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Assessment of Genetic Diversity for Storage Seed Protein in a Set of Released Varieties and their Diallel Set of Crosses in Indian Mustard [*Brassica juncea* (L.) Czern. & Coss.] Using SDS PAGE

Kuldeep Jangid*, K. Ram Krishna, Naresh Parashar, Bhawana Bhatt, Arpit Gaur and Vinod Kumar

Department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner
Sri Karan Narendra Agriculture University, Jobner, Jaipur – 303329, Rajasthan, India
**Corresponding author*

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A B S T R A C T

Brassica species are major oilseed crops which are an increasingly important part of the human diet worldwide. In present investigation 11 varieties and their 55 F_1's were characterized for storage seed protein profiling using sodium dodecyl sulphate – polyacryl amide gel electrophoresis (SDS - PAGE). The electrophoresis of the proteins revealed protein bands in the range of 80kD to less than 14 kD molecular weight (MW). Out of 15 bands 14 bands were more or less monomorphic and only single band was polymorphic. Gels were scored for the presence (1) and absence (0) for polymorphic band. The similarity coefficient among these genotypes ranged from 1 to 0.25. A large number of pairs assumed value of 1.0 (61.6 %) followed by 0.8 (35.4%) about 3% cases showed similarity coefficient of less than 0.8.

Introduction

Brassica species are major oilseed crops as well as vegetable crops like broccoli, cabbages, Chinese cabbage including leaf mustard which is an increasingly important part of the human diet worldwide. Among *Brassica* species, mustard (*Brassica juncea* L.) displays a great polymorphism and is a source of different types of vegetables, condiment and oilseeds. Mustard belongs to family Crucifereae (Syn. Brassicacae). It is natural amphidiploid having chromosome no (2n=36). It is self-pollinated but certain amount of cross-pollination (2-15 %) occurs due to insects and other factors. The place of origin of mustard is China and from there it was introduced to India (Prain, 1898 and Bailey, 1922.).

At present time, mustard has been identified as a good crop for supporting bee keeping activity. The oil of mustard possesses a sizable amount of erucic acid (38-57%), together with linolenic acid (4.7 to13.0%). The oleic and linoleic acids, which have a higher nutritive value, together constitute about 27%. The protein content in rapeseed and mustard normally ranges between 24-30% on the basis of whole seed basis and between 35-40% on meal basis. But the presence of toxic glucosinolates in the

mustard cake renders it unsuitable as a source of human protein.

In general, genetic improvement of crops can be accelerated when there is a broad genetic diversity and information on these genetic resources are available. The collection of these genetic resources and the assessment of genetic diversity within and between these resources should be given 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. In a common practice, genetic improvement is easy in those species/crops, which have broad genetic diversity, and the information regarding these hidden genetic resources is easily available. The electrophoretic profile of seed storage protein is a method to investigate genetic variation and to classify plant varieties (Isumera *et al.,* 2001). Seed protein is not sensitive to environmental fluctuations, its banding pattern is very stable and has been advocated for taxonomic study of various species (Vaughan and Denford, 1968; Yadava *et al.*, 1979 and Akhtar, 2001) and cultivars identification purpose. It has been widely suggested that such banding patterns could be important supplemental method for cultivar identification, particularly when there are legal disputes over the identity of a cultivar or when cultivars are to be patented (Tanskley and Jones, 1981).

Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice (Thanh and Hirata, 2002). However, the information on the SDS-PAGE on different species of *Brassica* for genetic diversity is still limited (Mukhlesure and Hirata, 2004). The objective of the present study is to check the variation in storage protein with the help of SDS-PAGE. Analysis of SDS-PAGE is simple and relatively less expensive, which are of added advantages for use in practical plant breeding.

Materials and Methods

Plant materials

A total of 11 varieties and their 55 F_1's of mustard (*Brassica juncea*) were evaluated in the present study. Experimental material was obtained from AICRP on oilseed (Taramira) Division, Department of Plant Breeding and Genetics, S.K.N. COA., Jobner.

