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Electrophoretic Characterization and the Relationship between Some Brassica Species

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Abstract

The objective of this study was to assess the genetic divergence available in different genotypes of oilseed *Brassica* based on analyses of seed storage proteins, for the identification of genetically diverse and agronomically superior genotypes of Brassica seed, which may generate putative transgressive segregates on hybridization. Diversity within species of Brassica can be analyzed at molecular level with the help of seed storage protein by using SDS-PAGE. On the basis of banding pattern zymogram (diagrammatical representation of different protein bands) were sketched, by which molecular weight of specie specific bands were calculated on the basis of Rf values of the bands on the gel. Through statistical analyses dendrogram was formed and genotypes were clustered into different groups by applying UPGMA (unweighed pair group mean analyses).

Keywords: *Brassica*, diversity, SDS-PAGE, seed protein.

1. Introduction

Brassica species are used as oil seed 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 [1]. Seed protein is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop. 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 [2]. Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice [3]. However, the information on the SDS-PAGE on different species of Brassica for genetic diversity is still limited [4].

Analyses of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding. In this study, a survey of seed protein was carried out to (a) assess the protein polymorphisms within and different cultivated species of Brassica (b) clarify the genetic nature of polymorphic bands.

2. Methods

Plant materials

Altogether, 30 cultivars of *Brassica rapa*, *Brassica juncea*, *Brassica carinata* and *Brassica napus* were used for this experiment (Table 1).

Electrophoresis

A single seed was grounded with a mortar and pestle and 10mg (0.01g) out of this seed flour was taken into a 1.5ml micro-tube. 400µl of the protein extraction buffer (62.5mM Tris-Hcl, pH 6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol, 5 M urea and 0.0001% bromo-phenol blue) was added and mixed well by vortexing. The crude homogenates were then centrifuged at room temperature with 15000rpm for 10 min. Thereafter, 6.5µl of the extract was directly analyzed by SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) using 15% (w/v) mini slab gel. Electrophoresis was carried out at 20V for stacking gel and 80V for separation gel in a buffer solution containing 0.025M Tris, 0.129M glycine and 0.125% SDS, until the dye front head migrated to

within 2mm of the end of the gel. Gels were stained with 0.5% coomassie brilliant blue (CBB) G-250 in acetic acid-methanol-water (3:22:25 volume ratio) for two hours and destained in acetic acid-methanol-water (5:20:75 volume ratio) for over night. Banding patterns were scored from at least two electrophoregrams for each cultivar.

Table 1. List of cultivars of oilseed *Brassicas* used in present study.

No.	Cultivar Name	Accession Number	Brassica Species	Breeding Institute/Origin
1	RL-18	22862	*B. juncea*	ORI, AARI, Faisalabad
2	Bahawalpur Raya	22852	*B. juncea*	RARI, Bahawalpur
3	S-9	19509	*B. juncea*	ARI, Tandojam, Sindh
4	Early Raya	23680	*B. juncea*	ARI, Tandojam, Sindh
5	Khanpur Raya	19510	*B. juncea*	ORS, Khanpur, R.Y.Khan
6	NIFA Raya	22854	*B. juncea*	NIFA, Peshawar
7	BARD-1	19493	*B. juncea*	NARC, Islamabad
8	Sultan Raya	19511	*B. juncea*	Unknown
9	Dacca Raya	1676	*B. juncea*	Bangladesh
10	Raya Anmol-87	22860	*B. juncea*	ORI, AARI, Faisalabad
11	Poorbi Raya	1673	*B. juncea*	ORI, AARI, Faisalabad
12	Canola Mustard	23695	*B. juncea*	NARC, Islamabad
13	Raya-2000	24168	*B. juncea*	ORI, AARI, Faisalabad
14	Chakwal Raya	22850	*B. carinata*	BARI, Chakwal
15	Peela Raya	22863	*B. carinata*	ORI, AARI, Faisalabad
16	DGL	22858	*B. napus*	ORI, AARI, Faisalabad
17	PR-7	1680	*B. napus*	ARI, Tarnab, Peshawar
18	NARC-22	1323	*B. napus*	NARC, Islamabad
19	Dure NIFA	22856	*B. napus*	NIFA, Peshawar
20	Shiralee	23633	*B. napus*	NARC, Islamabad
21	Dunkeld	23634	*B. napus*	Abroad
22	Haanza	23635	*B. napus*	Abroad
23	19-H	23636	*B. napus*	Abroad
24	Rainbow	23637	*B. napus*	Abroad
25	Abasin-95	22855	*B. napus*	NIFA, Peshawar
26	Chakwal Sarson	22851	*B. napus*	BARI, Chakwal
27	Pak.Cheen-89	23632	*B. napus*	ARI, Minora, Swat
28	Takwara	22853	*B. napus*	ARI, Dera Ismail Khan
29	Toria-A	23630	*B. rapa*	ORI, AARI, Faisalabad
30	BSA	23631	*B. rapa*	ORI, AARI, Faisalabad
31	Toria Sathi	22861	*B. rapa*	ORI, AARI, Faisalabad

