Korean J Crop Sci 49(4) 337~341(2004)

Morphological and Genetic Diversity of Korean Native and Introduced Safflower Germplasm

Kang-Bo Shim†, Seok-Bok Bae, Si-Kyu Lim, and Duck-Yong Suh

Upland & Industrial Crop Division, National Yongnam Agricultural Research Institute, RDA

ABSTRCAT: Morphological and genetic diversity of thirty nine safflower germplasm were collected and evaluated by Principal Component Analysis (PCA) and Random Amplified Polymorphic DNA (RAPD) method. Stem length and seeding to flowering days of the safflower germplasm showed 26 ~ 117cm and 76 ~ 179 days of variation respectively. USA originated germplasm showed higher oil content as 39%, but that of Japanese showed lower as 26%. PCA made three different cluster groups according to some agronomic characteristics of safflower. Korea originated germplasm showed similar cluster group with that of collected from USA in the PCA of stem length. But in the seeding to flowering days, it showed similar cluster pattern with that of collected from Japan rather than USA. In the experiment of RAPD analysis, total five primers showed polymorphism at the several chromosomal loci. Korea, China Japan and South Central Asia originated germplasm were differently classified with USA and South West Asia originated germplasm with lower similarity coefficient value (0.47). Most of Korea originated germplasm were grouped with South Central Asia originated germplasm with higher similarity coefficient value (0.74) conferring similar genetic background between both of them. China and Japan originated germplasm were dendrogramed with Korea originated germplasm at the 0.65 and 0.50 similarity coefficient values respectively. Some common results were expected from both of PCA and RAPD analysis, but lower genetic heritability caused by relative higher portion of environmental variance and environment by genotype interaction at the expression of those of agronomic characteristics made constraint to find any reliable results.

Keywords: safflower, PCA, RAPD, similarity coefficient, dendrogram

Safflower (*Carthamus tinctoris* L.) originated from Egypt and Afghanistan is annual herb, usually used as medicinal materials in Korea, China, and Japan (Lee, 1980). Safflower also cultivated as oil crop or important plant resources. Traditionally, it has been used as folk remedies for bone diseases such as fracture and osteoporosis etc. (An *et al.*, 1975). It's flower contains cartamin ($C_{12}H_{22}O_{11}$) which inhibits platelet coagulation and delays bleeding time (An *et al.*, 1975; Kee, 1993). Safflower seed contains several fatty acids, and linoleic acid was reported as function to reduce blood cholesterol content (Kim *et al.*, 2000). In recent, the scale of imported safflower mainly from China is gradually increased by the expansion of domestic requirement. But quality of imported safflower is very lower and cheaper than that of domestic, so many importers disguise imported safflower as domestic's which cause damage to many domestic safflower cultivators. Up to now, several researches relating to identification of imported or domestic safflower were conducted (Park, 1984; Kim *et al.*, 1999) usually in view of morphological difference or medicinal composition. But any experiment in view of comparing affinity and genetic relationship according to the origin of safflower were not conducted. This experiment was conducted to get basic information on the clustering and affinity of several agronomic characteristics for the identification of imported and domestic safflower by RAPD analysis and Principal Component Analysis (Williams *et al.*, 1990; Cooper *et al*, 1994).

MATERIALS AND METHODS

Agricultural Research Service Western Station, United States Department of Agriculture provided total thirty nine safflower germplasm collected from Korea, China, Japan, South Central Asia, South West Asia and USA. Those materials were sowed at pot (30 × 30cm) with three replications at the green house in October 20, 2002.

Some agronomic characteristics of safflower germplasm by PCA

It was surveyed some agronomic characteristics, such as stem length, seeding to flowering days and characteristics of leaf (shape, color, spine etc.) which showed much variable according to the different collected locations of safflower germplasm. And it was conducted PCA to confer variation or similarity among them.

†Corresponding author (Phone) +82-55-350-1241 (E-mail) shimkb@rda.go.kr
<Received August 19, 2004>

Oil content and fatty acid composition of safflower germplasm

Oil content of collected materials was analyzed by soxhlet method using ethyl ether extract solvent. Fatty acids content was also analyzed by several procedures, dissolving materials at hexane and methanol solution, cooling, neutralization by H_2SO_4, dehydration using sodium sulfate and quantification of fatty acids by gas chromatography (Varian 3400, Varian) with flame ionization detector at the condition of column temperature of 200 °C, injection and detector temperature of 220 °C.

