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STUDY OF TOTAL SEED STORAGE PROTEINS IN ETHIOPIAN MUSTARD (*BRASSICA CARINATA* A. BRAUN) GERMPLASM

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Abstract

The Ethiopian mustard (*Brassica carinata* A. Braun**)** germplasm, comprising 94 accessions was characterized for total seed storage proteins using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The germplasm accessions were obtained from PGRP Gene bank, Institute of Agri-Biotechnology and Genetic Resources (IABGR), NARC, Islamabad Pakistan. To our information, no studies have yet been carried out in Pakistan on the genetic evaluation of *B. carinata* genotypes based on total seed protein. Total seed proteins were electrophoretically separated on 12.25% polyacrylamide gels using standard protocols. A total of 31 polypeptide bands were observed, of which 14 (45.27%) were polymorphic and 17 (54.83%) were monomorphic. The molecular weight of various bands ranged from 8 to 180 KDa. Similarity coefficients varied from 0.50 to 1.00. The dendrogram based on dissimilarity matrix using unweighted pair group method with arithmetic averages (UPGMA) separated all accessions into five main groups. Overall low level of genetic variability was observed for SDS-PAGE (single dimension) in Pakistani local accessions, while medium to high level of genetic variability was observed for exotic material. As SDS-PAGE alone did not reveal high level of genetic variability, hence 2-D gel electrophoresis along with other advanced type molecular markers may reveal variability with more help. Our investigation suggests that more germplasm of Ethiopian mustard need to be acquired to broaden the genetic base for research and development.

Introduction

Amphidiploid species Ethiopian mustard (*Brassica carinata*) is evolved through inter-specific hybridization between *B. nigra* (L.) Koch (n = 8) and *B. oleracea* L. (n = 9) (U, 1935). The species was evolved in the highlands of Ethiopia and adjoining portion of east Africa and the Mediterranean coast (Simmonds, 1979; Hemingway, 1995). The poor people and smallholder farmers in Ethiopia produce the crop for different uses. They eat the leaf at its earlier stages of development either by thinning or topping and also harvest the seed for oil extraction and other uses. As compared to the other oil crops occupying the same ecological nitch in Ethiopia, it gives the highest yield (Hiruy *et al*., 1983), but it possesses high erucic acid in the oil and high glucosinolates in the oil free meal. Researchers in Canada, India and Spain had showed interest to this crop due to its tolerance to biotic and abiotic stresses under semi-arid condition (Rakow, 1995).

Brassica species are used as oilseed crops (*B. napus* and *B. juncea*), leafy vegetables and turnip (*B. oleracea* and *B. rapa*), and are cultivated worldwide. Especially, in East Asia, many varieties of *B. rapa* are used as agronomically important vegetables. In general, genetic improvement of crops can be accelerated when broad genetic diversity and the information of these genetic resources are available. Research on brassica germplasm could enhance the edible oil production and nutritional benefits of these crops. The collection of these genetic resources and the assessment of genetic diversity within and between landraces should have priority for varietal improvement. At the same time it is necessary to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation and collection of germplasm and to increase the utilization of plant genetic resources. The electrophoresis of seed storage protein is a method to investigate genetic variation and to classify plant varieties (Isumera *et al*., 2001, Akbar *et al*., 2012).

The technique of Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is commonly used for separation of seed storage proteins (Ullah *et al*., 2010). An immense work of research has been inattentive, especially members of the mustard family (*Brassicaceae* or *Cruciferae*) such as species of *Brassica*. Widespread reviews did not show much attention on seeds of various plants with respect to their oil extracts. Food value and its shelf life increase with high amount of protein and high percentage of seed oil (Munazza *et al*., 2009, Shah *et al*., 2011). Similarly, certain amounts of important drugs are also present in plants having high food values. Among the oilseed crop, oilseed rape and related species are now the second largest oilseed crop in the world providing 13% of the world supply. The world demand mainly depends on two species, *B. napus* L. and *B. rapa* L. on a large scale. In the seed of these species the oil and protein percentage are 40% or more and 30 to 35%, respectively (Nasar *et al*., 2006). Seed protein is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for taxonomic study of various species (Vaughan & Denford, 1968; Yadava *et al*., 1979; Akhtar, 2001) and cultivars identification purpose in crop (Kour & Singh, 2008). It has been widely suggested that such banding patterns could be important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivar or when cultivars are to be patented (Tanskley & Jones, 1981). Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice (Thanh & Hirata, 2002). However, the information on the SDS-PAGE on different species of brassica for genetic diversity is still limited (Rahman & Hirata, 2004). In a common practice, genetic improvement is easy in those species/crops, which

