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SDS-PAGE BASED GENETIC DIVERGENCE IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.)

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Abstract

Safflower (*Carthamus tinctorius* L.) germplasm, comprising of 116 accessions was characterized using Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of seed storage proteins. The germplasm was acquired from different countries of the world. Total seed proteins were separated through electrophoresis polyacrylamide gels using standard protocols. Eighteen (60%) of the protein bands detected were polymorphic, the rest being monomorphic. Eight bands (14, 17, 18, 19, 20, 23, 24 and 25) were more than 80% common in all accessions. Similarity coefficients among the accessions ranged from 0.00 to 1.00. Accessions 16327 and 26752 were the most divergent genotypes having maximum dissimilarity with all the other accessions used. Unweighted Pair Group Method with Arithmetic averages (UPGMA) was used which is based on dissimilarity matrix. The dendrogram obtained separated all accessions into four main clusters (I, II, III and IV) and two independent individual genotypes. Four major clusters comprised of 23, 75, 8 and 8 accessions, respectively. This technique did not reveal genetic variability of significant value in safflower genotypes, hence advanced molecular and biochemical markers are recommended for further studies. This study will be helpful for the future breeding program of safflower accessions.

Introduction

Safflower (*Carthamus tinctorius* L.) mainly grown under dry land conditions as an oilseed crop (Fernandez-Martinez & Knowles, 1978) belongs to the family compositae (Asteraceae). It is a thistle-like annual, diploid ($2n = 24$) herbaceous crop that thrives in hot dry climates and is capable of surviving on minimal surface moisture. Other members of this family are artichoke, chrysanthemum, niger and sunflower (Yuan *et al*., 1989). However, it is considered as a minor oilseed crop as compared to other oilseed crops grown worldwide. Major area of production of Safflower is Mexico, Ethiopia, Kazakhstan, USA, Australia and India. In these countries it is grown on an area of 85000, 72000, 63000, 54000 and 35000 (each for last two) ha respectively. In Pakistan it has minor acreages (Ashri, 1975). Safflower leaves have numerous spines. Its fruits are white and shiny, with thick pericarp of weight ranging upto 0.1 g (Fernandez-Martinez & Knowles, 1978). Besides oilseed purpose, safflower has found its uses in pharmaceutical industry and cure in traditional folklore since old times. Safflower is used for various kind of medicinal properties, specifically for various issues related to bones (Seo *et al*., 2000; Lee *et al*., 2005). Mundel *et al*., (2004) reported that high value proteins as pharmaceutical & Industrial enzyme can be obtained from Safflower through genetic modification. In Pakistan, it is believed that seed decoction with sugar can be effective laxative to clear urinary tract while in Bangladesh it is used to reduce rheumatic pains. In India and Afghanistan its 'tea' is considered good to avoid abortion or to get cured of infertility (Weiss, 1983).

To have a variety of better traits of any crop we need first hand information about its genetic diversity. (Shinwari *et al.*, 2013). For breeding programs to be successful, we have to assess the available germplasm for knowing its diversity (Sultan *et al*., 2013). Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used as the biochemical techniques, due to its lenience and usefulness for telling the genetic construction of crop germplasm (Nisar *et al.*, 2011). This technique separates the seed storage proteins of the crop being analyzed (Khan *et al*., 2013) which helps in resolving the taxonomic and evolutionary relationships of the crop and its relatives both wild and cultivated (Das & Mukharjee, 1995; Khan *et al*., 2010).

Assessments of genetic diversity based on biochemical markers of different crops have been studied by different researchers. Akbar *et al*., (2012) used SDS-PAGE technique to study genetic diversity of *Sesamum indicum* for total seed protein and got satisfactory results. Khurshid & Rabbani, (2012) employed SDS-PAGE for electrophoretic comparisons of seed protein profiles from seed of different brassica cultivars. Similarly based on SDS-PAGE, Shinwari *et al*., (2013) examined the genetic diversity of 102 *Eruca sativa* genotypes collected from different ecological areas of Pakistan. Khan *et al.*, (2013) studied total seed proteins in rice (*Oryza Sativa* L.) genotypes of Pakistan through SDS-PAGE. Likewise, Zada *et al.*, (2013) utilized this method to study protein-based genetic diversity in *Brassica carinata* A. Braun (Ethiopian mustard) germplasm. The present study focuses on evaluating genetic variability of 116 safflower accessions from diverse ecologies of the world by SDS-PAGE analysis.