Genetic variation of safflower germplasm by RAPD analysis

DNA separation and extraction were conducted using Wizard Magnetic 96 DNA Plant System kits(Wizard Magnetic 96, Labsystem co.). DNA was quantified by Fluorometer 96 (Fluoroskan Ascent FL, Labsystem co.) under the standard curve (Jonshon *et al.*, 2002). Bulked DNA solution was prepared to compare DNA band variation by dividing about 2 μl of DNA of each germplasm from total twelve samples to make 24 μl of bulked DNA solution. Optimum PCR analysis condition for total 25 μl solution for GeneAmp PCR System 9700 is 15.3 μl of DdH_2O, 2.5 μl of 10X buffer, 3.0 μl of $MgCl_2$, 0.2 μl of Taq, 1.0 μl of primer, 1.0 μl of template DNA by 94 °C for 4 min. 30 sec., 35 °C, 15 sec., 72 °C, 2 min. 30 sec., 40 °C for 15 sec. to conduct total forty cycles. Amplified DNA product was conducted band separation at 2% of agarose gel by 0.5X of TBE buffer solution and identify separated band under ethidium bromide solution NTSYS-PC, version 2.12d was used to score reproducible DNA band by presence (1) or absence (0) to make dendrogram under PGMA (paired group mean arithmetic average). Similarity Coefficient was also analyzed by Similarity for Quantitative Data (SIMQUAN) using S(x,y)= 2Nxy/(Nx + Ny) formula (Nei *et al.*, 1979). At the formula, Nx and Ny mean RAPD band at x, y, Nxy means covalent band of x, y.

RESULTS AND DISCUSSION

Some agronomic characteristics of safflower germplasm

It is important to compare morphological agronomic characteristics to confer genetic variation or those affinity indirectly. We surveyed some characteristics of different originated safflower germplasm (Table 1)

Korea originated safflower germplasm showed longest stem length as 88 cm, but that of collected from South Central Asia was shortest as 51 cm. General architectural type of safflower germplasm were branching and erecting. Predominant color of flower petal was red, orange, and leaf type was lanceolate, spine type. Days from seeding to flowering showed that South Central Asia and South West Asia originated germplasm were about 92 ~ 100 days, but that of Korea was 80 days.

Oil content and fatty acids composition of safflower germplasm

No significant statistical difference on the oil content and fatty acids composition among safflower germplasm was showed. But, USA originated germplasm contained higher oil content as 39%. Linoleic, oleic and palmitic acid by which generally occupy 95% of total fatty acids are main components in safflower. In the comparison of fatty acids composition among safflower germplasm, China originated germplasm showed high linoleic acid composition as much

Table 1. Several agronomic and morphological characteristics of different originated safflower germplasm.

Originated country	Stem length ♪ (cm)	Branch type	Growth type	seeding to flowering days ♪	Color of flower petal	Leaf type	Spine
China	75±7.3	Branch	Erect	90±4 7	Red	Lanceolate, oblong	Fewer spine, spine
Japan	77±3 0	Branch	Erect	86±1 2	Red	Lanceolate	Spine
Korea	88±8 5	Branch	Erect	80±2 2	Red	Oblong, lanceolate	Spine, fewer spine
South Central Asia	51±18.2	Branch	Sprawl, erect	100±34.8	Orange	Lanceolate	Spine
USA	60±3.6	Branch	Erect	90±0 9	Yellow, orange	Lanceolate	Spine
South West Asia	84±21 9	Branch	Erect	92±1 9	Orange,red	Oblong, lanceolate	Spine, fewer spine

♪ value · Mean ± standard deviation

Table 2. Comparison of oil content and fatty acids composition of different originated safflower germplasm.

