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GENETIC DIVERSITY FOR STORAGE SEED PROTEIN PROFILE IN MUSTARD [*BRASSICA JUNCEA* (L.) CZERN. & COSS.] GENOTYPES

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ABSTRACT

Brassica species are major oilseed crops which are an increasingly important part of the human diet worldwide. In present investigation 45 mustard (*Brassica juncea*) genotypes/ varieties 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 80 kD to less than 14 kD molecular weight (MW). Out of 15 bands, only 7 bands were polymorphic. Gels were scored for the presence (1) and absence (0) of every polymorphic band. The similarity coefficient among these genotypes ranged from 1 to 0.33. Only about 5% cases showed similarity coefficient of less than 0.6. The genotypes studied were divided into five major clusters at more than 75% similarity coefficient through constructing the dendrogram on the basis of dissimilarity matrix using UPGMA. The study revealed genotype clustering instead of geographical clustering. Clustering seemed to be independent of the seed traits such as oil and protein content studied. This evaluation will significantly help for identification and differentiation of mustard genotypes and for best utilization in mustard varietal improvement program in India.

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INTRODUCTION

Brassica species are major oilseed crops as well as vegetable crops like broccoli, cabbages, Chinese cabbage including leaf mustard which are an increasingly important part of the human diet worldwide. Oilseed crops are next to cereals in production of agricultural commodities in India which occupy a place of prime importance in Indian economy. Indian mustard [*Brassica juncea* (L.) Czern and Coss] is the second most important oilseed crop of the world as well as India after groundnut. Indian mustard (Brassica juncea (L.) Czern and Coss) is a natural amphidiploid (2n = 36) of *Brassica campestris* (2n = 20) and Brassica nigra (2n = 16). It is self-compatible and largely selfpollinated crop (85-90%) (Shekhawat *et al.*, 2014). India is one of the largest rapeseed-mustard growing country occupying first position with 20.23% area and second position with 11.7% share to the global production (Mishra and Kolte, 2014). Among the oilseeds in India, *Brassica* ranks second in area and production after groundnut and contributes 26% after total vegetable oil seed output (Choudhary *et al.,* 2015). 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 broad genetic diversity and the information of these genetic resources are available. The collection of these genetic resources and the assessment of genetic diversity within and between landraces should have priority for varietal improvement. Quantitative genetic markers are helpful in the estimation of genetic variation. It is helpful in appropriate use of gene pool in specific programs (Sadia *et al.,* 2009). The electrophoresis of seed storage protein is a method to investigate genetic variation and to classify plant varieties (Isumura *et al.,* 2001; Turi *et al.,* 2010). Seed protein is not sensitive to environmental fluctuations and banding patterns therefore are very stable, and can be used for cultivar identification purpose (Tanksley and Jones, 1981; Nasr *et al.,* 2006; Kour and Singh, 2008). However, the information on the use of SDS-PAGE on different species of *Brassica* for genetic diversity is still limited (Rahman and Hirata, 2004). Seed protein is not sensitive to environmental fluctuations, its banding pattern is very stable. 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 (Tanksley 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 or assess the protein polymorphism and determine the genetic diversity in mustard genotypes 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 45 genotypes of mustard (*Brassica juncea*) were evaluated in the present study. 39 varieties were obtained from AICRP on oilseed, ZRS, Sri-ganganagar (Raj.) and rest of 6 promising genotypes were obtained from AICRP on oilseed (Taramira) Division, Department of Plant Breeding and Genetics, S.K.N. COA., Jobner, Jaipur.

Protein extraction and Gel electrophoresis

For the extraction of proteins, the 100 mg. of seed coat removed samples were ground using a 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 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 10ºC. 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 (Sneath and Sokal, 1973) 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).

