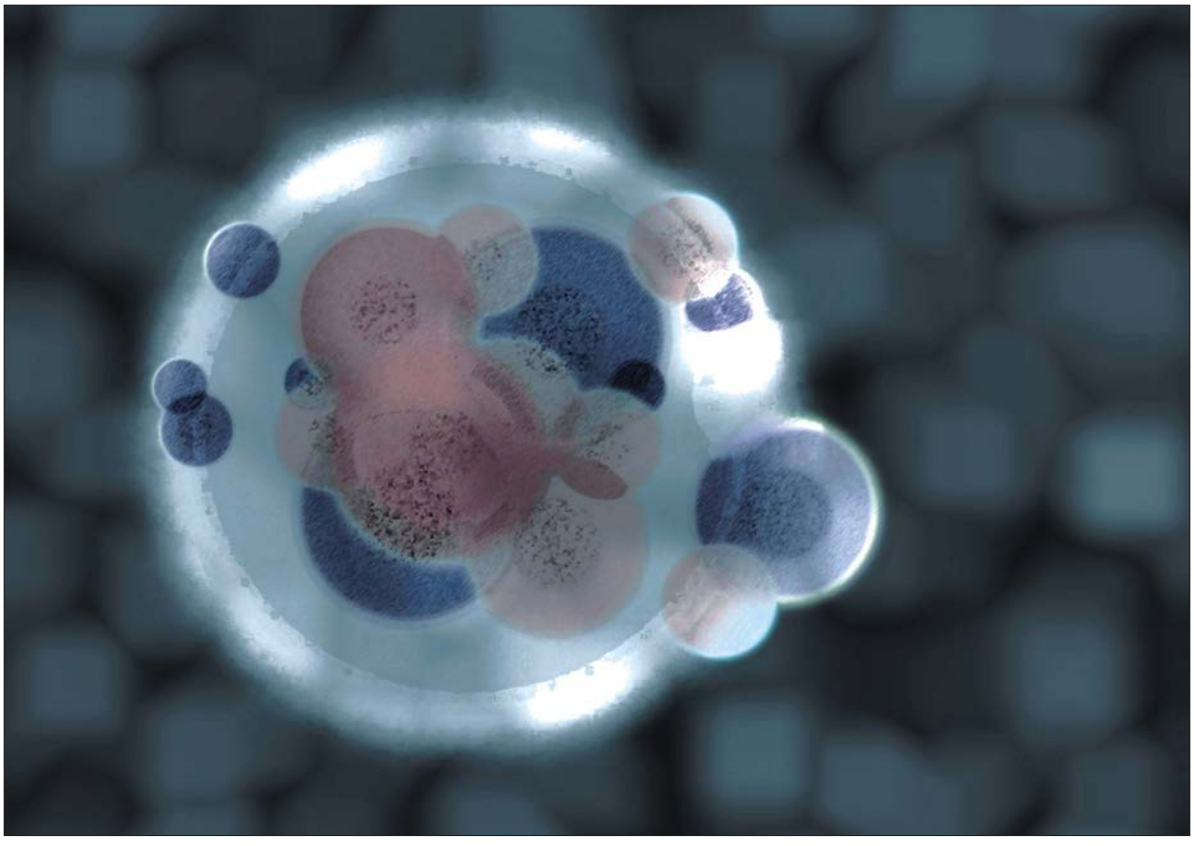

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Genetic Diversity in *Brassica* Species Using SDS-PAGE Analysis

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Abstract: Eighty five different cultivars of *Brassica rapa, B. juncea, B. napus, B. carinata, B. oleracea* and hexaploid *Brassica*, collected from Bangladesh, Japan, China and Denmark, were analyzed for seed and leaf protein variations by SDS-PAGE to identify the polymorphic genetic markers for evaluation of genetic resources. Ten polymorphic markers were identified from seed protein and no identifiable polymorphic band was found from leaf protein. However, polymorphic markers clearly distinguished these *Brassica* species. *Brassica rapa* var. 'yellow sarson' of Bangladesh origin showed uniquely identifiable four polymorphic bands for seed protein in contrast to the other *B. rapa* of brown-seeded type. The Bangladeshi and Japanese cultivars of *B.* rapa differed among protein quantity. Analytical results of SDS-PAGE for seed protein showed that hexaploid *Brassica* has the highest indices, such as % of polymorphic band, the degree of phenotypic diversity (Ho), diversity value for genetic marker (H_{EP}) and the sum of the effective number of alleles (SENA). The genetic diversity values of hexaploid *Brassica* were followed by amphidiploid (*B. napus, B. juncea, B. carinata*) and diploid (*B. oleracea, B. rapa*) species, respectively.