Originated country	Oil content ♪ (%)	Fatty acid composition (%) ♪		
		Palmitic acid	Oleic acid	Linoleic acid
China	27 1±6 1	5.0±0.4	10.6±1 0	80 4±1.1
Japan	25 7±1 2	5 0±0 2	11.0±0.4	79 4±0 6
Korea	29 8±1.5	9.4±0 4	12.4±0 3	73 4±0.6
South Central Asia	27.9±3 8	4.9±0 5	30 6±28.4	59 1±28 5
USA	38 6±2.7	5 1±0 8	36.3±34.9	54 6±34.3
South West Asia	26.6±0 7	5.4±0.6	11 0±1.3	78 6±1 4

♪ value : Mean ± standard deviation

as 80%, but germplasm collected from USA showed about 30% lower than that of China. In contrast, germplasm collected from USA showed higher oleic acid as much as 25% compared to China (Table 2).

Agronomic characteristics by PCA

In the PCA for stem length of safflower germplasm with different origins, total three groups are clustered; germplasm from China, Japan grouped one, that from Korea, USA grouped another, and that from South Central Asia, South West Asia grouped other one (Fig. 1). Therefore it was concluded that different genetic inheritance or main factor affecting genetic response was diverse according to the collected locations. Interpretation ratio for IPCA1 and IPCA2 was 62%, 22% respectively showing total 84% of genetic explanation by them. Especially, the fact that germplasm from China and Japan placed same group at the plot of PCA was under the same understanding that some safflower germplasm from Japan was expected to show high similarity to the that of China in view of molecular variation by RAPD analysis.

In the comparison of seeding to flowering days of safflower germplasm at the PCA analysis, three groups are made, but those general response was different from that of stem length. Interpretation ratio of seeding to flowering days for IPCA1 and IPCA2 is 63%, 22% respectively (Fig. 2). According to the result of PCA for seeding to flowering days, Korea originated germplasm showed high similarity to that of Japan germplasm, and both of USA and South Central Asia originated germplasm showed similar response We also expected similar result that both of them would show higher similarity value at the RAPD analysis.

In the PCA for comparison of oil content of safflower germplasm, Japan, USA and South West Asia originated germplasm showed similar response and that of Korea showed different response to others. Interpretation ratio of oil content for IPCA1 and IPCA2 is 63%, 23% respectively (Fig. 3). At the Fig. 3, germplasm from USA and South West Asia showed similar response in view of above results, similarity test at the RAPD analysis also was expected that both of USA and South West Asia originated safflower population would show high similarity value

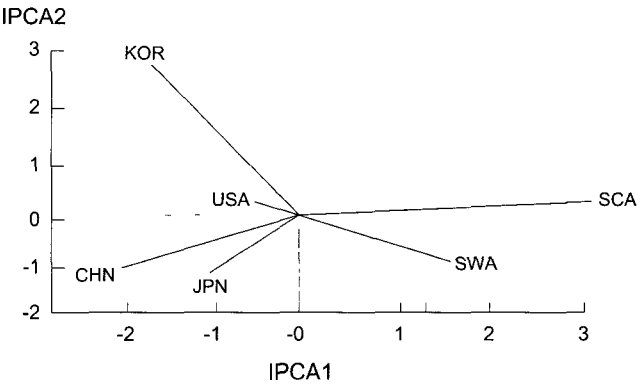

Fig. 1. Plot of the first two principal components for stem length of six different originated safflower CHN China, JPN : Japan, KOR Korea, SCA : South Central Asia, SWA : South West Asia, USA . U.S A.

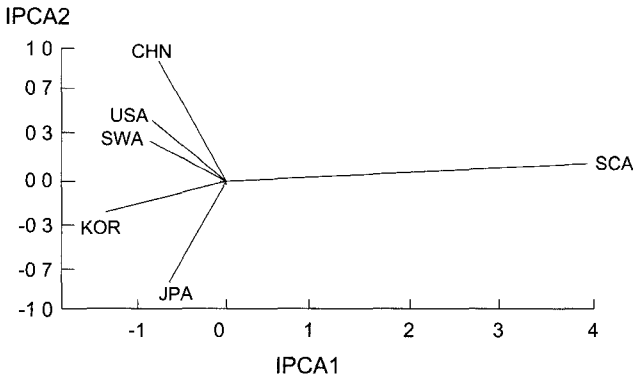

Fig. 2. Plot of the first two principal components for seeding to flowering days of different originated safflower germplasm