Protein extraction and gel electrophoresis

For the extraction of proteins, the 100 mg. of seed coat removed samples were ground using pestle and mortar. The seed powder of each variety was taken in an Eppendorf tube and 1ml. of defatting solution (Chloroform, methanol and acetone in 2:1:1 ratio) was then poured in each tube. After thorough shaking, the Eppendorf tubes were left for 3 hr. The supernatant was decanted and samples were then kept for some times for drying. Then after, 1ml. of extraction buffer (0.0625M Tris-HCl at p^H 6.8, 8M Urea, 2% SDS, 5% 2-Mercaptoethanol) was added and Eppendorf tubes were kept overnight at 10^0C. The next day, the samples were centrifuged at 10,000 rpm for 20 min. Supernatant (10µL.) was used for protein separation. SDS-PAGE was conducted according to the procedure of Laemmli (1970) with minor modifications described by Mukhlesure and Hirata (2004). 10µl of these samples were loaded into the wells of the polyacrylamide gel slab prepared for electrophoresis. The electrophoresis was carried out on BioRAD vertical gel electrophoresis equipment (Model: Protean II Xi Cell) along with its cooling unit with a power supply maintained at 20 mA for four and half hours. Two separate gels were run under similar electrophoretic conditions in

order to check the reproducibility of the results. After electrophoresis gels were stained with Coomassie brilliant blue R 250 overnight; followed by distaining overnight and finally washing in tap water.

Data analysis

Gels were scored for the presence (1) and absence (0) of every protein band. These binary data were analyzed using NTSYS-pc (Numerical Taxonomy System, Version 2.1, Rohlf 2000). The SIMQUAL sub-programme was used to calculate the Jaccard's coefficient using following formula (Jaccard, 1908).

Jaccard's coefficient= $N_{AB}/ (N_{AB}+N_A+N_B)$

Where, N_A and N_B represents no. of bands in sample A and sample B, respectively. N_{AB} is the number of bands shared in the samples. Similarity matrices as computed by the programme were used to construct the UPGMA (un-weighted pair group method with arithmetic average dendrograms to elucidate the diversity among the genotypes studied. Statistical stability of the branches in the cluster was estimated by bootstrap analysis with 1000 replicates, using Winboot software programme (Yap and Nelson, 1996).

Results and Discussion

The present investigation was carried out to distinguish between 11 parents and their 55 diallel crosses of Indian mustard (*Brassica juncea* L.) on the basis of storage seed protein profile using SDS-PAGE. The parents / genotypes used in the study were different released varieties, therefore, it was thought justified to determine their differences among themselves on the basis of the profile of their seed storage proteins. Characterization of genotypes based on seed storage protein/subunits is well documented in different groups of crops such as blackgram (Ghafoor and Ahmad, 2005; Ghafoor *et al.*, 2002), *Capsicum annuum* L. (Anu and Peter, 2003), *Solanum* (Menella *et al.*, 1999), *Vignaspp* (Rao *et al.*, 1992; Sharma, 2012; Chaoudhary, 2013) and Wheat (Siddiqui and Naz, 2009), including mustard (Rabbani *et al.*, 2001; Geetha and Balamurugan, 2011; Mukhlesure and Hirata, 2004; Parashar, 2014).

Sodium Dodecyl Sulphate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) as described by Laemmeli (1970) is a powerful and dependable technique for characterization of proteins. In present investigation, the total acid soluble proteins (Tris-HCL soluble) were extracted from cotyledons of the seeds following the procedure of Mukhlesure and Hirata (2004). Four different aliquots of proteins extract were examined for loading on the gel. It was found that loading of 10 uL of protein extract produced more sharper bands as compared to 5, 20 or 30uL of sample aliquots, therefore in all other experiments, 10 uL of protein extract was loaded during electrophoresis (fig-1).

The protein extracts from the cotyledons of the seeds of mustard genotypes were prepared and 14 samples loaded on a gel plate along with marker protein. The comb used in these experiments could develop 15 wells for loading the samples. The electrophoregram of the gel revealing banding pattern of the mustard genotypes is shown in figures-2.

It may be seen that the protein bands represented a Mol. Wt. of 80 kD to less than 14 kD. Further these appeared to represent four different regions. In the same figure alphabetic designations have been assigned to different protein bands. A perusal of protein subunits bands through Figure-2 revealed that four different recognizable regions of protein profiles were resolved. Parashar (2014) has also described a similar picture while

studying SDS-PAGE of storage seed proteins of 45 different genotypes of mustard. Further, similar studies performed by Rabbani *et al*., (2001) and Turi *et al.,* (2010) have also demonstrated 4 regions or darkly stained regions of protein bands.