Key words: Genetic diversity, *Brassica*, SDS-PAGE, seed protein, leaf protein

INTRODUCTION

Brassica species are major oilseed crop as well as vegetables like broccoli, cabbages, Chinese cabbage and leaf mustard are an increasingly important part of the human diet worldwide. 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 proteins 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 an important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivars or when cultivars are to be patented[2]. Variation of seed storage proteins has also been analyzed to estimate the center of genetic diversity and possible dissemination pathway in common bean[3]. Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice[4]. However, the information on the SDS-PAGE on different species of *Brassica* for genetic diversity is still limited.

Analysis of SDS-PAGE are simple and inexpensive, which are added advantages for use in practical plant breeding. In this study, a survey of leaf and seed protein were carried out to (I) to assess the protein polymorphisms within and different cultivated species of *Brassica.* (ii) To investigate the geographical distribution of their electrophoretic banding types. (iii) To clarify the genetic nature of polymorphic bands.

MATERIALS AND METHODS

Plant materials: Eighty five different cultivars of *B. rapa, B. juncea, B. napus, B. carinata, B. oleracea* and hexaploid *Brassica* were used for this experiment

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Table 1: Number of cultivars showed presence or more stained to the respective banding types

							E							J		
Species	Origin	Culti-vars	A	B	C	D	E_1	E_2	E_3	F	G	H	I	J_1	J_2	J_3
YS *B. rapa*	Bangladesh	6	0	0	6	6	6	0	0	6	0	0	6	0	6	0
BS *B. rapa*	Bangladesh	6	0	0	1	0	6	0	0	6	6	0	6	6	0	0
	Japan	12	0	12	0	7	12	0	0	0	12	0	12	12	0	0
	China	8	0	2	1	0	8	0	0	2	8	0	8	8	0	0
B. juncea	Bangladesh	10	0	10	0	10	0	10	0	0	10	10	10	0	10	0
	Japan	18	0	12	0	18	0	18	0	0	18	18	18	0	18	0
	Denmark	2	0	2	0	2	0	2	0	0	2	2	2	0	2	0
B. napus	Bangladesh	16	16	6	0	10	0	0	16	12	16	0	16	0	0	16
	Japan	2	2	2	0	2	0	0	2	2	2	0	2	0	0	2
B. carinata	Denmark	1	1	1	0	1	0	1	0	0	1	1	0	0	0	1
	Japan	1	1	1	0	1	0	1	0	0	1	1	0	0	0	1
B. oleracea	Japan	2	2	2	0	2	0	2	0	0	2	0	0	0	0	2
Hexaploid *Brassica*	Denmark	1	1	1	0	1	0	0	1	1	1	1	1	0	0	1
Total		85														

N.B.: YS= Yellow sarson, BS= Brown seeded

Table 2: Polymorphism of SDS-PAGE for seed protein in different species.

	Between different species	Within species					
		B. rapa	*B. juncea*	*B. napus*	*B. carinata*	*B. oleracea*	Hexaploid *Brassica*
SDS-PAGE	31.4	21.2	3.2	6.3	0	0	0

(Table 1). All the materials were collected from Bangladesh, Japan, China and Denmark.

Electrophoresis: Leaf protein and seed storage proteins were extracted from young leaf and cotyledon of single seed, respectively and ground with (200 µl for leaf protein and 50-70 µl for seed protein) extraction buffer of 0.0625 M Tris-HCl (pH 6.8), 8 M Urea, 2% Sodium dodecylsulfate (SDS) and 5% 2-Mercaptoethanol and then kept for over night. The crude homogenates were then centrifuged at room temperature with 14000 rpm for 20 min. Thereafter, 8.5 µl of the crude extract was directly analyzed by SDS-Polyacrylamide gel electrophoresis (SDS-PAGE) using 12% (w/v) mini-slab gel. 0.1% Bromophenol blue (BPB) was used as marker dye to the cathode buffer. Electrophoresis was carried out at 10 mA for stacking gel and 20 mA for separation gel in a buffer solution containing 0.277 M glycine, 0.058 M Tris and 0.125% SDS, until the dye front head migrated to within 2 mm of the end of the gel. Gels were stained with 0.5% Coomassie Brilliant Blue (CBB) G-250 in acetic acid-ethanol-water (2:5:5 volume ratio) for one hour and distained in acetic acid-methanol-water (7:20:73 volume ratio) for over night. Banding patterns were scored from at least two electrophoregrams for each cultivar. When ambiguous band patterns were obtained, electrophoresis was further carried out by changing the gel concentration and/or electrophoresis time to determine the protein type.