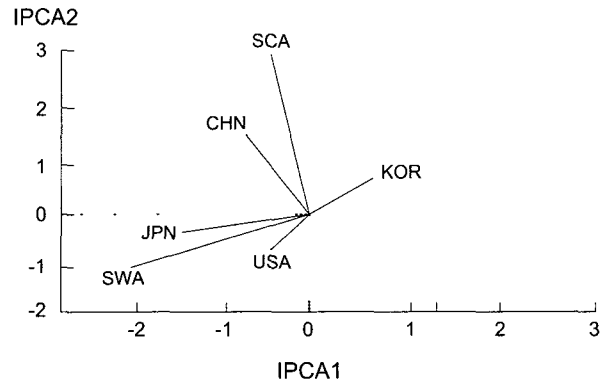

Fig. 3. Plot of the first two principal components for oil content of six different originated safflower germplasm.

RAPD analysis of different originated safflower germplasm

Total five primers among sixty primers(OPAA120, OPB120, OPF120) showed polymorphism at the screen of safflower germplasm. Those selected primers were used for the analysis of genetic variation of DNA level using RAPD method. one or two specific DNA bands at the same locus were identified at the bulked solution from China and USA, in which it is conferred that USA and China originated safflower germplasm have similar genetic background (Fig. 4).

According to similarity analysis by Similarity for Quantitative Data (SIMQUAN), Korea, China, Japan and South

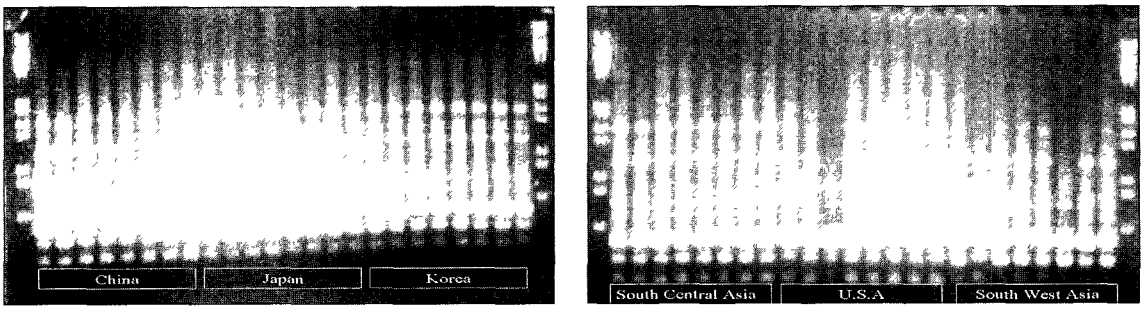

Fig. 4. Representation of polymorphic DNA band on bulked DNA solution using selected primers