A perusal of protein subunit bands of 11 parent genotypes and their 55 reciprocal crosses revealed that there were 15 well recognizable protein bands. However when compared, the A to N protein bands were more or less similar (monomorphic) whereas only ‘’O ‘’ band showed polymorphism. On contrary, Parashar (2014) in his studies on 45 mustard genotypes, (which includes the parents of present study also) observed that out of 15 bands observed, 7 were polymorphic. Depending on the intensity of stain in the band, the binary data was generated where value of ‘1’ was assigned to presence of darker stained protein band and value of ’0’ assigned to the presence of protein band with very light stain. The genotype 20 (a cross between Bio-902 x RH-819) was distinct from the rest of 65 genotypes as it scored ‘0’ values (being less stained) for all the protein bands. The presence or absence of band was used to generate binary data for each of the 66 genotypes. Using these binary data and NTSYSpc software, the Jaccard’s similarity coefficients were obtained. The similarity coefficient assumed only values of 0.25, 0.31, 0.8 and 1.00.

Table.1 Pedigree of the genotypes used in the study

Sr.No.	Genotype	Pedigree	Source/Origin
1.	**RB-50**	**LAXMI x RH 9617**	**CCS HAU Bawal**
2.	**PBR-97**	**(DIR 202 x PR 34 x V3) x (RLM 619 x VARUNA)**	**PAU RS Bathinda, Punjab**
3.	**RH-819**		**CCS HAU Hisar**
4.	**ROHINI**	**Selection from natural population of VARUNA**	**CSAUT Kanpur (UP)**
5.	**BIO-902**	**Somaclonal variation of VARUNA (1993)**	**IARI, New Delhi**
6.	**NRCDR-2**	**MDOC-43 x NBPGR-36**	**DRMR, Bharatpur**
7.	**RGN-73**	**RGN 8 x PUSA BOLD**	**RAU, Sriganganagar**
8.	**GEETA**	**Spontaneous mutant of cultivar RH-30**	**CCS HAU Hisar**
9.	**NPJ-112**	**SEJ-8 x PUSA JAGNNATH**	**IARI, New Delhi**
10.	**KRANTI**	**selection from VARUNA**	**GBPUA&T, Pantnagar**
11.	**LAXMI**	**PR-15 (KRANTI) x RH-30**	**CCSHAU Hisar**

Fig.1 A close-up view of electrophoregram showing the resolution of protein subunit bands in loading of different aliquots of samples

