were detected in individuals from other country cultivars. We found seven phenetic bands for determining the specific marker of cultivars with SCAR markers. Though the number of individuals sampled for analysis was small and probably not fully representative of the total available diversity in *C. tinctorius*, this study demonstrates that the regions (Morocco, Syria, and Turkey) of the Mediterranean Sea were more variable than other regions with the exception of India. In this result, although only simple result of RAPD is difficult to assert the center of species diversity of *C. tinctorius*, the regions of the Mediterranean Sea may be the most probable candidate for the origin of safflower. India was also the candidate of the center or secondary center of species diversity of *C. tinctorius*. RAPD markers were effective in classifying cultivar levels of safflower.

Key words : *Carthamus tinctorius*, RAPD, safflower, genetic diversity

Introduction

Safflower (*Carthamus tinctorius* L.) is a highly branched, herbaceous, thistle-like annual, usually with many long sharps pines on the leaves [2]. Plants are 30 to 150 cm tall with globular flower heads (capitula) and commonly, brilliant yellow, orange or red flowers which bloom in July. Each branch will usually have from one to five flower heads containing 15 to 20 seeds per head [4].

Safflower is one of humanity's oldest crops. Chemical analysis of ancient Egyptian textiles dated to the Twelfth dynasty identified dyes made from safflower, and garlands made from safflower were found in the tomb of the pharaoh Tutankhamen. Chadwick [2] reported that the Greek name for safflower occurred many times in Linear B tablets, distinguished into two kinds: a white safflower, which is measured, and red which is weighed. The explanation is that there are two parts of the plant which can be used; the pale seeds and the red florets [2].

Traditionally, safflower was used for medicines, making red (carthamin), and yellow dyes, especially before cheaper aniline dyes became available [19]. In colouring textiles, safflower's dried flowers are used as a cultivar textile dye. Cultivar dyes derived from plants are not widely used in industry but it is getting more important world because of naturality and fashion trends. The colourful matter in safflower is benzoquinone-based Carthamin, so it is one of the quinone type natural dyes. It is a direct dye (CI Natural Red 26) and soluble.

Safflower was also known as carthamine in the 19th century [4]. It is a minor crop today, with about 600,000 tons being produced commercially in more than sixty countries worldwide. India, United States, and Mexico are the leading producers, with Ethiopia, Kazakhstan, China, Argentina and Australia accounting for most of the remainder [9].

Studies to clarify the overall status of several countries of this species were not conducted. Thus, the aim of this paper was to determine the compositions of the components of *C. tinctorius* for the first time and to compare it with the composition of *C. tinctorius* from the other countries.

Throughout this century a large number of samples from

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*Corresponding author

Tel : +82-51-890-1521, Fax : +82-51-890-1529
E-mail : mkhuh@deu.ac.kr

landraces of cultivated plants and from related wild species have been collected, and these are now available to breeders aiming at enlarging the genetic basis of these plant materials [10]. However, emphasis has been put on the general under uses of this potentially useful diversity in breeding programs [12]. This situation is partly due to the lack of a systematic genetic evaluation of plant collections. Information on germplasm diversity and genetic relationships among plant materials is therefore essential for breeders.

DNA-based RAPD (random amplified polymorphic DNA) has the advantage of being and easy, allowing resolution of complex patterns of genetic variation while the DNA sequence information is not available [11]. Although RAPD has the disadvantage of low reproductivity of marker bands and dominant of band markers, it is a preferable approach for safflower. In this paper, we have used the RAPD technique to investigate the genetic diversity of *C. tinctorius* and provide baseline information for crop management. The results from RAPD analysis may cast a light on the issue

of genetic differentiation of the safflower and the origin of this species.

Materials and Methods

Plant materials and DNA extraction

Wild seeds were collected from 149 cultivated populations (or accessions) of *C. tinctorius* of 26 countries including Korea (Table 1). All seeds of samples were obtained from National Agrobiodiversity Center, NAAS, PDA (Suwon, Gyeonggi Province, Korea) which plants were collected from 26 countries in the world. The 100 collected seeds per population or cultivar were sown in to outdoor plots with same environmental conditions. We classified into 26 cultivars because same lineages were found among them.