Evaluation

Gels were evaluated by eye on a light box. Determination of the apparent molecular weight of individual protein subunits was carried out using molecular weight marker proteins: β-galactosidase, 116.0 KD; Bovine serum albumin, 45.0 KD; Lactate dehydrogenase, 35.0 KD; REase Bsp 981, 14.4 KD. (Protein molecular wt. Marker, Fermentas), applied on each gel and Rf values, the relative mobility of protein subunits was calculated.

Statistical analyses

All the monomorphic and polymorphic bands visible to the eye were scored and only unambiguously scored bands were used in the analyses. Each band was given score of 1 for presence or polymorphism and 0 for absence. Data analyses were conducted using NTSys-pc, version 2.2 (Exeter soft ware, Setauket,N.Y.). Similarities between cultivars were estimated using Dice coefficient of similarity (Rohlf 1992). Cluster analyses were conducted on similarity estimates using the unweighed pair- group method for arithmetic averages (UPGMA) and the resulting clusters were expressed as dendrograms.

3. Results and Discussion

The protein patterns of 30 Brassica cultivars after SDS-PAGE are shown in Figure 1. In total 29- 31 bands per cultivars were detected in electrophoregrams. Of these polymorphic bands appear at 13 positions designated as A, B, C, D, E, F, G, H, I, J, K, L and M respectively (Figure 2). Bands at position 'E' were divided into three patterns (E1, E2 and E3). These showed polymorphism on the basis of difference in protein intensity among genotypes. Bands at positions G and J showed presence- or- absence type polymorphism. At position G, some genotypes expressed a single band and others showed a pair of bands and two levels of mobility of the bands were detected. These banding patterns were recognized in the position as G1 and G2 respectively. *B. rapa* exhibited a specie-specific band at position G1 (M.wt-32.6 KD), *B. napus* produced a specie-specific band at G2 (M.wt-30.7KD), *B. carinata* exhibited both of these bands at positions G1 and G2 where as *B. juncea* possessed none of these. Eruca sativa species exhibited a characteristic band at position 'J' (M.wt-20 KD).

Three groups or clusters obtained from Dendrogram, clustering of population follow their genetic similarity (Figure 3). Cluster 1 comprises of fifteen genotypes where as cluster 2 comprises thirteen and cluster 3 comprises of three genotypes (Table 2). In this study intra and inter specific variation of seed storage proteins of 30 cultivars of oilseed Brassicas from Pakistan were analyzed. No report on SDS-PAGE of seed protein of Brassica varieties of Pakistan is available, so it seems to say that SDS-PAGE technique has proven to be a useful tool in supporting classical taxonomy studies [5]. It was possible to distinguish certain genotypes based on seed protein. Landraces are a useful source of genetic variation and the greater the variation, the greater the chances of a landrace possessing genes of gene combinations of interest to plant breeders [6]. The high stability of seed protein profile and its additive nature make seed protein electrophoresis a powerful tool in elucidating the origin and the evolution of cultivated plants [7].

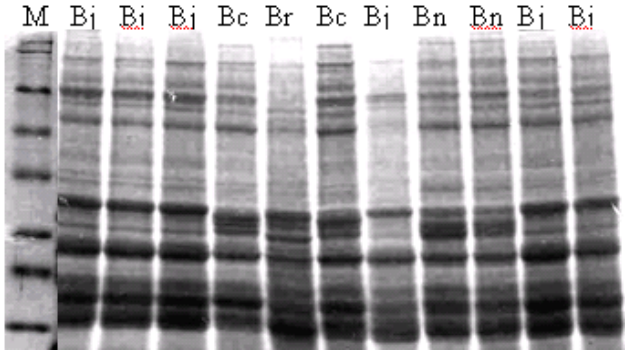

Figure 1. Electrophoretic patterns of various cultivars of oilseed Brassicas based on SDS-PAGE of total seed proteins. M-Molecular weight marker, Bc-Brassica carinata, Bj-Brassica juncea, Bn-Brassica napus and Br-Brassica rapa (campestris).