Materials and Methods

Plant material: One hundred and sixteen safflower accessions of diverse origin were used during present investigation.

Buffers preparations: Protein extraction buffer was prepared from 0.5M Tris-HCl of pH8, 5M Urea, 0.2% SDS and 1% 2-mercaptoethanol. Bromophenol blue was added as a dye to pursue protein in the gel. Tris-glycine (9.0g Tris-HCl and 43.2g glycine per 3 liters buffer solution at pH 8.9) and 0.1% SDS (3g) was used to prepare the electrode buffer.

Protein extraction: Total seeds were powdered to extract proteins. Protein extraction buffer (400 µl) was added to1.5 ml tube, having 0.1 gm of seed flour. It was mixed using glass rod. and mixed precisely in eppendorf tube with the help of a small glass rod. Samples were homogenated by centrifugating for 12-14 minutes at 12k rpm at room temperature. The samples were stored at -4^{o}C.

Preparation of electrophoretic gel: SDS PAGE was conducted in 12.2% polyacrylamide slab gels following Laemmli (1970). Vertical slab gel was prearranged in a glass sandwich. Twenty and 0.135% by weight acrylamide and N.N-methylene-acrylamide respectively was used in 0.5M Tris-HCl buffer having pH 8.8, with 0.27% SDS to prepare the separating gel. Ten % Ammonium per sulphate and 15µl TEMED (Tetramethylen-diamine) was added to polymerize the gel. While the staking gel was prepared from 30% acryamide and 0.8% N.N-methylene-bis-acrylamide in 0.25M Tris-HCl buffer (pH 6.8) which contained 0.2% SDS. Similarly Staking gel was polymerized. Using micropipette 8 µl sample was loaded in the stacking gel in each well.

Electrophoresis: It took around 3 hours at 100V to the bromophenol blue marker to reach the bottom of the gel. To determine Molecular weights of alienated polypeptides, standard Unstained Protein Molecular Weight Marker ranging from 10 to 220 kDa (Cat. No.10747-012; Invitrogen Life Technologies) was co-electrophoresed. The gels stained with 2% coomassie blue solution for 30 to 40 minutes. Gels were washed with a solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and distilled water in the ratio of 5:20:75 (v/v) to destain it. It was washed till such time till the color of background disappeared and bands could be observed.

Data analysis: Clear bands were scored for statistical analysis on the basis of polypeptide bands presence (1) or absence (0) to prepare a binary data matrix. Similarity index (s) was determined for all likely pairs of protein band types by the formula ($S = w / (a + b – w)$. This was converted into a dissimilarity matrix for dendrogram construction via method of Un-weighted Pair-group by Means of Arithmetic Averages following Sneath & Sokal, (1973).

Whereas
w = number of bands of ordinary mobility
a = number of type ‘a’ protein bands
b = number of type ‘b’ protein bands

All the analyses were performed using statistical package NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA).