We randomly selected ten plants per cultivar for the analyses within and among populations. Genomic DNA was isolated from one unexpended leaf of one plant. DNA was extracted with the plant DNA Zol Kit (Life Technologies Inc.,

Table 1. Codes of countries and measurements of genetic variation for 26 countries of *C. tinctorius*

Code	Country	Np	Pp	A	A_E	H	I	Rank
AFG	Afghanistan	10	8.13	1.081	1.058	0.034	0.049	
ARM	Armenia	11	8.94	1.089	1.063	0.037	0.054	
AUS	Australia	11	8.94	1.089	1.063	0.037	0.054	
AZE	Azerbaijan	8	6.50	1.065	1.046	0.027	0.039	
CAN	Canada	6	4.88	1.049	1.035	0.020	0.030	
CHN	China	5	4.07	1.041	1.029	0.017	0.025	
EGY	Egypt	6	4.88	1.049	1.035	0.020	0.030	
ETH	Ethiopia	12	9.76	1.098	1.069	0.040	0.059	7
HUN	Hungary	10	8.13	1.081	1.058	0.034	0.049	
IND	India	16	13.01	1.130	1.092	0.054	0.079	1
IRN	Iran	12	9.76	1.098	1.069	0.040	0.059	7
KAZ	Kazakhstan	14	11.38	1.114	1.081	0.047	0.069	5
KOR	Korea	9	7.32	1.073	1.052	0.030	0.044	
MAR	Morocco	16	13.01	1.130	1.092	0.054	0.079	1
MEX	Mexico	4	3.25	1.033	1.023	0.014	0.020	
PAK	Pakistan	14	11.38	1.114	1.081	0.047	0.069	5
RUS	Russia	8	6.50	1.065	1.046	0.027	0.039	
SDN	Sudan	6	4.88	1.049	1.035	0.020	0.030	
SYR	Syria	16	13.0	1.130	1.092	0.054	0.079	1
TKM	Turkmenistan	10	8.13	1.081	1.058	0.034	0.049	
THA	Thailand	4	3.25	1.033	1.023	0.014	0.020	
TJK	Tadzhikistan	9	7.32	1.073	1.052	0.030	0.044	
TUR	Turkey	16	13.01	1.130	1.092	0.054	0.079	1
UKR	Ukraine	6	4.88	1.049	1.035	0.020	0.030	
USA	USA	12	9.8	1.098	1.069	0.040	0.059	7
UZB	Uzbekistan	9	7.32	1.073	1.052	0.030	0.044	
Mean		10.0	8.1	1.081	1.057	0.034	0.049	

Rank: The nine highest genetic diversities among 26 countries.

Grand Island, New York, USA) according to the manufacturer's protocol. Extracted DNA was quantified using Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's instructions.

RAPD analysis

RAPD primers were obtained from Operon Technologies Inc. (USA). All the reactions were repeated twice and only reproducible bands were scored for analyses. 40 primers (OPC01-OPC20 and OPD01-OPD20) used for a preliminary RAPD analysis. All the reactions were repeated twice and only the reproducible primers were used for analyses. The 13 primers produced good amplification products in quality, reproducibility, and variability (Table 2).

Amplification reactions were performed in total 50 ul mixed solution containing 2.5 μl of the reaction buffer, 10 mM Tris-HCl, 1.25 mM dNTP, 5.0 pM primer, 2.5 units Taq DNA polymerase, and 25 ng of genomic DNA. DNA was amplified on a Takara PCR Thermal Cycler DICE (TP600, Japan) using the following programme. Initial denaturation was carried out for 1 min at 94°C, followed by 35 cycles of 50 sec at 94°C, 30 sec at 45°C, 2 min at 72°C, and a final 10 min extension at 72°C.

A 100 bp ladder DNA marker (Pharmacia) was used in the end of for the estimation of fragment size. The amplification products were separated by electrophoresis on 1.5% agarose gels, stained with ethidium bromide, and photographed under UV light using Alpha Image TM (Alpha Innotech Co., USA).