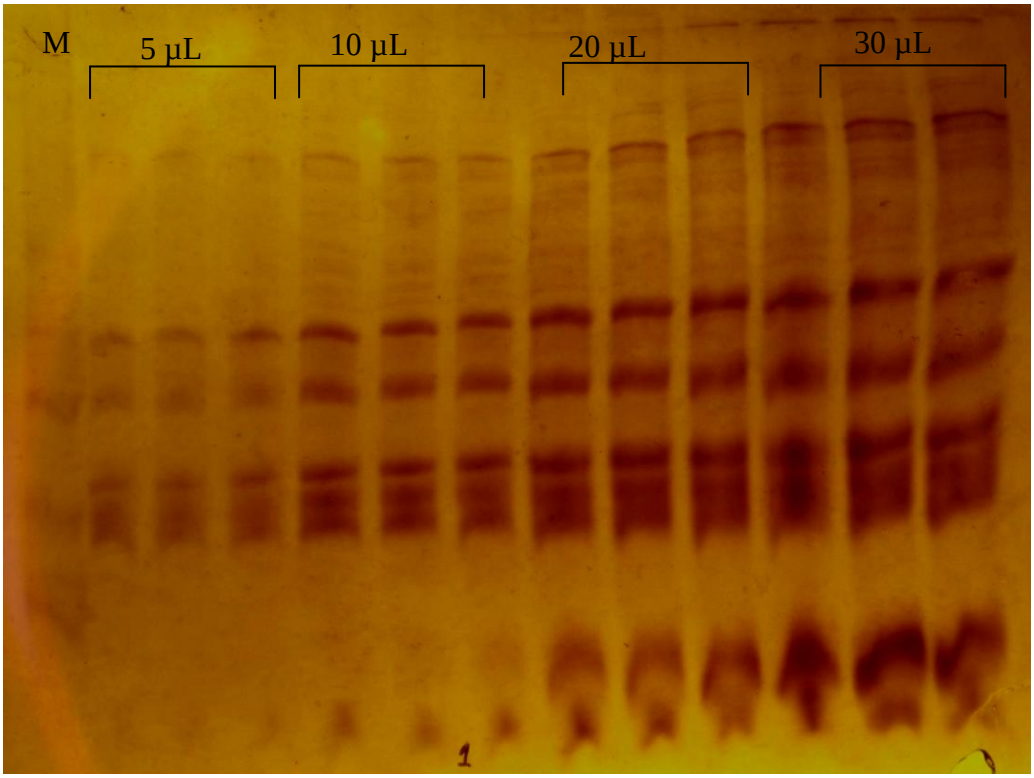


Fig.2 A close up view of electrophoregram of mustard genotypes

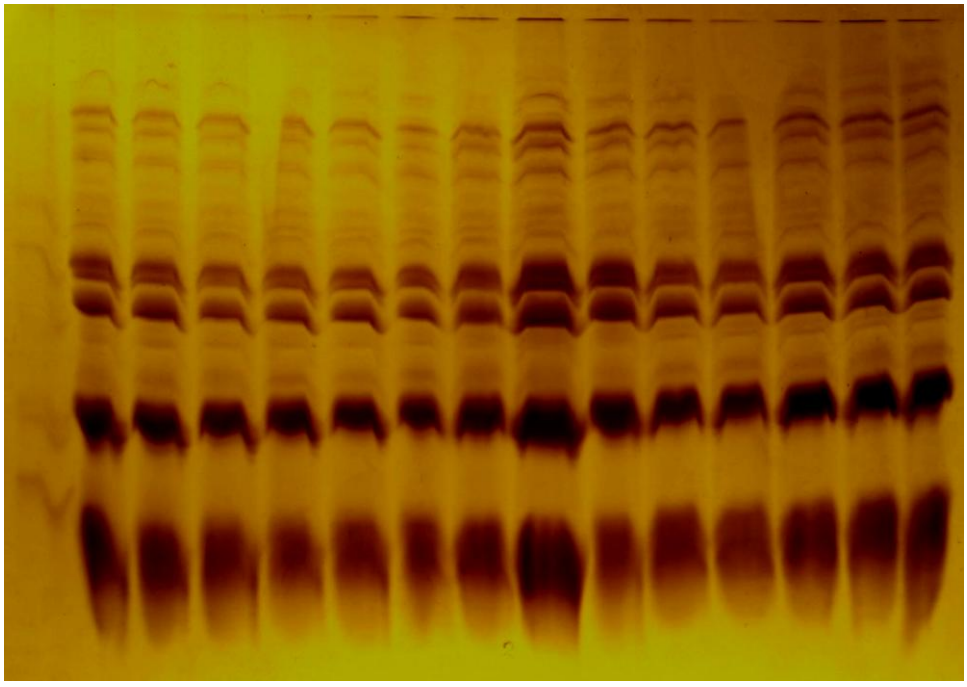
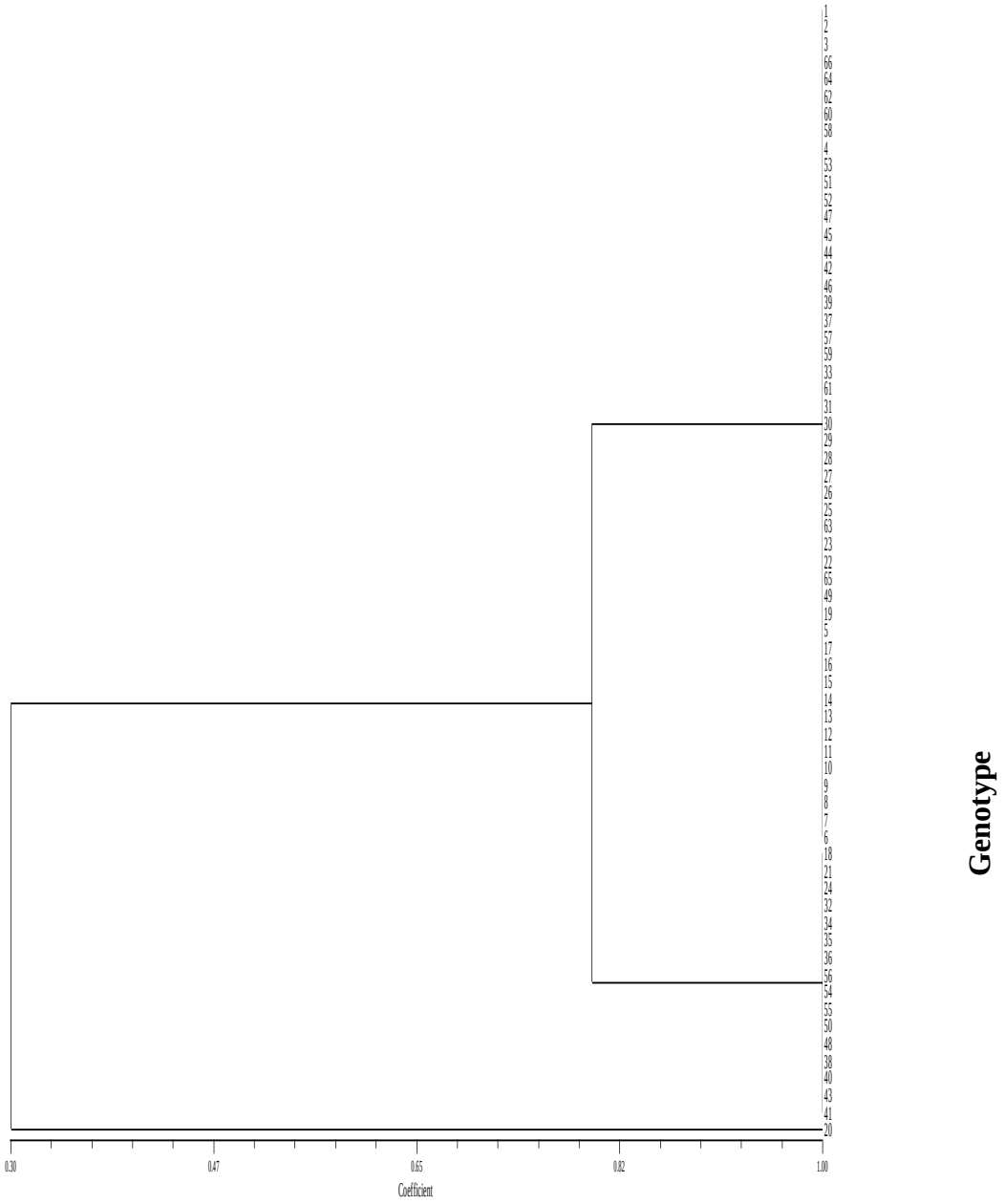