Results

As shown in Fig (1) we could observe 30 protein bands in the assessed accessions. Out of 30 bands, 18 (60%) were polymorphic and 12 (40%) were monomorphic. Size of the protein bands ranged from 5 to 232 kDa. Bands number 14, 17, 18, 19, 20, 23, 24 and 25 were more than 80% common in all safflower genotypes and considered as monomorphic. Accessions with minimum proteins bands were 16337 (Afghanistan), 16360 (India), 16278 (India), 26753 (Beijing, China), 16234 (India) and 16270 (Pakistan). They have 3, 3, 8, 8, 9 and 9 protein bands, respectively. Some accessions showed maximum protein bands, such as 26741 (China), 26739 (Sichuan, China), 26740 (China) and 26742 (Shandong, China) showed 26, 25, 24 and 23 bands, respectively. Minor protein bands of the gel had more variations. Several bands had aggregate protein peptides at a definite molecular weight. The range of similarity coefficients was from 0.00 to 1.00. Minimum similarity was observed between 16327 and 26752. Accessions 16327 and 26752 were the most divergent genotypes having maximum dissimilarity with all the other accessions used in the study. Based on dissimilarity matrix using UPGMA, dendrogram distributed 116 genotypes into four main clusters (I, II, III and IV) and two independent individual accessions (Fig. 2). Four major clusters comprised of 23, 75, 8 and 8 accessions, respectively and could be further sub-divided into a number of sub-groups. Two groups could be recognized in cluster I. Group (I-1) consisted of 22 accessions including check cultivar (Thori-78), whereas second group (I-2) contained only one accession, 16276. Cluster II was the biggest group with 75 genotypes of safflower, which was further sub-divided into two sub-groups. There were 19 accessions in sub-group II-1, while sub-group II-2 of this cluster consisted of 56 genotypes. Cluster III was a smaller group and could also be further sub-divided into two sub-groups. Sub-group (III-1) comprised of seven accessions, whereas sub-group III-2 had only one accession, 26790. Cluster IV was also a smaller one and included eight accessions. Accessions 26752 and 16327 were the most diverse genotypes, forming two independent and isolated individual clusters (Table 1).

Discussion

Plants have different types of proteins on the basis of which they are diverse from each other and this could be a source of identifying diversity at protein & seed level using SDS-PAGE. (Rehman & Hirata, 2004; Shah *et al.,* 2011). SDS-PAGE technique is mostly thought as a reliable mean and has proved to be an important way of revealing the differences and relations between and within taxa; and is mainly free of environmental variations (Iqbal *et al*., 2005). Genetic diversity can be easily evaluated through biological markers (Jan *et al*., 2011). Our investigation showed a limited level of intra-specific diversity in the germplasm of safflower which were supported by Obreht *et al*., (2002) who reported that cultivated genotypes of *C. tinctorius* had same electrophoretic patterns and differ from the wild species of *C. lanatus*. Zilic *et al.,* (2010) also reported a very similar seed proteins profiling pattern in sunflower protein bands (thirty two). This identical pattern is in fact the use of same species accessions of the safflower. Based on the major proteins bands of safflower according to our grouping pattern ranged from 25 to 5 kDa, variations were observed in a few accessions (29 and 30 number bands) mainly collected from China (26738, 26740, 26741), Turkey (26752) and USA (26747, 26748), while in minors proteins bands like major bands ranged from 232 to 26 kDa in bands number 1 and 2, diversity were available mostly in accessions from Iran (16306), Germany (16320), India (16367), China (26740, 26741) and USA (26750). Low level of differences in major bands in different accessions specifies that genes coding for these proteins are preserved in the species (Ali *et al.,* 2007).

The present results indicates that this technique present a means for studying genotypes discrimination based on genetic variation in seed protein in the accessions of safflower. Safflower germplasm which showed the same banding pattern may be duplicated; it should be confirmed through the use of advanced molecular markers. In the present investigation limited intra-specific differences were observed and it was noticed that SDS-PAGE technique alone is not sufficient to study intra-specific variation. Besides, it is suggested that diverse germplasm should be collected from geographically dissimilar regions, to make a large based gene pool with maximum diversity. Our research work will be helpful to provide seed protein based safflower genetic variation information to different researchers, academia and genetic resources managers dealing with the crop.

