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Research Article

Evaluation of Genetic Variation among Indian Mustard (*Brassica juncea* L.) Genotypes by SDS-PAGE Method

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Abstract: A comparative study of total seed protein profile was carried out to determine the extent of genetic variability amongst 53 genotypes of Indian mustard [(*Brassica juncea* L.) germplasm using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) technique. Total seed protein was estimated. To estimate genetic diversity, 12 different types of protein bands were obtained based on the banding pattern of all the genotypes; seven (58%) were polymorphic and five were monomorphic. The protein size base polymorphism revealed the range of protein bands based on their molecular weights which ranged from ~10 kDa to ~180 kDa. Dendrogram was constructed using UPGMA method which divided the genotypes into five groups: group I had 18 genotypes, group II 17 genotypes, group III 3 genotypes, group IV 13 genotypes and group V 2 genotypes. Among the studied genotypes, similarity coefficient values ranged from 17% to 100%. Highest similarity coefficient (i.e., 100%) was recorded between Bj765 and Bj785, Bj790 and Bj791, Bj790 and Bj794. The observed clusters based on this study exhibited a genetic diversity among these genotypes of Indian mustard on Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) level. Thus, this study revealed that the SDS-PAGE method plays a key role in the study of protein based variation among different genotypes of plant species.

Keywords: *Brassica juncea* L., SDS-PAGE, genetic diversity, cluster analysis

1. INTRODUCTION

Brassica juncea L., commonly known as Indian mustard, is one of most economically important agricultural commodities grown in more than 50 countries including Asia, Australia, America and Europe [1-5]. Indian mustard 2n=AABB=36 is the most common source of oil seed, vegetable and condiments [5]. *Brassica juncea* L. had originated through natural crossing between *Brassica nigra* (2n=BB=16) and *Brassica campestris* (2n=AA=20) [6].

To study genetic variability among different plant species, various morpho-biochemical markers are used [7-10]. The Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) is employed for estimation of molecular weight of proteins and polypeptides. Introduced by Laemmli [11], SDS-PAGE has become a most firmly established laboratory procedure. Thus, SDS-PAGE analysis is also a useful tool to investigate genetic diversity and classify plant varieties in *Brassica* species [12]. The SDS-PAGE analysis is used to determine protein variation among different organisms, which can detect various kinds of protein subunits in different species [7, 13]. SDS-PAGE technique provides efficient and quick protein profiling of different plant species and it is absolutely environmental-friendly [14, 15]. Total seed protein based variation gives accurate genetic diversity among crop genotypes. It is used as a tool for crop improvement and to assist in plant domestication for phylogenetic relationship. Jan et al. [16] and

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Table 1. *Brassica juncea* L. genotypes used in the study (obtained from PGRI, NARC, Islamabad).

Accession Number	Accession Number	Accession Number
Bj603	Bj780	Bj838
Bj608	Bj785	Bj839
Bj624	Bj788	Bj840
Bj642	Bj790	Bj849
Bj645	Bj791	Bj852
Bj655	Bj794	Bj860
Bj688	Bj795	Bj887
Bj716	Bj796	Bj895
Bj724	Bj797	Bj902
Bj732	Bj798	Bj906
Bj741	Bj799	Bj910
Bj742	Bj800	Bj913
Bj761	Bj803	Bj914
Bj762	Bj814	Bj927
Bj765	Bj823	Bj929
Bj770	Bj825	Bj935
Bj772	Bj829	Bj937
Bj779	Bj835	

Shinwari et al. [17] detected maximum protein bands at mass ranges from 15-220 kDa in important *Eruca sativa* L. genotypes through SDS-PAGE technique. Similarly, Zada et al. [18] characterized 94 genotypes of *Brassica carinata* L. through this method; they observed both polymorphic and monomorphic protein bands. Jan et al. [12] reported protein based polymorphism in different *Brassica rapa* L. genotypes. Akbar et al. [19] performed SDS-PAGE for different *Sesame indicum* L. genotypes and reported protein based polymorphism. This method has been also utilized by Jan et al. [8] for protein profiling in *Brassica rapa* sub species brown *Sarson*. Saleem *et al.* [20] characterized 100 genotypes of *Brassica juncea* L. genotypes through SDS-PAGE method. Protein based polymorphism varies with type of species [14, 21]. The SDS-PAGE gives accurate protein profiling for all genotypes of all species. Therefore, the objective of the current study was to characterize and identify the diverse genotypes of *Brassica juncea* L. by employing SDS-PAGE method.

2. MATERIAL AND METHODS

2.1 Plant Material

Seeds of *Brassica juncea* were obtained from Plant Genetic Resources Institute (PGRI), National Agricultural Research Centre (NARC), Islamabad, Pakistan. 53 genotypes of *Brassica juncea* L. were characterized using SDS-PAGE technique at the evaluation lab of PGRI. Accession numbers of these genotypes are listed in Table 1.