Statistical analysis: 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.

The degree of polymorphism was quantified using Shannon's index of phenotypic diversity[5,6]:

$H_O = -f^{\circ} p_i \log p_i$ where p_i is the frequency of a particular band *I*.

Diversity value (H_{EP}) for genetic markers may be calculated from the sum of the squares of phenotypic frequencies by the formula:

$H_{EP} = 1-f^{\circ} p_i^2$; where p_i is the frequency of the *i*th band[7].

The sum of effective number of alleles (SENA) was calculated by determining the effective number of alleles for each locus[8], namely

$$SENA = f^{\circ} \{(1/f^{\circ} p_i^2)-1\}$$

RESULTS

Leaf protein: The cultivars of *B. rapa, B. juncea, B. napus, B. carinata, B. oleracea* and hexaploid *Brassica* were tested for leaf protein analysis by SDS-PAGE. The result does not distinguish well even between different species.

Seed protein: In total, 34-35 bands per cultivars were detected in SDS-PAGE electrophoregrams. Of these, polymorphic bands appear in ten positions designated as 'A', 'B', 'C', 'D', 'E', 'F', 'G', 'H', 'I' and 'J', respectively (Table 1 and Fig. 1). Bands in the position 'A', 'G', 'H' and 'I', showed presence-or-absence type

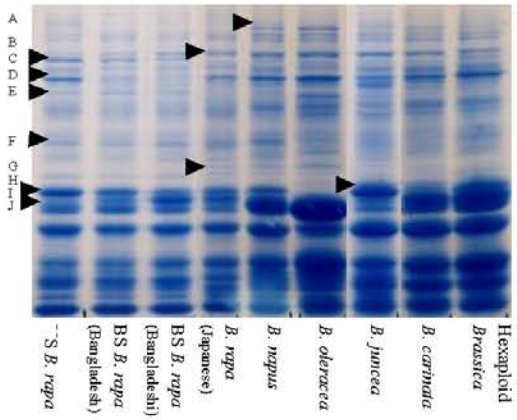

Fig. 1: Electrophoregram types identified by SDS-PAGE of seed protein of different species of *Brassica*

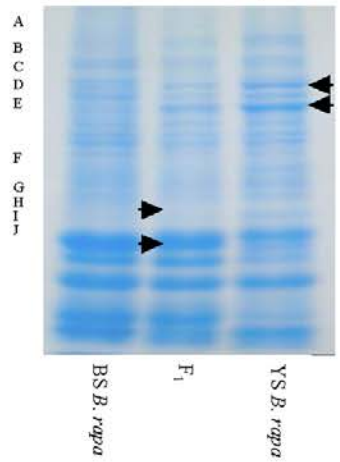

Fig. 2: Electrophoregram type of F_1 of the cross of yellow sarson (Agrani) and brown seeded (R-307) cultivar of *B. Rapa*

Table 3: Average of Sharonnon's information index (Ho), sum of effective alleles (SENA) and genetic diversity (Hep) of the cultivars of different species

Species	H_O	SENA	H_{EP}
Hexaploid *Brassica*	3.279	25.35	0.962
B. napus	3.215	23.86	0.960
B. carinata	3.155	22.63	0.958
B. juncea	3.147	22.15	0.957
B. oleracea	3.111	21.12	0.955
Y.S. *B. rapa*	3.090	20.96	0.954
B.S. B. rapa	3.040	19.60	0.951
Range	2.940-2.279	19.17-25.35	0.951-0.962

Y.S.= Yellow sarson, B.S.= Brown seeded

polymorphisms. In position 'E', some genotypes expressed a single band and other showed a pair bands and two levels of mobility of the bands were detected. These banding pattern were recognized in the position as 'E_1', 'E_2' and 'E_3', respectively. Bands in the positions 'B', 'C', 'D' and 'F' differed in the protein intensity among genotypes. Bands in position 'J' were also divided into three patterns ('J_1', 'J_2' and 'J_3'), based on size and number of bands in cluster; 'J_1' represented two medium thick bands, 'J_2' as three relatively thin bands and 'J_3' as one very thick band.

The number of cultivars that exhibited polymorphic banding patterns in each position is shown in Table 1 and Fig 1. Seed storage protein of all eighty-five cultivars were tested by every twenty individual seeds for a cultivar. In this study, 31.4% polymorphic bands were obtained among different species. In the case of polymorphism of different cultivars of the same species the cultivars of *B. rapa* showed the highest percentage of polymorphism (21.2%) followed by *B. napus* (6.3%) and *B. juncea* (3.2%) (Table 2).