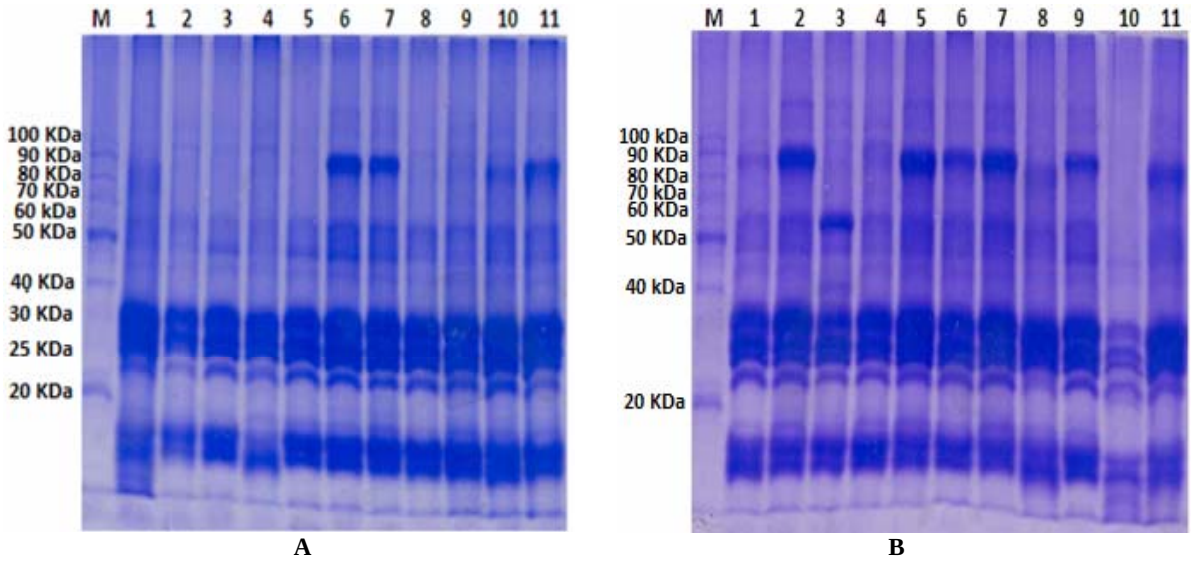

Fig. 1. Electrophoretic banding pattern produced by SDS-PAGE of total seed proteins of safflower accessions.
A: 1=16279, 2=16280, 3=16282, 4=16283, 5=16284, 6=16285, 7=16288, 8=16290, 9=16291, 10=16292, 11=16293
B: 1=16297, 2=16304, 3=16306, 4=16307, 5=16314, 6=16316, 7=16317, 8=16318, 9=16320, 10=16324, 11=16326

Table 1. Grouping of 116 safflower genotypes based on SDS-PAGE analysis.

Group	Sub-group	Accessions	No. of genotypes	Percent (%)
I	1	Thori-78, 26800, 26799, 26793, 26763, 26765, 16274, 16271, 16270, 26797, 16235, 16214, 16360, 16342, 16326, 16234, 16333, 16337, 16358, 16343, 16210 and 16200	22	18.97
	2	16276	1	0.86
II	1	26778, 26779, 26777, 26780, 26784, 26785, 26782, 26781, 26783, 26776, 16265, 16263, 16260, 16253, 16252, 16266, 16249, 16262 and 16240	19	16.38
	2	16320, 26741, 26740, 26739, 26738, 26737, 26732, 26736, 26735, 26734, 26733, 16367, 16318, 16324, 16318, 16324, 16316, 16314, 16307, 16306, 16304, 16317, 16293, 26753, 26763, 26762, 26761, 26759, 26756, 26754, 26750, 26749, 26792, 26791, 26771, 26770, 26769, 26768, 26767, 26766, 26764,26755, 26292, 26297, 26285, 26284, 26290, 26291, 26283, 26288, 26282, 26280, 26279, 26278, 26246 and 26245	56	48.28
III	1	26747, 26748, 26746, 26745, 26744, 26743 and 26742	7	6.03
	2	26790	1	0.86
IV	-	26775, 26774, 26773, 26772, 26758, 26757, 26751 and 26760	8	6.90
V	-	26752	1	0.86
VI	-	16327	1	0.86