have immense genetic diversity, and the information regarding these hidden genetic resources is easily available. Research on brassica germplasm could increase the edible oil production and its nutritional benefits. Therefore, collection of these high value genetic resources and the estimation of genetic assortment within and between landraces should have priority for varietal improvement. Further, it is necessary to develop better methods of characterization and evaluation of germplasm, in order to improve strategies for conservation and collection of germplasm and increase the utilization of plant genetic resources. As a result, electrophoresis of seed storage protein is a method used to explore genetic difference and classify plant varieties. The objective of the present study is to check the genetic variation in the local collections and acquired from abroad for seed storage protein with the help of SDS-PAGE. Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding.

Materials and Methods

Plant material: Plant material consisted of 94 accessions of Ethiopian mustard (*B. carinata*). Details are given in Table 1. The germplasm accessions were obtained from PGRP Gene bank, Institute of Agri-Biotechnology & Genetic Resources (IABGR), NARC, Islamabad, Pakistan. Most of the accessions were acquired from abroad, whereas few were also collected from diverse ecologies of Pakistan.

Table 1. List of *Brassica carinata* accessions used in present study for SDS-PAGE analysis.

No.	Source of acquisition/collection	No. of accessions
1.	Local collection	10
2.	Centre for Genetic Resources (CGN), Wageningen, The Netherlands	74
3.	Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany	10
	Total	**94**

Protein extraction: For the extraction of total seed proteins, whole seeds were powdered with mortar and pestle. To extract proteins from flour10 mg flour was put into 1.5ml micro-tube; protein extraction buffer (400µl) was mixed thoroughly and vortexed. The extraction buffer contained 0.5M Tris-HCl (pH 8.0), 0.2%SDS, 5M Urea, and 1% 2-mercaptoethanol. Bromophenol blue was added to extraction buffer as a dye to show the movement of protein in the gel. The homogenate samples centrifugation at 15,000 rpm for 10 minutes at room temperature (RT). Supernatant (10 µl) was used for protein separation.

Gel electrophoresis: SDS-PAGE of total seed protein was carried out in 12.25% polyacrylamide slab gels in discontinuous buffer system according to method of Laemmli (1970). Ten microliters of sample was loaded into the wells of stacking gel. Electrophoresis was carried out at 75V for 3 hours until bromophenol blue marker crossed bottom of the gel. Pre-stained protein marker, ranging from 10 to 170 kDa (Fermentas Life Sciences) was run for reference to molecular weight of respective protein bands in kd. After electrophoresis, the gels were stained with 2% commassie blue solution for one hour and destained by solution containing 5% (v/v) acetic acid, 20% (v/v) methanol and distilled water in the ratio of 5:20:75 (v/v) for two hour**.**

Data analysis: Depending upon the presence or absence of polypeptide bands, band migration and intensity, similarity index was designed for all potential pairs of protein types. The score was 1 for the presence and 0 for absence of bands. Band intensity was not used for determining diversity. Based on outcome of electrophoretic band spectra, similarity index (s) was deliberated for all possible pairs of protein type electrophoregrams by using the following formula (Sneath & Sokal, 1973):

$$S = w / (a + b – w)$$

where, S = similarity index, w = number of bands of common mobility, a = number of bands of protein a, b = number of bands in protein type b. The similarity matrix thus generated was converted into a dissimilarity matrix (dissimilarity = 1 – similarity) and used to build dendrogram by unweighted pair-group method with arithmetic averages (Sneath & Sokal, 1973). All the analyses were carried out using statistical package NTSYS-pc, version 2.1 (Applied Biostatistics Inc., USA).