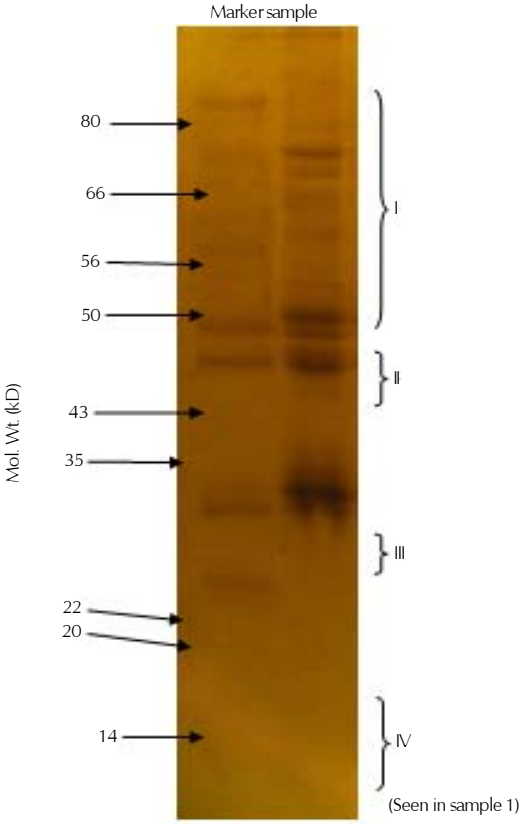

Figure 1: Marker protein bands and identification of regions in an electrophoretic gel in a sample of mustard seed storage protein

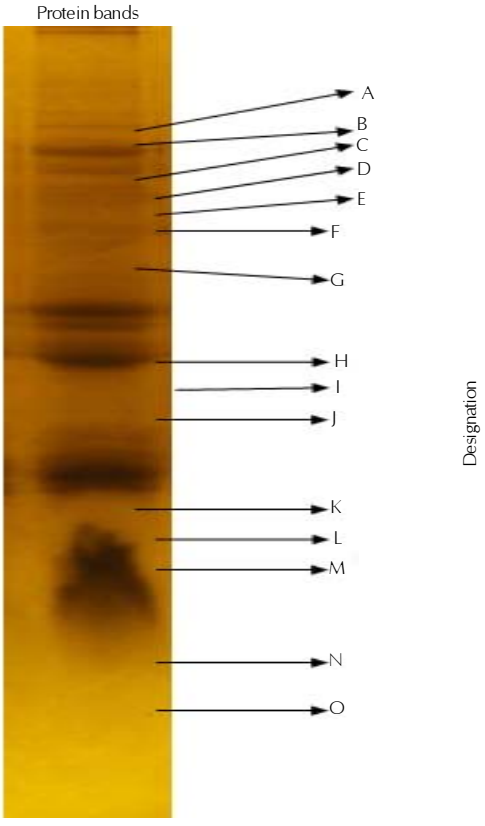

Figure 2: Designations (arbitrary) assigned to the protein bands of mustard samples

RESULTS AND DISCUSSION

The present investigation on SDS-PAGE was carried out on different varieties of Indian mustard (*Brassica juncea)* with a view to find possible differences among the genotypes on the basis of the protein subunit band profiles. There were 45 genotypes of mustard which represented different and geographically wide regions of collection and varietal development. Of these 39 were released varieties and 6 promising genotypes of mustard included in the present study. Being developed from different regions, the genotypes were apparently expected to have considerable differences; therefore, it was thought justified to determine the same on the basis of the profile of their seed storage protein. Characterization of genotypes based on seed storage proteins/ subunits is well documented in different groups of crops such as black gram (Ghafoor and Ahmad, 2005), *Vigna sp* (Sharma, 2012; Choudhary, 2013), wheat (Siddiqui and Naz, 2009) and mustard (Rabbani *et al.,* 2001 ; Mukhlesure and Hirata, 2004; Geetha and Balamurugan, 2011).

In the present investigation, the total soluble proteins (Tris-HCl soluble) were extracted from cotyledons of the seeds following the procedure of Mukhlesure and Hirata (2004). Initially, three different aliquot volumes of proteins extracts were examined on gel. Loading with 10 μL of protein extract revealed more sharper bands as compared to 20 or 30 μL of sample aliquotes loaded (Fig. 1). Fig. 1 shows the protein profile of a sample (protein extracted from single genotype) along with marker protein lane.

The pattern of protein bands in the present study were more close to those observed by Geetha and Balamurugan (2011).