To convert the selected RAPD band to a SCAR (sequence characterized amplified region) marker, the bands excised, cloned and sequenced following the procedure outlined in Jung et al. [8]. The excised PCR band was sequenced and the SCAR marker sequences were designed by identifying the original 10 bp sequence of the RAPD primer. The specific SCAR primer were used different individuals of same cultivars and 25 different bulk cultivars which were not shown the band. The 5.0 ul of amplification products were primarily separated by electrophoresis on 2.0% agarose gels, stained with ethidium bromide, and quantified the yield of total DNA using Bio-Rad.

Statistical analyses

All RAPD bands were scored by eye and only unambiguously scored bands were used in the analyses. Because RAPDs are dominant markers, they were assumed that each band corresponded to a single character with two alleles, presence (1) and absence (0) of the band, respectively. Loci were named based on the primer and observed band size.

The following genetic parameters were calculated using a POPGENE computer program (ver. 1.31) developed by Yeh et al. [18]: allele frequencies, the percentage of polymorphic loci (P_p), mean numbers of alleles per locus (A), effective number of alleles per locus (A_E), gene diversity (H), and Shannon's index of phenotypic diversity [14].

Nei's gene diversity formulae (H_T, H_{TS}, and G_{ST}) were used to evaluate the distribution of genetic diversity within and among cultivars [14]. The indirect estimates of gene flow, N_m (the number of migrants per generation), were based on G_{ST} [17].

Nei's genetic identity was calculated for each pairwise

Table 2. Lists of decamer oligonucleotide utilized as primers, their sequences, and associated bands

No. of primer	Sequence (5'-3')	No. of bands	Cultivar-specific bands	
			Band	Cultivar
OPC07	GTCCCGACGA	6		
OPC11	AAAGCTGCGG	7		
OPC14	TGCGTGCTTG	7		
OPC18	TGAGTGGGTG	8	OPC18-01	SYR
OPC19	GTTGCCAGCC	6		
OPC20	ACTTCGCCAC	11	OPC20-7, 11	ETH
OPD01	ACCGCGAAGG	12	OPD01-12	UZB
OPD04	TCTGGTGAGG	10		
OPD07	TTGGCACGGG	12	OPD07-12	IND
OPD10	GGTCTACACC	10		
OPD12	CACCGTATCC	13		
OPD16	AGGGCGTAAG	12	OPD16-04	PAK
OPD20	ACCCGGTCAC	9	OPD20-08	SYR
Total	-	123	7	5

Table 3. Estimates of genetic diversity of *C. tinctorius*. Total genetic diversity (H_T), genetic diversity within cultivars (H_S) proportion of total genetic diversity partitioned among cultivars (G_{ST}), and gene flow (N_m)

Primer	H_T	H_S	G_{ST}	N_m
OPC07	0.266	0.029	0.765	0.043
OPC11	0.268	0.001	0.807	0.036
OPC14	0.316	0.030	0.905	0.059
OPC18	0.361	0.042	0.907	0.058
OPC19	0.155	0.019	0.448	0.038
OPC20	0.240	0.044	0.726	0.200
OPD01	0.189	0.015	0.784	0.030
OPD04	0.257	0.024	0.732	0.042
OPD07	0.229	0.028	0.678	0.044
OPD10	0.232	0.030	0.619	0.052
OPD12	0.169	0.015	0.650	0.137
OPD16	0.280	0.061	0.671	0.236
OPD20	0.264	0.085	0.607	0.214
Total	0.248	0.033	0.715	0.091

combination of cultivars [14]. Homogeneity of variance among accessions was tested by Bartlett's statistics.

The Mantel test was examined the correlation between the matrix of genetic distance and spatial distance within a site.

A maximum parsimony tree (MP) was inferred using heuristic search, branch-swapping options and tree bisection-reconnection. Confidence values for individual branches were determined by a bootstrap analysis with 1000 repeated sampling of the data. In addition, a phenetic relationship was constructed by the neighborjoining (NJ) method [16] using the NEIGHBOR program in PHYLIP version 3.57 [5].