Results

Among 94 accessions tested (Table 1) very close relationship was found between the *B. carinata* accessions that were indigenous to Pakistan. However, the electrophoretic seed protein profiles of exotic accessions were diverse to moderate extant as they belonged to a wide range of geographic origin. A total of thirty-one bands were scored among the 94 accessions of *B. carinata* evaluated by SDS-PAGE. Of these 31 bands, 14 (45.27%) were polymorphic and 17 (54.83%) were monomorphic. Size of the protein bands generated by SDS-PAGE ranged from 8 to 180 KDa. One new band in accessions 25957 and 25967, and two new bands were present in accession 25960. (Fig. 1). One band was observed absent in accessions 25961, 25966 and 25967. One band with low intensity was found in accessions 25959, 25964, 25965, 25968 and 25970, while accession 25971 has 2 bands with low intensity. One band with fast migration was observed in accessions 25959, 25963, 25964, 25965, 259667, 2596825970 and 25971, while accession 25961 has 2 bands with fast migration. (Fig. 1). Over all variability in intensity was viewed in many protein bands, but not considered for determining diversity of protein profile.

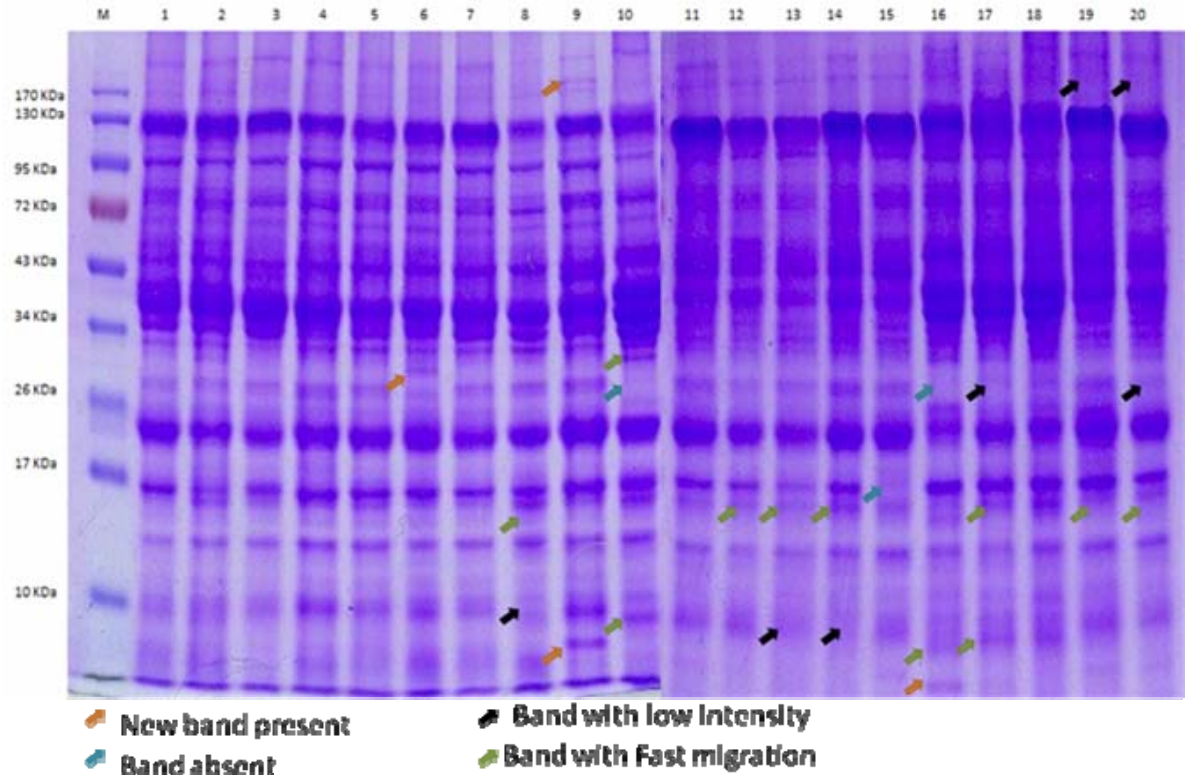

Fig. 1. Electrophoretic banding pattern generated by SDS-PAGE of seed storage proteins of some of *B. carinata* accessions. M = Protein ladder, 1 = 259952, 2 = 25953, 3= 25954, 4 =25955, 5 =25956, 6 =25957,7= 25958, 8= 25959, 9= 25960, 10= 25961, 11=25962,12=25963, 13=25964, 14=25965, 15=25966, 16=25967, 17=25968, 18=25969, 19=25970, 20= 259971.

Table 2. Grouping of 94 *B. carinata* accessions based on cluster analysis using SDS-PAGE analysis.