2.2. Protein Extraction and Purification

Seeds were crushed and grounded with the help of mortar and pestle using CTAB Method [22]. The protocol of Jan et al. [12] was used for rapid protein extraction and purification by minor modifications. The grounded material was taken

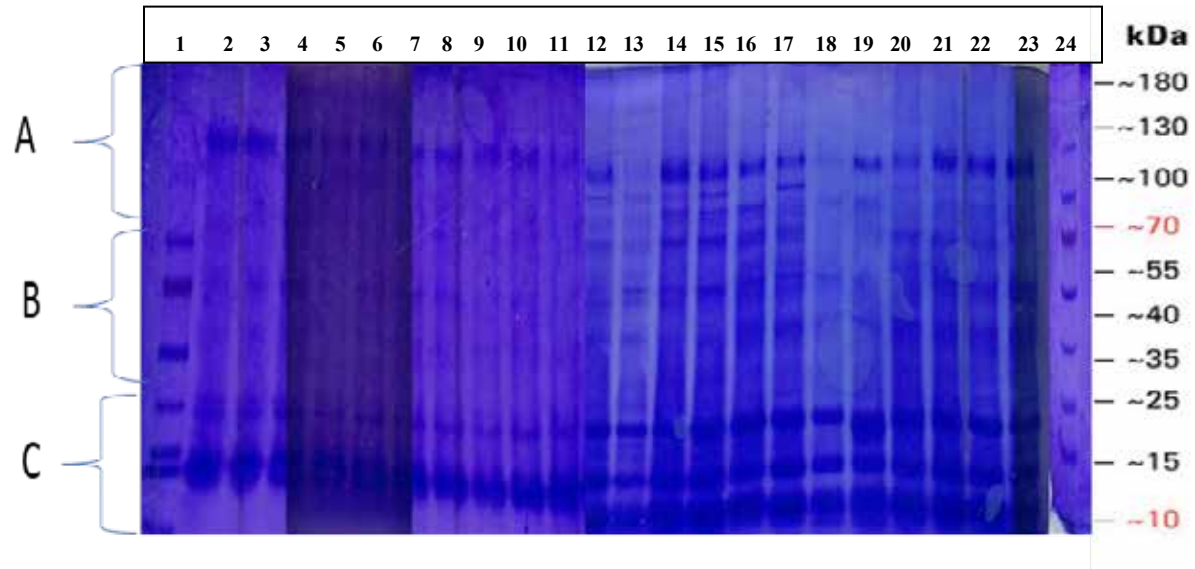

Fig. 1. Banding patterns of various genotypes of *Brassica juncea* L. based on SDS-PAGE of total seed proteins. L-1 Protein ladder, L-2 Bj765, L-3 Bj770, L- Bj772, L-5 Bj762, L-6 Bj779, L-7 Bj780, L-8 Bj785, L-9 788, L-10 Bj790, L-11 Bj791, L-12 Bj795, L-13 Bj796, L-14 Bj797, L-15 Bj799, L-16 Bj800, L-17 Bj803, L-18 Bj927, L-19 Bj935, L-20 Bj814, L-21 Bj823, L-22 Bj825, L-23 Bj829, L.24 Protein Ladder.