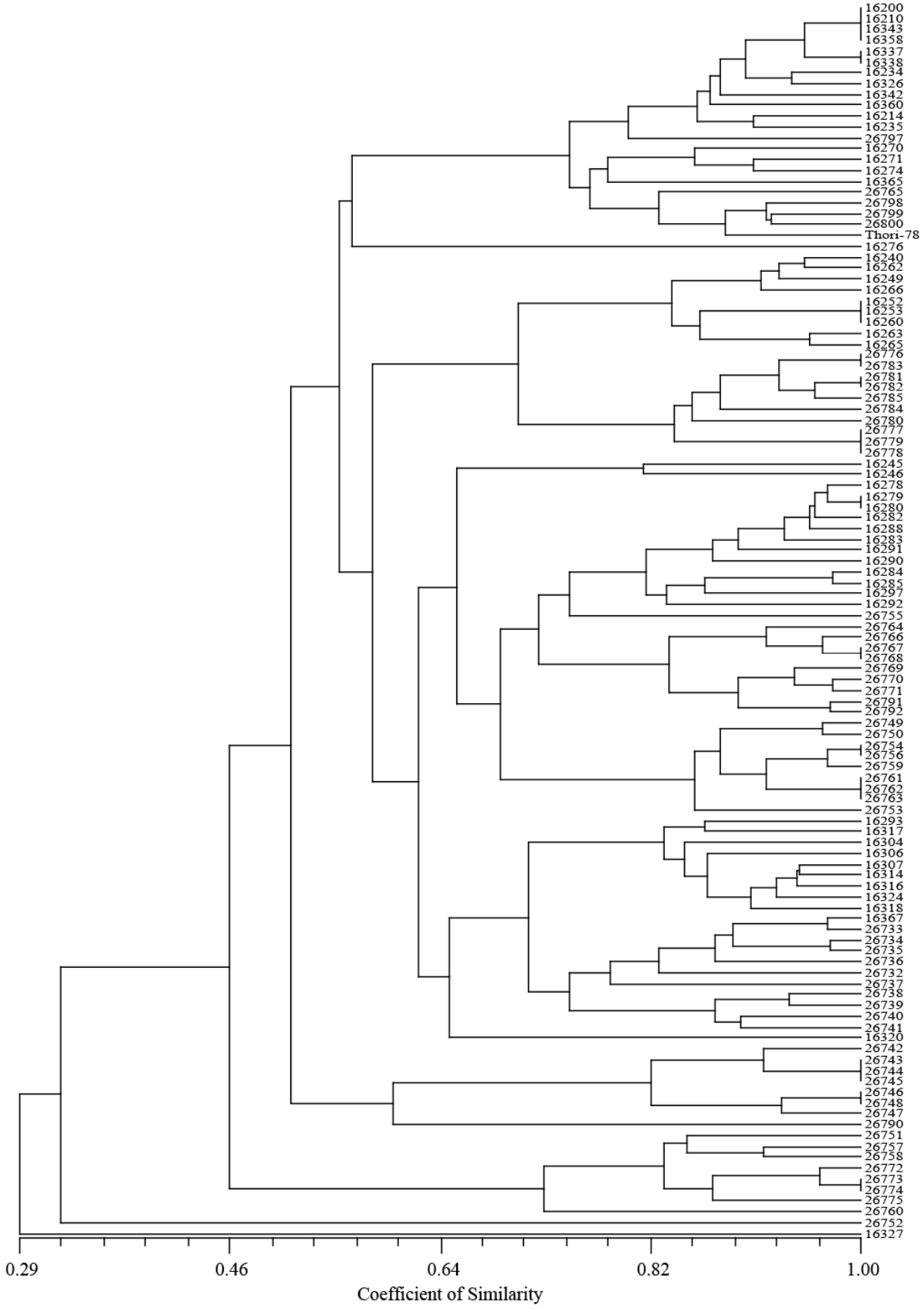

Fig. 2. Dendrogram showing the relationship among safflower accessions based on SDS-PAGE of total seed storage proteins.

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