Results

We obtained 123 bands from all the cultivars (Table 2). The average number of bands was 9.5 per primer. Of all cultivars, 82.9% were polymorphic among all cultivars. In a simple percentage of polymorphic bands, the Mexico cultivar and Thailand cultivar exhibited the lowest variation (3.3%). The India, Morocco, and Syria cultivars showed the highest (13.0%) (Table 1). Mean number of alleles per locus (A) from 1.033 to 1.130 with a mean of 1.081. The effective number of alleles per locus (A_E) ranged from 1.023 to 1.092. The phenotypic frequency of each band was calculated and used for estimating genetic diversity (H) within cultivars. The safflower maintained a low level of genetic diversity for polymorphic primers. The total H was 0.034 across cultivars. Shannon's index of phenotypic diversity (I) of safflower ranged from 0.020 to 0.079 with a mean of 0.049. The

nine highest genetic diversities among 26 countries were India, Moroco, Syria, Turkey, Kazakhstan, Pakistan, Ethopia, Iran, and USA (Table 2).

Total genetic diversity values (H_T) and interlocus variation in the within-cultivar genetic diversity (H_S) were 0.248 and 0.033, respectively (Table 3). On a per-locus basis, the proportion of total genetic variation due to differences among cultivars (G_{ST}) was 0.715. This indicated that about 71.5% of the total variation was among cultivars. These values indicate that most of the genetic diversity of safflower is found among cultivars and there is a high among-cultivar differentiation. The remaining genetic variation (28.5%) was occurring within cultivars. The estimate of gene flow, based on G_{ST}, was slightly low among cultivars of safflower (N_m=0.091).

Values of genetic distance (D) were <0.463 (Table 4). Genetic identity values among pairs of cultivars ranged from 0.630 to 0.907.

The Mantel test was used to test for correlations between the matrix of genetic diversity and spatial distance. Genetic diversity did not almost correlate with spatial distance in safflower cultivars.

The OPC18-01 band is the specific marker for the Syria cultivar, whereas no products were detected in individuals from other country cultivars (Fig. 1). The specific DNA fragment seemed to be useful to discriminate among cultivars and was used to develop the SCAR marker. Figure 2 gave rise to the yield of total DNA using Bio-Rad. We found many phenetic bands for determining the specific marker of cultivar with SCAR markers (Table 2). Seven bands (OPC18-01,

Table 4. Genetic identity (above diagonal) and genetic distances (below diagonal) of 27 cultivars of *Carthamus tinctorius* (Compositae) based on RAPD