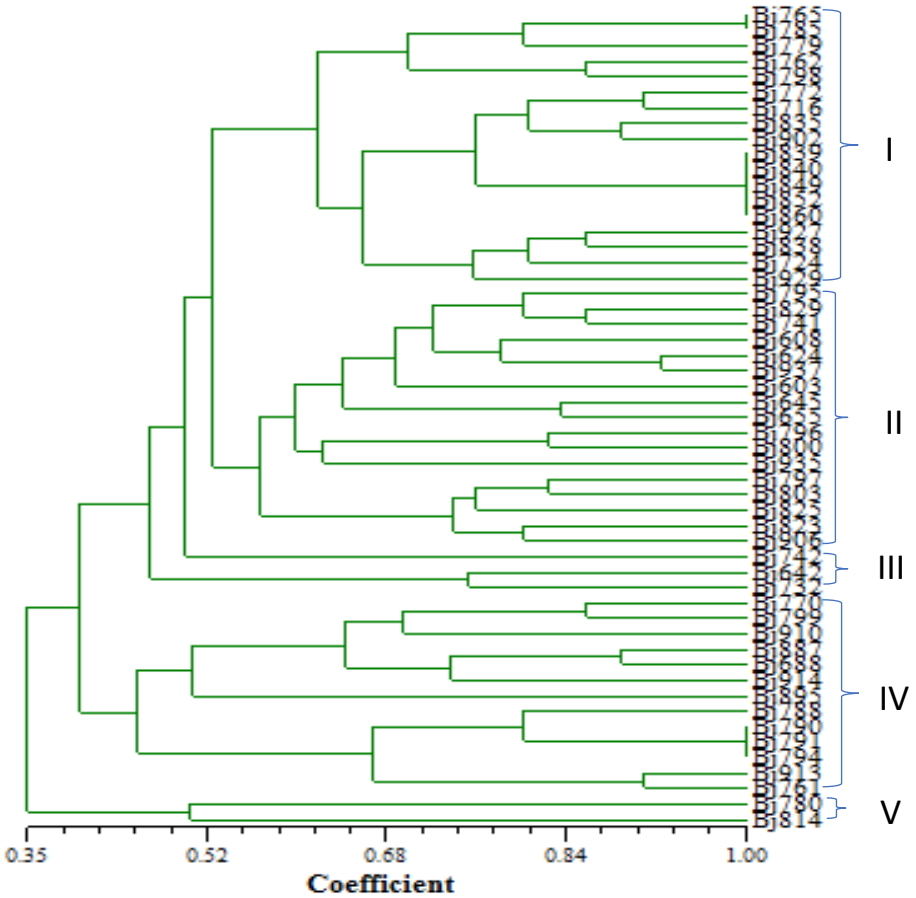

Fig. 2. Dendrogram showing the relationship among 53 genotypes of *Brassica juncea*L. Based on SDS-PAGE of total seed proteins.

into 10 mL test tube. A volume of 5 mL of chloroform, methanol and acetone mixture (2:1:1) was added and mixed well by vertexing. Then samples were kept at room temperature for overnight. After centrifugation the samples solvent was removed and defatted seed powder was taken and placed in 1.5 mL centrifuge tubes.

Then the protein extraction buffer (0.05 M Tris-HCl buffer-pH 8.0 mixed 0.2% SDS, 5 M urea and 1% β-mercaptoethanol) was added. Little bit Bromophenol blue was added to extraction buffer as a dye. After mixing all these chemicals 400 µL of homogenized solution was added to each sample in 1.5 mL centrifuge tube and mixed thoroughly by vertexing and centrifuged at 10,000 rpm for ten minutes at room temperature (RT). After centrifugation process the crude protein recovered as clear supernatant on the top of the tube, the supernatant was transferred into new 1.5 mL centrifuge tube. Then protein profiling of samples was performed using SDS-PAGE as described by Laemmli [11].

2.3. Electrophoresis

Crude protein samples were directly analyzed by SDS-PAGE using 12.5% separation gel and 4.5% stacking gel. 20 µL protein samples were loaded into the wells of stacking gel with the help of micropipette. Electrophoresis was carried out at 100 Volts until the bromophenol blue (BPB) reached to the bottom of gel plate.

2.4. Staining and Destaining

When the blue line reached at the bottom of gel plates, electric supply was disconnected. Gel is separated from gel plates with spatula. Separating gel was put in the box which contained staining solution and put on the shaker for two hours then staining solution was exchanged by de-staining solution and box was shaked gently almost overnight until the background of the gel disappeared to absorb excess CBB and the electrophoresis bands on gel clearly visible.

2.5. Data Analysis

For polymorphism, the protein bands were scored as 1 for presence and 0 for absence. The Jaccard's similarity index was calculated using NTSYSpc version 2.1 Applied Biostatistics Inc., USA) software was used to compute pairwise Jaccard's similarity coefficients and this similarity matrix was used in cluster analysis using an unweighted pair group method with arithmetic averages (UPGMA) and sequential, agglomerative, hierarchical and nested (SAHN) clustering algorithm to obtain dendrogram.

3. RESULTS AND DISCUSSION

Seed samples of 53 *Brassica juncea* L. accessions were used for total protein comparisons. The banding patterns of some of the total seed protein showed close relationship and some showed a range of geographic difference among studied genotypes (Table 2, Fig. 1). Total of 12 bands were detected and seven bands were observed in all genotypes. The ratio of polymorphic bands was (58%).

After the study of banding pattern in these genotypes three zones were observed (A, B, C) showing variations. Thus, Zone A was having protein weight from 70-180 kDa. Zone A having four bands in which 3 bands were polymorphic and protein bands in zone B ranged from 35-69 kDa. Protein bands observed in B region were five among them four were polymorphic. Zone B Consists of both dark & light stained bands. While, zone C Ranged from 10-30 kDa. Three Protein bands were detected in this region and only one Protein band showed polymorphism (Table 2). Similar results were found by [12, 23, 8, 18] who also reported the same banding pattern in *Brassica* Species. Similarly [24, 25, 26] detected the same results. The results extracted from SDS-PAGE (Fig. 1) provides a powerful tool for reliable variety identification based on genetic differences in seed storage protein composition among different cultivars of *Brassica juncea* L. The genotypes Bj796 showed the highest protein bands (12) followed by Bj797 (9); Bj799, Bj803 8; Bj800, Bj829, Bj624, Bj655 and Bj741 showed 7 bands.