Table 1: Grouping of 45 mustard genotypes based on cluster analysis using SDS-PAGE analysis

Cluster	Sub-cluster	Genotype numbers	Mustard genotypes	Origin	Protein content (%)	Oil content (%)
I	I A	1	VARUNA	CSAUA&T, Kanpur	25.68	39.2
		5	ROHINI	CSAUA&T, Kanpur	23.68	39.0
		8	RH-749	CCSHAU, Hisar	24.70	38.2
		9	NPJ-113	IARI, New Dehli	25.67	38.2
		13	PBR-357	ZRS, Bhatinda, Punjab	24.80	38.5
		40	RH-819	CCSHAU, Hisar	25.89	38.6
		14	PUSA-AGARINI	IARI, New Dehli	25.35	39.2
		24	NRCDR-2	DRMR, Bharatpur	25.71	41.4
	I B	3	VARDAN	CSAUA&T, Kanpur	24.43	37.6
		4	GEETA	RRS, Bawal	24.37	35.7
		45	RB-50	RRS, Bawal	25.50	39.8
		10	PBR-378	ZRS, Bhatinda, Punjab	24.18	39.2
II	II A	2	KRANTI	CSAUA&T, Kanpur	26.87	39.2
		6	RH-406	CCSHAU, Hisar	23.11	38.6
		11	RGN-303	ARS, Sriganganagar	25.78	42.0
		12	RN-393	Navgoan, Alwar	25.60	39.2
	II B	15	PUSA-BOLD	IARI, New Dehli	25.87	38.9
		38	MAYA	CSAUA&T, Kanpur	25.40	40.2
		32	RGN-73	ARS, Sriganganagar	25.11	39.6
		30	RGN-229	ARS, Sriganganagar	24.62	40.2
		28	RGN-306	ARS, Sriganganagar	25.80	39.2
		36	RH-30	CCSHAU, Hisar	24.33	40.0
		33	RGN-48	ARS, Sriganganagar	24.91	40.0
		44	PBR-1334-61-1	DRMR, Bharatpur	24.86	38.7
		21	RH-50	CCSHAU, Hisar	25.17	38.1
		20	RGN-281	ARS, Sriganganagar	25.62	41.2
		19	GM-3	SKDAU, S.K. Nagar, Gujrat	25.30	38.6
		18	RGN-253	ARS, Sriganganagar	25.43	40.0
		17	RLM-619	PAU, Ludhiana	24.50	38.0
		31	RGN-145	ARS, Sriganganagar	24.68	38.8
		29	RGN-298	ARS, Sriganganagar	25.33	40.2
		35	LAXMI	CCSHAU, Hisar	25.41	39.7
		39	RGN-236	ARS, Sriganganagar	25.17	39.2
		37	PCR-7	CSAUA&T, Kanpur	25.77	36.8
		34	RGN-13	ARS, Sriganganagar	24.87	39.2
III		23	NAVGOLD	Navgoan, Alwar	25.90	39.9
IV	IV A	7	RL-1359	PAU, Ludhiana	23.37	36.4
		16	BIO-902	IARI, New Dehli	26.67	39.5
		43	PBR-1492-56-3	DRMR, Bharatpur	23.67	39.7
		41	PBR-1676-65-1	DRMR, Bharatpur	24.65	38.4
		42	PBR-1480-9-3	DRMR, Bharatpur	23.78	39.3
		27	RGN-277	ARS, Sriganganagar	25.70	40.3
		22	CS-52	CSSRI, Karnal	24.85	41.6
	IV B	26	CS-234-2	CSSRI, Karnal	24.57	39.4
V		25	JMWR-08-3	ZARS, Morena	25.87	37.7