Code	AFG	ARM	AUS	AZE	CAN	CHN	EGY	ETH	HUN	IND	IRN	KAZ	KOR
AFG	-	0.832	0.853	0.853	0.765	0.771	0.770	0.830	0.671	0.777	0.792	0.765	0.750
ARM	0.184	-	0.775	0.898	0.848	0.770	0.734	0.796	0.692	0.765	0.784	0.728	0.810
AUS	0.159	0.254	-	0.775	0.784	0.795	0.732	0.819	0.699	0.781	0.767	0.788	0.724
AZE	0.159	0.108	0.256	-	0.806	0.755	0.763	0.791	0.672	0.733	0.765	0.710	0.670
CAN	0.244	0.165	0.244	0.216	-	0.818	0.735	0.758	0.737	0.835	0.778	0.829	0.709
CHN	0.260	0.261	0.229	0.281	0.201	-	0.745	0.737	0.709	0.828	0.749	0.775	0.721
EGY	0.261	0.309	0.312	0.311	0.308	0.295	-	0.793	0.632	0.797	0.834	0.808	0.663
ETH	0.186	0.228	0.199	0.235	0.278	0.305	0.232	-	0.630	0.767	0.824	0.800	0.683
HUN	0.399	0.369	0.359	0.397	0.306	0.343	0.460	0.463	-	0.663	0.692	0.674	0.723
IND	0.253	0.268	0.248	0.311	0.180	0.189	0.227	0.265	0.412	-	0.765	0.790	0.709
IRN	0.234	0.244	0.266	0.268	0.251	0.289	0.182	0.193	0.368	0.268	-	0.809	0.651
KAZ	0.268	0.317	0.238	0.342	0.188	0.255	0.214	0.223	0.395	0.236	0.213	-	0.707
KOR	0.288	0.345	0.324	0.401	0.343	0.327	0.410	0.381	0.325	0.344	0.429	0.346	-
MAR	0.328	0.220	0.284	0.309	0.193	0.292	0.408	0.260	0.399	0.237	0.337	0.262	0.357
MEX	0.299	0.218	0.247	0.260	0.218	0.289	0.415	0.251	0.386	0.321	0.363	0.229	0.367
PAK	0.259	0.210	0.221	0.274	0.231	0.253	0.273	0.240	0.341	0.246	0.251	0.276	0.386
RUS	0.233	0.156	0.194	0.188	0.174	0.266	0.325	0.173	0.358	0.255	0.244	0.205	0.349
SDN	0.231	0.154	0.254	0.218	0.177	0.290	0.361	0.217	0.406	0.253	0.258	0.242	0.357
SYR	0.196	0.071	0.255	0.109	0.169	0.246	0.306	0.199	0.401	0.268	0.244	0.276	0.386
TKM	0.261	0.217	0.232	0.260	0.207	0.269	0.285	0.293	0.379	0.189	0.305	0.278	0.351
THA	0.222	0.240	0.225	0.306	0.210	0.191	0.231	0.275	0.380	0.179	0.261	0.278	0.373
TJK	0.304	0.185	0.245	0.247	0.128	0.228	0.334	0.287	0.343	0.215	0.278	0.244	0.312
TUR	0.266	0.216	0.224	0.299	0.206	0.289	0.321	0.230	0.401	0.240	0.272	0.256	0.357
UKR	0.196	0.236	0.197	0.214	0.283	0.272	0.267	0.210	0.450	0.317	0.288	0.284	0.303
USA	0.255	0.187	0.246	0.233	0.219	0.296	0.363	0.256	0.422	0.248	0.290	0.262	0.397
UZB	0.168	0.218	0.142	0.181	0.282	0.282	0.271	0.162	0.445	0.253	0.267	0.277	0.313

Code	MAR	MEX	PAK	RUS	SDN	SYR	TKM	THA	TJK	TUR	UKR	USA	UZB
AFG	0.720	0.742	0.772	0.792	0.794	0.822	0.770	0.801	0.738	0.766	0.822	0.775	0.845
ARM	0.805	0.804	0.810	0.856	0.858	0.931	0.805	0.7875	0.831	0.806	0.790	0.830	0.804
AUS	0.753	0.781	0.802	0.824	0.776	0.775	0.793	0.798	0.783	0.800	0.821	0.782	0.868
AZE	0.735	0.771	0.761	0.829	0.804	0.897	0.771	0.737	0.781	0.742	0.808	0.792	0.835
CAN	0.825	0.804	0.794	0.840	0.838	0.845	0.813	0.810	0.880	0.815	0.754	0.803	0.755
CHN	0.747	0.749	0.777	0.767	0.748	0.782	0.764	0.826	0.796	0.742	0.762	0.744	0.754
EGY	0.665	0.660	0.761	0.722	0.697	0.736	0.752	0.794	0.716	0.756	0.766	0.695	0.763
ETH	0.771	0.778	0.786	0.841	0.805	0.820	0.746	0.760	0.751	0.794	0.811	0.774	0.850
HUN	0.671	0.680	0.711	0.699	0.666	0.670	0.684	0.684	0.709	0.670	0.638	0.656	0.641
IND	0.789	0.726	0.782	0.775	0.776	0.765	0.828	0.836	0.807	0.786	0.729	0.781	0.776
IRN	0.714	0.695	0.778	0.784	0.773	0.784	0.737	0.770	0.758	0.762	0.750	0.748	0.765
KAZ	0.769	0.795	0.759	0.815	0.785	0.759	0.757	0.758	0.784	0.774	0.753	0.769	0.758
KOR	0.700	0.693	0.680	0.706	0.700	0.680	0.704	0.689	0.732	0.700	0.739	0.672	0.732
MAR	-	0.840	0.779	0.850	0.871	0.807	0.765	0.750	0.820	0.809	0.706	0.879	0.740
MEX	0.174	-	0.769	0.881	0.818	0.788	0.757	0.724	0.801	0.792	0.746	0.810	0.759
PAK	0.250	0.263	-	0.829	0.796	0.792	0.868	0.850	0.812	0.874	0.771	0.807	0.816
RUS	0.162	0.127	0.188	-	0.871	0.871	0.816	0.811	0.836	0.820	0.805	0.869	0.831
SDN	0.138	0.201	0.228	0.138	-	0.880	0.757	0.794	0.816	0.802	0.728	0.892	0.787
SYR	0.215	0.239	0.234	0.138	0.128	-	0.793	0.777	0.794	0.805	0.792	0.849	0.815
TKM	0.268	0.279	0.142	0.203	0.279	0.232	-	0.865	0.820	0.881	0.784	0.771	0.789
THA	0.287	0.323	0.163	0.209	0.231	0.253	0.145	-	0.839	0.837	0.742	0.765	0.774
TJK	0.198	0.222	0.208	0.179	0.203	0.231	0.198	0.176	-	0.790	0.771	0.798	0.768
TUR	0.212	0.234	0.134	0.199	0.220	0.217	0.127	0.178	0.236	-	0.730	0.783	0.769
UKR	0.348	0.294	0.260	0.217	0.318	0.234	0.243	0.299	0.261	0.315	-	0.760	0.907
USA	0.129	0.211	0.214	0.141	0.114	0.164	0.260	0.268	0.226	0.245	0.274	-	0.791
UZB	0.301	0.275	0.204	0.185	0.240	0.205	0.238	0.256	0.264	0.262	0.098	0.234	-