Based on similarity index among studied genotypes the similarity coefficient values were 17

Table 2. Presence and absence of protein bands in the SDS-PAGE analysis of *Brassica juncea* L.

Protein region	Protein bands	No. of Genotypes	
		Presence	Absence
Group A	1	50	3
	2	27	26
	3	15	38
	4	15	38
	5	8	45
Group B	6	20	33
	7	14	39
	8	22	31
	9	11	42
	10	16	37
	11	36	17
	12	21	32
Group C	13	31	22
	14	47	6

Table 3. Cluster pattern of *Brassica juncea* L. genotypes on genetic divergence.

Protein region	Genotypes
Group-I	Bj765, Bj785, Bj779, Bj762, Bj798, Bj772, Bj716, Bj835, Bj902, Bj839, Bj840, Bj849, Bj852, Bj860, Bj927, Bj838, Bj724 and Bj929
Group-II	Bj795, Bj829, Bj741, Bj608, Bj624, Bj937, Bj603, Bj645, Bj655, Bj796, Bj800, Bj932, Bj797, Bj803, Bj825, Bj823, and Bj906
Group-III	Bj742, Bj642 and Bj732
Group-IV	Bj770, Bj799, Bj910, Bj887, Bj688, Bj914, Bj895, Bj788, Bj790, Bj791, Bj794, Bj913 and Bj761
Group-V	Bj780, and Bj814

– 100%. The highest similarity coefficient (100%) was recorded between Bj765 and Bj785, Bj790 and Bj791, Bj790 and Bj794 followed by Bj624 and Bj937 (92%), Bj716 and Bj772 (91%) respectively. The lowest similarity index was recorded Between Bj914 and Bj800 (17%) followed by 18% in Bj 796 and Bj780. So, the genotypes Bj914 and Bj800 are highly diverse from rest of the genotypes. Based on Euclidean distances the dendrogram was constructed (Fig. 2) which classified the genotypes into five groups. The group I consists of 18 genotypes i.e. Bj765, Bj785, Bj779, Bj762, Bj798, Bj772, Bj716, Bj835, Bj902, Bj839, Bj840, Bj849, Bj852, Bj860, Bj927, Bj838, Bj724 and Bj929; Group II includes 17 genotypes i.e. Bj795, Bj829, Bj741, Bj608, Bj624, Bj937, Bj603, Bj645, Bj655, Bj796, Bj800, Bj932, Bj797, Bj803, Bj825, Bj823, and Bj906; Group III includes three genotypes i.e. Bj742, Bj642 and Bj732; Group IV includes 13 genotypes i.e. Bj770, Bj799, Bj910, Bj887, Bj688, Bj914, Bj895, Bj788, Bj790, Bj791, Bj794, Bj913, Bj761 and Group V includes only two genotypes (Bj780 and Bj814). In group, I the genotypes Bj839, Bj840, Bj849, Bj852 and Bj860 showed the least genetic distance and consequently have the most genetic

linkage. Furthermore, Group I have the most linkage with Group II, III and the least linkage with group IV and V.

Thus, per the results in this study and the results of others we can assume that the use of seed storage protein can be recommended for genetic diversity and linkage studies. To study the generic and species level SDS-PAGE is a useful method for classical taxonomic method [27]. Moreover, protein kinds and their differences using SDS-PAGE analysis helps the breeders to select the diverse genotypes for breeding programs at seed level and to crack up the record of transparency of genetic resources [28]. It is clear from cluster analysis that there is a fair diversity in genotypes. The study findings are further strengthened by the reports of [23, 25, 29] they also concluded the comparable results. The less divergent genotypes may be preserved in the gene bank for use in breeding programs and the diverse genotypes having different banding patterns are suggested to be used in future breeding programs [30, 31].

4. CONCLUSIONS

Total seed protein profiling could be a useful tool for diversity analysis and genotype identification between and within *Brassica* species. Assessment based on protein and section of desirable genotype is of immense importance for crop breeders. The seed storage protein profiling based on SDS-PAGE is an effective method to examine genetic diversity. In this study, out of 12 bands, 7 bands were detected polymorphic. A dendrogram was constructed based on SDS-PAGE which divided the genotypes into five groups. The genotype Bj796 exhibited maximum protein bands (12). Similarity index in these 53 genotypes of Indian mustard ranged from 17% to 100%. The diverse genotypes identified in this study can be used in future breeding programs for the development of new varieties.

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