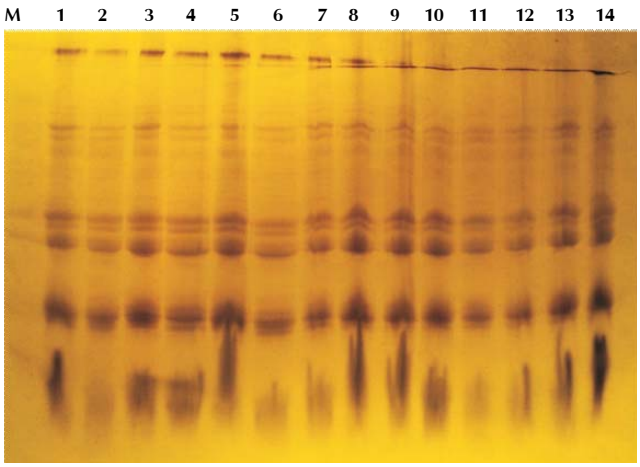

Figure 3: A closeup view of electrophoregram of mustard genotypes 1-14

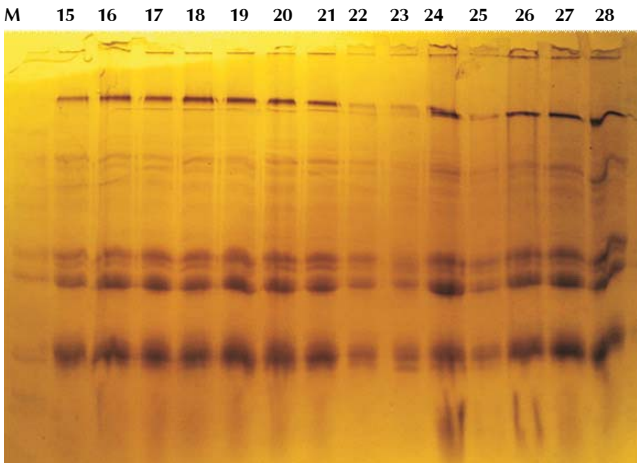

Figure 4: A closeup view of electrophoregram of mustard genotypes 15-28

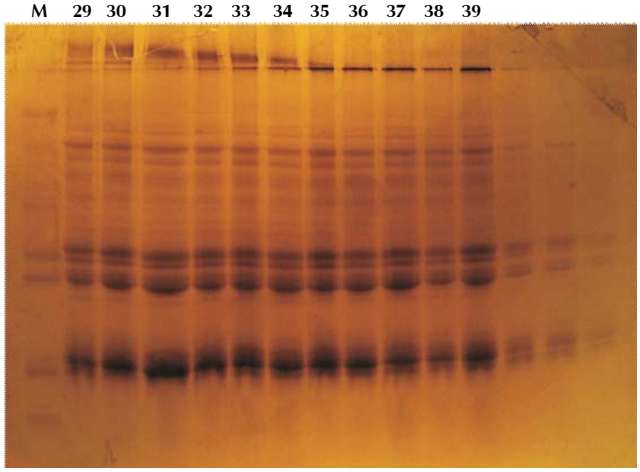

Figure 5: A closeup view of electrophoregram of mustard genotypes 29-39

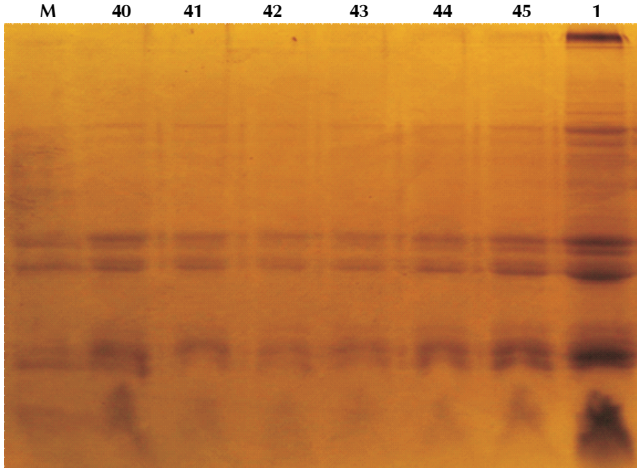

Figure 6: A closeup view of electrophoregram of mustard genotypes 40-45

However, they reported a total of 7 bands out of which only 3 were polymorphic. On the contrary, in other investigations (Mukhlesure and Hirata, 2004) involving many species of genus *Brassica*, 10 polymorphic bands from the seed protein were reported. In *Brassica juncea* accessions, involving collections of Pakistan, Rabbani *et al.* (2001) have reported a total of 20 protein subunit bands. Such differences can be attributed to the genotypic differences existing in the collections.