Fig. 1. The specific band of SYR (Syria) with the OPC18 primer. Abberations of AFG, ARM, AUS, and so on are the same as Table 1. M is the 100 bp ladder DNA marker.
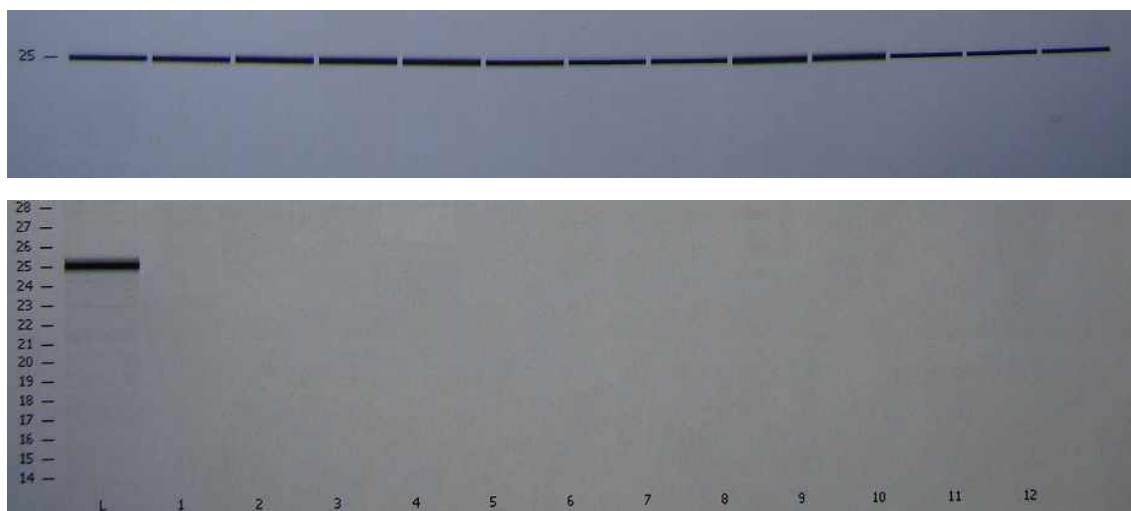

Fig. 2. The quantified specific band to 13 different Syria individuals (upper). and the specific band of Syria (L) is not amplified other country bulk samples (1-12) (below).

OPC20-07, 11, OPD01-12, OPD07-12, OPD16-04, and OPD20-08) are specific for one cultivar.

Clustering of accessions was performed based on the matrix of calculated distances using the NJ algorithm (Fig. 3). In dendrogram, many cultivars were well separated from each other. Thus, RAPD markers are effective in classifying cultivar levels of safflower though 26 countries could not distinguish each other perfectly on the basis of phenotypic traits. Although many cultivars were separated from each other, there were numerous discrepancies among the clades obtained here.