Group	# Of accessions	Accession names/numbers	Local/Exotic
I	60	25952, 25953, 25970, 25957, 25958, 25959, 25962, 25963, 25965, 25967, 25968, 25954, 25955, 25956, 25971, 25964, 25966,25969, 25960, 25961, 25981, 25982, 25983, 25984, 25985, 26015, 26016, 26018, 26021, 26019, 26012, 26013, 26014, 26020, 25987, 25988, 25989, 25999, 25991, 25993, 26002, 26005, 26003, 26006, 26004, 25997, 25998, 26008, 26008, 26009, 26011, 26010, 26017, 25999, 26000, 26001, 25986, 25994, 25995 and 25996	Both Local and Exotic
II	9	25992, 2595, 25973, 25974, 25980, 25976, 25977, 25978 and 25979	Exotic
III	1	25992	Exotic
IV	20	26022, 26023, 26025, 26024, 26025, 26024, 26035, 26026, 26033, 26034, 26189, 26027, 26029, 26028, 26190, 26191, 26192, 26194, 26193, 26030, 26031 and 26032	Exotic
V	4	26195, 26196, 26197 and 26198	Exotic

The cluster diagram revealed five major groups i.e. I, II, II, III, IV and V (Fig. 2). Group I consisted of 60 accessions (including all the 10 local and 50 exotic accessions), group II consisted of 9 exotic accessions, group III consisted of only one accession (exotic), group IV consisted of 20 (exotic) accessions and group V comprised of 4 (exotic) accessions (Table 2). Similarity coefficients ranged from 0.50 to 1.00.

The results obtained from SDS-PAGE electrophoresis showed that the method provides a powerful tool for reliable intra-varietal diversity identification based on genetic differences in seed storage protein among different accessions. The present study outlines the narrow genetic diversity in the different *B. carinata* based on SDS-PAGE for local germplasm.