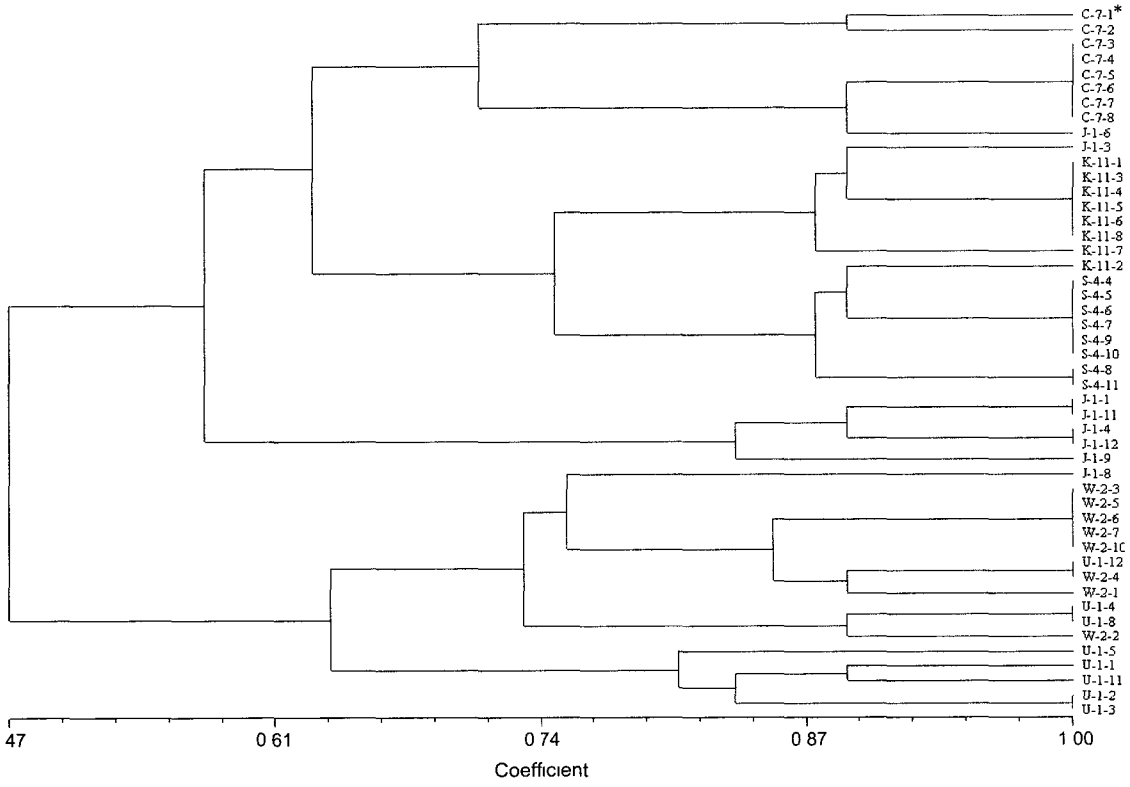

Fig. 5. Dendrogram of 48 different safflower accessions by PGMA C- China, J- . Japan, K- Korea, S- : South Central Asia, W- : South West Asia, U- . U.S.A.

Central Asia originated germplasm were classified differently with those from USA and South West Asia at the 0.47 value of similarity coefficient. Most of Korea originated germplasm was grouped with that of South Central Asia at the 0.74 value of similarity coefficient showing higher genetic similarity between both of them. China and Japan originated germplasm drew dendrogram with that of Korea at the 0.65, 0.50 similarity coefficient values respectively.

In the principal component analysis of stem length, seeding flowering days and oil content, different responses were displayed according to safflower originated locations because much interaction of some genotype by environmental factors was affected to express those related heritability. Also, it was not easy to find any co-existent variation between principal component analysis and AFLP analysis. To get more reliable experimental results of genetic background or variation for morphological characteristics of different originated safflower germplasm, it is necessary to reduce any environmental variation parts among total variations and apply suitable biotechnology (Yan *et al.*, 1998).

REFERENCES

An, D K and C S Yuk 1975 Present medecinal plants In : Safflower Kommon Publishers 358-359

Cooper, M. and I H Delacy 1994 Relationships among analytical methods used to study genotype variation and genotype by environment interaction in plant breeding multi-environment experiments The Appl Genet. 88 561-572

Jonshon, R. C , W J Johnston , C. T. Golob , M. C. Nelson, and R J. Soreng. 2002 Characterization of the USDA *poa pratensis* collection using RAPD markers and agronomic descriptors Genetic Resources and Crop Evolution 49 : 349-361.

Kee, C. H. 1993 The pharmacology of chinese herbs CRC, press 249

Kim, J. H., D. T. Kwak, D Y Choi, and K D. Moon. 1999 Comparison of the chemical compositions of korean and chinese safflower seed Korean J. Food Sci Technology 31(4) . 912-918

Lee, C. B 1980 Picture Book of Korean Plants In . Safflower. Baekyang Publishers. 779-800.

Nei, W. and W H Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Nat'l Acad Sci 76 : 5269-5273

Park, S. W. 1984 Studies on cultural practice and useful composition of korean local safflower Ph.D. thesis Univ. of Konkuk, Seoul, Korea.

Williams, J G K , A R Kubelik , K. J Livak , J A. Rafalski, and S V. Tingey 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic acids Res 18 : 6531-6532.

Yan, W. and L A Hunt 1998. Genotype by environment intraction and crop yield Plant Breed. Rev. 16 135-178.