The protein extracts of the seeds of mustard genotypes were prepared and 14 samples were loaded on a gel plate along with marker protein in the first lane. The comb used in these experiments could develop 15 wells for loading the samples. The electrophoregrams of the gels, each revealing banding pattern of the mustard genotypes are shown in figures 3 to 6. A typical banding pattern has been shown in Fig. 2. The banding pattern revealed four distinct regions of protein subunits (fig.1). The first region corresponded to Mol. Wt. between 80-50 kD whereas the second region spanned over 43-35 kD, third region was around 22-20 kD and last region corresponded to less than 14 kD of Mol. Wt.

A persual of protein bands of 45 genotypes (through figures 3, 4, 5 and 6) revealed that out of 15 bands obtained only 7 were polymorphic and rest were monomorphic. Using binary data and NTSYSpc software, the Jaccard's similarity coefficient were obtained. The similarity coefficient ranged between 0.33 to 1.0. This indicated that no two genotypes were exactly identical. Among the 45 genotypes, higher coefficient of values were associated with large number of pairs. Only about 5% case showed similarity coefficient of less than 0.6. Thus it may be inferred that large number of genotypes are closely similar, atleast 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 45 genotypes of mustard employing NTSYSpc programme (Fig. 7). The cluster analysis of the genotypes revealed 5 distinct clusters at more than 75% similarity coefficient (Table 1). The clustering was more apparent after performing the bootstrap analysis using Winboot programme at 1000 cycles.

The salient features of the clustering are described under :-

Cluster III included only one genotype Navgold (YRN-6) from Alwar (Raj.) which was also the only yellow seeded variety included in tha study.

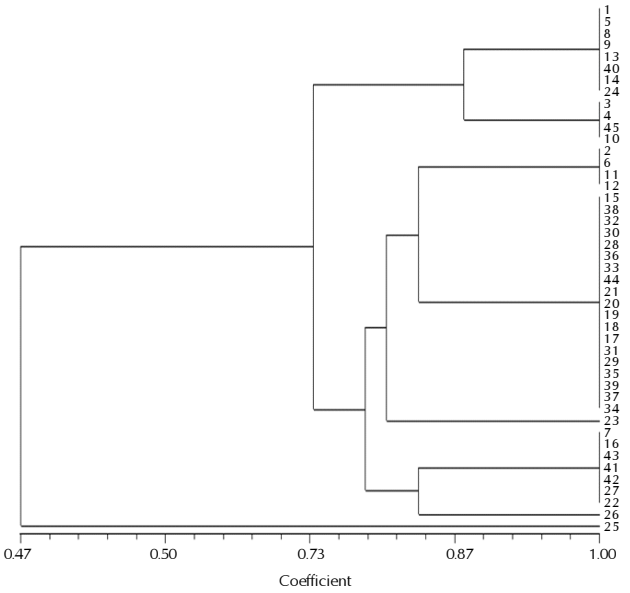

Figure 7: Dendrogram of the 45 mustard genotypes revealed by UPGMA cluster analysis of SDS-PAGE based genetic similarity estimates

Cluster II included maximum number of genotypes (51%).

Cluster I and IV included 12 and 8 genotypes, respectively.

There was only one genotype from ZARS, Morena i.e. JMWR-08-3 which appears to be most diverse from rest of the collection and was placed singly in cluster V.

In case of clusters I, II and IV, it was seen that these were not associated with genotypes of specific region, however, all the genotypes of Rajasthan origin (bearing RGN number) were characteristically placed in cluster II.

Both the seed traits, protein percentage and oil content also seems to bear no association with clustering, hence seed protein profile was independent of seed protein and oil content.

On the basis of results of present investigation it may be concluded that SDS-PAGE on seed storage protein in mustard may reveal usable protein band polymorphism to study the diversity of the genotypes. Some of the genotypes may be identified on their specific banding pattern and information could help make decisions regarding the choice for selecting parents for improvement of mustard productivity through hybridization.

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