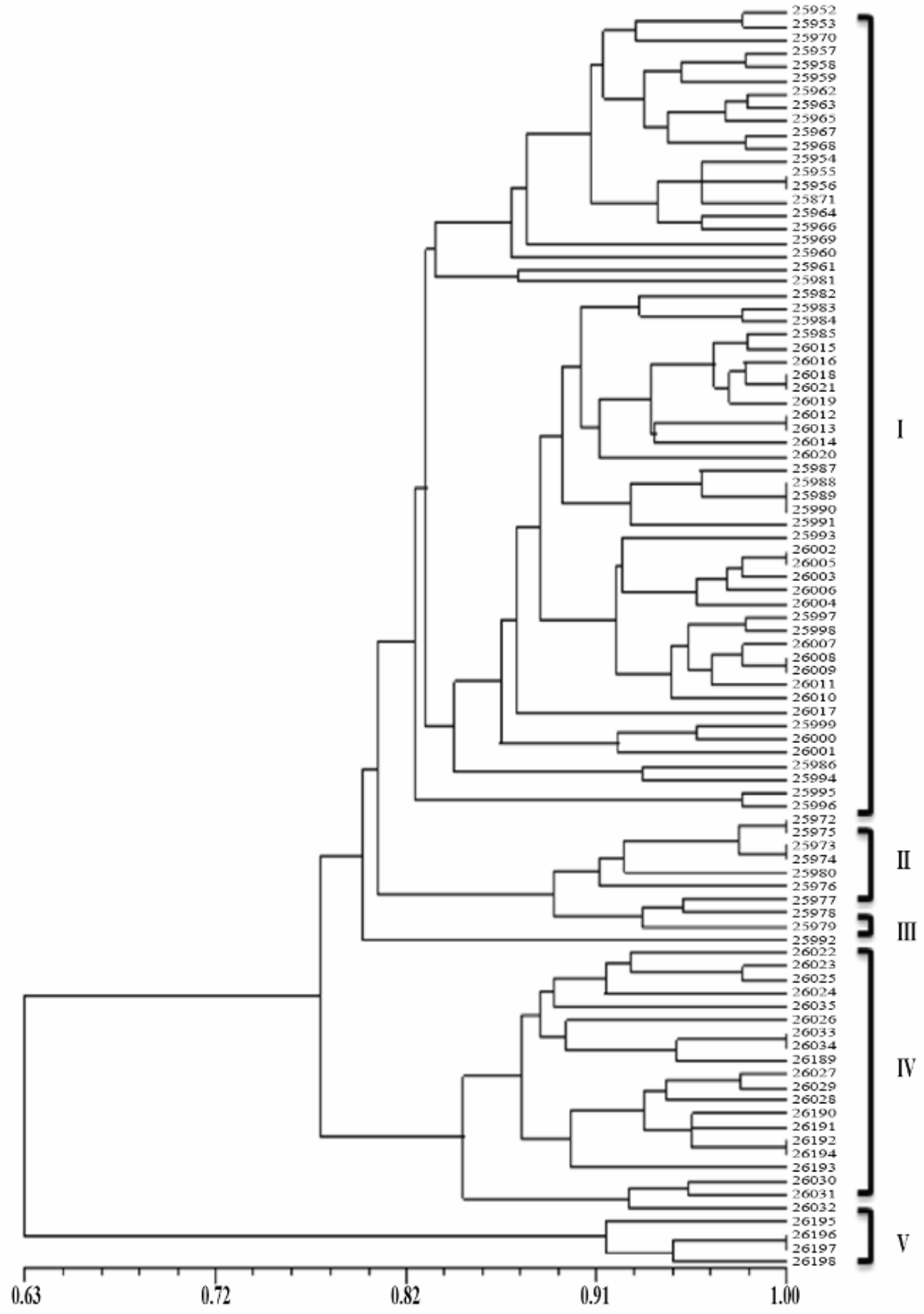

Fig.2. Dendrogram showing the relationship among *B. carinata* genotypes based on SDS-PAGE of total seed proteins

Discussion

Seed protein analysis by SDS-PAGE has proved to be an important way of revealing the differences and relationships between and within taxa; and is mainly free of environmental variations (Javaid *et al*., 2004; Iqbal *et al*., 2005). The high stability of the seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants (Ladizinsky & Hymowitz, 1979). Our results also gave good insight to diversity of *B. carinata* germplasm. The present study, outlines the narrow genetic diversity in the different local material of *B. carinata* based on SDS-PAGE for local germplasm; as all the accessions were grouped in one cluster (Fig. 2). Hence it directs to a need to acquire more germplasm to combat the limited level intra-specific diversity present in the evaluated indigenous *B. carinata* germplasm. Alipour *et al*., (2002) evaluated the seed protein level of 5 accessions and observed 30 protein bands; with less variation in local accessions; however, the sample size was very small in his study. In our study variations based on major bands were present in a few accessions, but diversity based on minor bands was available in most of the accessions. Our results were supported by the findings of Mehrani (2002) and Ghafoor *et al*., (2003) who reported a limited level of intra-specific variation for seed protein among in pea and chickpea. However, Ali *et al*. (2007) and Nisar *et al*., (2007) reported a high level of intra-specific variation for seed protein among pea and chickpea germplasm, respectively. The disagreement is in fact due to use of different gene pools both from the exotic and local resources.

In case of exotic germplasm of *B. carinata* , based on SDS-PAGE protein banding pattern, their grouping in 5 major clusters indicate moderately high variability; which may be attributed to their geographic origin; though Rabbani *et al*., (2001) observed narrow genetic diversity in Indian mustard having different geographic origin. Similarly, Munazza *et al*., (2009) evaluated 30 accessions of different *Brassica* species for genetic diversity of the total seed protein, though SDS-PAGE and found no genetic diversity among these genotypes on protein level. Raymond *et al*., (1991) also found that the cluster pattern for sunflower genotypes showed variation having no relation with its locality. Similarly, Sihag *et al*., (2004) also investigated that there is no direct relation between genetic diversity and geographic distribution. These studies propose that the variation may be genetic and may not be related to geographical origin; this aspect needs to be explored in future studies.

However, in this regard the conclusion of Ghafoor *et al*., (2002) that SDS-PAGE will be the best tool in the case of inter-specific variation rather than intra-specific variation. The results are further strengthened by the early findings of Javaid *et al*., (2004) who also reported minimum genetic diversity in groundnut for SDS-PAGE and suggested two-dimensional (2-D) electrophoresis. Therefore the accessions, having alike banding patterns are suggested to be studied in the future with the help of 2-D electrophoresis and DNA markers. This may be due to the fact that these differences have some important hidden qualities and we can preserve these precious materials in genebank for use in breeding program (Celis & Bravo, 1984; Beckstrom, 1989). By studying these local and exotic accessions, it is concluded that these materials have genetic diversity particularly in the exotic material that may be utilized for crop improvement of local germplasm. Present evaluation offered first details documented for *B. carinata* genotypes in Pakistan based on total seed storage protein markers.

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