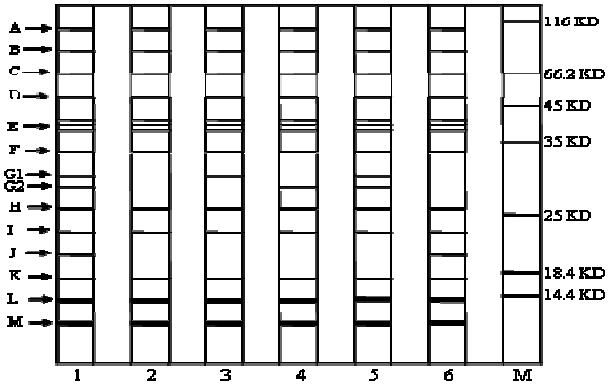

Figure 2. Summarizing spectrum of seed storage proteins (1) all species, (2) *Brassica juncea*, (3) *B. rapa*, (4) *B. napus*, (5) *B. carinata*, (6) *Eruca sativa*, (M) Molecular weight protein marker.

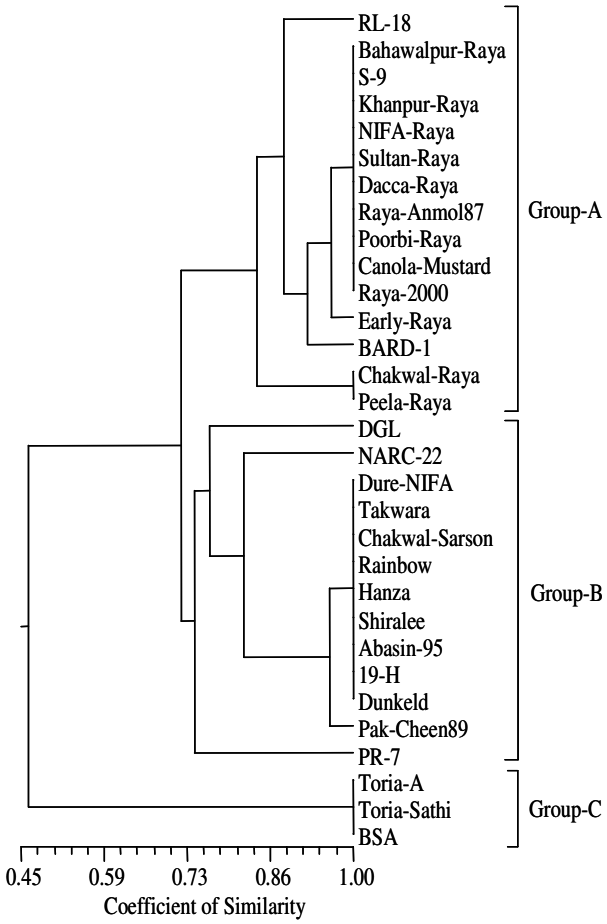

Figure 3. Dendrogram showing the relationships among 31 cultivars of oilseed Brassica based on SDS-PAGE of seed storage proteins.

Table 2 Cluster pattern of oilseed Brassica cultivars based on their genetic divergence.

Cluster	***Cultivar***	***Species***
Group-A	RL-18, Bhawalpur-raya, S-9, Khanpur-raya, NIFA-raya, Sultan-raya, Dacca-raya, Raya- Anmol, Poorbi-raya, Canola-mustard, Raya-2000, Early-raya, BARD-1	*B. juncea*
	Chakwal-raya, Peela-raya	*B. carinata*
Group-B	DGL, NARC-22, Dure-NIFA, Takwara, Chakwal-sarson, Rainbow, Hanza, Shiralee, Abasin-95, 19-H, Dunkeld, Pak/Cheen-89, PR-7	*B. napus*
Group-C	Toria-A, Toria-sathi, BSA	*B. rapa*

4 Conclusions

The present investigation revealed high variation in different cultivars of oil seed mustard in Pakistan with regard to their total seed protein profiles. Regarding interspecific variation among cultivars this investigation revealed no variation. The genetic affinities within cultivars of same species generally

corroborated the morphological analysis [8]. Phenotypically, most of the oilseed cultivars belonging to same specie also showed close association. This uniformity in interspecies cultivars also agreed with the findings of Ladizisky and Alder [9] and Ahmad and Slinkard [10], who examined different cultivars of cultivated chickpea and concluded that seed protein was a very conservative trait in chickpea. Similarly, Raymond et al. [11] and de vries [12] also reported similar electrophoretic patterns of protein among the cultivars of sunflower and lettuce, respectively.The result of differentiation of yellow sarson and brown seeded types of Brassica shows a similar agreement with the report of Das et al. [13] where the result clearly separated the yellow seeded and brown seeded varieties.

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