Discussion

We analyzed 26 countries of *C. tinctorius* with the internal transcribed spacer (ITS) region of nuclear ribosomal DNA using primers ITS1 and ITS4 [1]. However they were same sequences with 703 or 704 bp. Only one nucleotide was changed and one was inserted. Thus, we could not use the detailed information of differences of 26 countries within this species.

To better define the morphological traits in various countries, associations among 24 traits, were investigated by principal coordinate analysis (PCA) (Fig. 4). The locations of countries were defined by the two first principal coordinates, PC1 and PC2, which explained 32.7% and 16.8% of total variation in morphological traits. All the 26 countries, Australia and Russia were situated adjacent to one another though geographical distance is very remote. The Korean cultivar is isolated from cultivars of other countries.

This study demonstrates that RAPDs offer a suitable method for detection of genetic variability in safflower. The degree of polymorphism revealed in the three *C. tinctorius* cultivars by amplification with arbitrary primers is extensive. Partitioning of genetic variability indicates that, on average, 71.5% is distributed between, and 28.5% within, cultivars. These findings are not in agreement with the observation that out-breeding plants retain considerable variability and most variation is exhibited within cultivars [7]. Though the number of individuals sampled for analysis was small and probably not fully representatives of the total available diversity in *C. tinctorius*, our study demonstrates
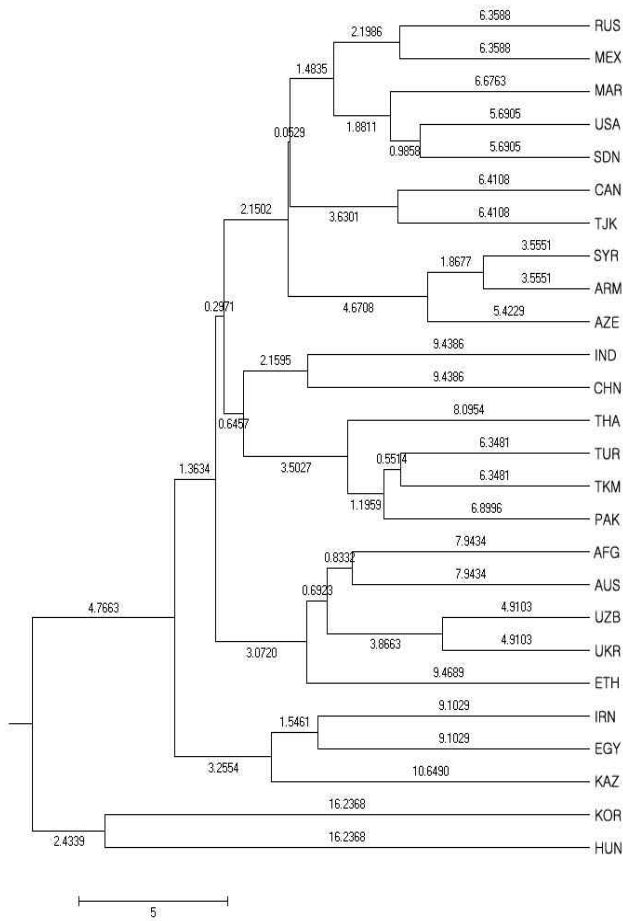
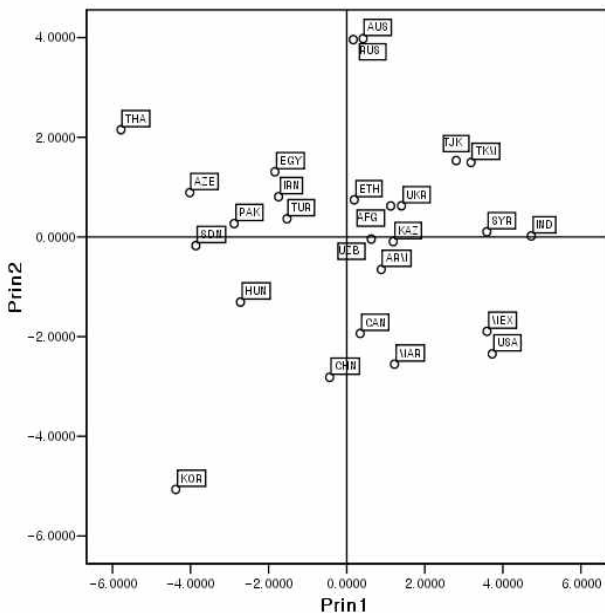Fig. 3. A Phylogenetic tree for 26 countries based on RAPD analysis.