Fig.3 Dendrogram of 66 genotypes of mustard revealed by UPGMA cluster analysis of SDS-PAGE based genetic similarity estimates



Higher coefficient of values was associated with large number of pairs. A perusal of similarity coefficient revealed that a large number of pairs assumed value of 1.0 (61.6 %) followed by 0.8 (35.4%) about 3% cases showed similarity coefficient of less than 0.8. Thus it may be inferred that large number of genotypes are closely similar, at least on the basis of seed storage protein profile. A dendrogram was constructed using Jaccard's similarity coefficients obtained for protein band binary data observed on 66 genotypes of mustard employing NTSYSpc program (Fig. 3). The cluster analysis of the genotypes revealed 3 distinct clusters at more than 75% similarity coefficient of the 3 branching observed, genotype 20 (Bio-902 x RH-819) was quite distinct from rest of the two clusters. It is interesting to note that all the parents fell in cluster 1 along with most of

their F1 crosses (38 crosses) whereas rest of the 16 crosses fell in cluster 2. Although, Parashar (2014) observed 5 clusters but only at above 80% similarity coefficient indicating that the clusters are genetically relatively close to each other. The observations of the present study may seek support from the origin of the parents. A perusal of the pedigree of the parents involved in the present study indicated that an old ruling variety of Indian mustard "*Varuna"* was involved at one or the other stage of their development (Table 1). This may be a plausible reason why the parents of the present study are indistinguishable on the basis of SDS-PAGE of storage seed protein profile. Rabbani *et al.*, (2001) also failed to distinguish 52 accession on the basis of storage seed protein profile and based on this arrived on the same conclusion.

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