Fig. 4. Results of principal component analyses of 24 morphological data in *C. tinctorius*.

that the regions (Morocco, Syria, and Turkey) of the Mediterranean Sea are more variable than other regions except India (Table 1). In this result, although only simple result of RAPD is difficult to assert the center of species diversity of *C. tinctorius*, the regions of the Mediterranean Sea may be the most probable candidate for the origin of safflower. India was also the candidate of the center or secondary center of species diversity of *C. tinctorius*.

If the RAPD analyses are reliable in their ability to detect variability among *C. tinctorius* cultivars, then this method can be used to study the diffusion routes of cultivated plants because variability among cultivates should reflect their phylogenetic relationship after domestication. This investigation of the diffusion routes of safflower by comparing RAPD markers among cultivars is based on several assumptions. The cultivars that were used in the analyses are more or less the same as those that diffused in the past or are the direct descendants of the plants that diffused. Diffusion took place stepwise without long distance migration.

If Korean safflower cultivars, for example, consist of mixture of those that diffused with Chinese and India accessions in ancient times and those that diffused recently, or some cultivars came directly from remote areas as a result of long distance migration, then some cultivars will have quite different positions in the phylogenetic tree (Fig. 3) to their geographical positions, and the phylogenetic relationship among Asian cultivars will be confusing as similar cases.

Although the amount variability detected with RAPD analysis is dependent upon the selection of appropriate primers and problems with reproducibility have plagued the use of this technique in the past, this method has the advantage of being inexpensive and simple to perform, and does not require a previous knowledge of the genome [6]. However, we were able to limit these problems in this study, as evidenced by the results of our duplicate analysis, by careful DNA preparation, strict adherence to amplification protocols and a rigorous interpretation of the results. We also found the results of RAPD to be faster and easier to interpret than ITS techniques studied, making it an ideal technique for use in the identification of *C. tinctorius* accessions or cultivars. Based on the results study, RAPD markers were the least polymorphic markers of those evaluated, and consequently had the least resolving power.

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초록 : RAPD 마커에 의한 수집된 홍화자원에서 계통관계와 유전적 다양성

성정숙 · 조규택 · 이기안 · 백형진 · 허만규[1]*

(농촌진흥청 농업유전자원센터, [1]동의대학교 분자생물학과)

홍화(*Carthamus tinctorius* L.)는 세계 여러 나라에 분포하고 있는 초본류이다. 이 종은 경제적으로 중요한데 홍화는 약용, 적색소, 노랑 색소로 이용된다. RAPD 기법으로 홍화의 26 집단 간 유연관계와 유전적 다양성을 조사하였다. 모든 집단에서 123개 밴드를 얻었으며 시발체(primer) 당 평균 9.5개 밴드를 나타내었다. 홍화의 유전적 다양도는 집단 내에 대부분 귀속되며 높은 집단 간 분화를 나타내었다. OPC18-01 밴드는 시리아 그룹에 특이 밴드였으며 다른 나라 집단에서는 발견되지 않았다. 이런 7개 특이 마크(SCAR)를 발견하였다. 비록 홍화의 분석한 개체 수가 적고 각 나라의 대표성을 의미하지 않지만 본 연구 결과 지중해의 지역(모로코, 시리아, 터키)이 인도를 제외한 다른 지역보다 변이가 높았다. 단순히 RAPD만으로 단정하기 어렵지만 홍화의 기원 센터의 후보군으로 지중해 연안으로 추정된다. 인도 역시 홍화의 2차 센터의 후보군이다. RAPD 마커는 홍화의 자연 집단을 분류하는데 효과적이었다.