The six Bangladeshi yellow sarson cultivars of *B. rapa* showed four uniquely identifiable banding character at band 'C', 'D', 'G' and 'J', respectively (Table 1 and Fig. 1). The Bangladeshi cultivars of brown seeded *B. rapa* showed distorted pattern i.e. presence-or-absence type of band in four polymorphic position of the same cultivar of different seeds with almost equal frequency, where the Japanese and Chinese cultivars didn't show any distorted pattern (result has not shown here). Highly dense band 'B' was specially expressed in Japanese and few Chinese cultivars of brown seeded *B. rapa.* Where Bangladeshi cultivars were detected as high intense band on 'F'. All the cultivars of *B. rapa* (yellow sarson and brown seeded types) exhibited a band at 'I' and failed to produced any band at 'A' and 'H' and also were 'E_1' types.

The cultivars of *B. juncea* exhibited a band in 'G', 'H' and 'I' and didn't show any band at 'A'. These cultivars showed protein rich band at 'D'. The cultivars were 'C'-less and 'F'-less with 'E_2' and 'J_2' types. Polymorphism of different cultivars of *B. juncea* was obtained at band 'B'.

In the case of *B. napus*, bands existed at band 'A', 'G' and 'I'; and failed to produced any band at 'H' and the cultivars were 'C'-less, 'E_3' and 'J_3' types of banding pattern. Polymorphism of different cultivars of *B. napus* were obtained at 'D' and 'F' bands.

In this study each two cultivars of *B. carinata* and *B. oleracea* were used to compare the bands with other species. However, the cultivars of *B. carinata* exhibited a band at position 'A', 'G' and 'H' and failed to show any band at position 'I'. The cultivars were 'B'-rich, 'F'-less, 'E_2' and 'J_3' types. Cultivars of *B. oleracea* produced band at position 'A', 'G' and null of band at position 'H' and 'I'. These cultivars were 'B'-rich, 'C'-less, 'F'-less, 'E_2' and 'J_3' types.

The trigenomic hexaploid was the harbor of all three genomes of *Brassica*, exhibited the highest number of band per assay. It produced band 'A', 'G', 'H' and 'I'.

This cultivar was 'B'-rich, 'D'-rich, 'F'-rich, 'C'-less, 'E_3' and 'J_3' type.

Statistical analysis: Phenotypic frequency of each band was calculated and used in estimating average Shannon's index of diversity (Ho) of different species (Table 3). The Ho values of different cultivars of yellow sarson and brown seeded *B. rapa, B. napus, B. carinata* and *B. oleracea* were not significantly different, but significant different (paired t-test) was observed among different species as well as yellow sarson and brown seeded cultivars of *B. rapa* (Table 3).

The highest phenotypic diversity (Ho) was observed in hexaploid *Brassica,* followed by *B. napus, B. carinata, B. juncea, B. oleracea,* yellow sarson and brown seeded *B. rapa,* respectively. The similar trend were observed in the genetic diversity value (H_{EP}) and sum of the effective number of alleles (SENA) (Table 3).

Banding nature of F_1 of yellow sarson and brown seeded *B. rapa:* The Bangladeshi yellow sarson cultivar (Agrani) and Japanese brown seeded cultivar (JR-310) were polymorphic at six banding positions. The F_1 showed the sum of all polymorphic banding types of the respective banding position of parents (Fig. 2).

DISCUSSION

In this study, the intra-and-interspecific variation and geographical distribution of leaf protein and seed storage protein of eighty-five cultivars of *Brassica* were analyzed. Banding pattern of SDS-PSGE of leaf protein of even different species of *Brassica* cultivars did not show any better significant different. No report on SDS-PAGE for leaf protein of *Brassica* is available, so it seems to say that more diverged cultivars and species are necessary to find out polymorphism in leaf protein SDS-PAGE technique has proven to be a useful tool in supporting classical taxonomy studies[9]. Protein types and their variation differed among different species, which information will help us to early identification of the species at seed level as well as to get the information on purity of genetic resources. The genotypes of *B. rapa* from Bangladesh, Japan and China also differ in position 'B' and 'F'. This information may help us to establish the diversity origin of the Asian germplasm. Thanh and Hirata[4] observed seed storage protein diversity in wild and cultivated rice. Only two genotypes of *B. juncea* from Denmark and two genotypes of *B. napus* from Japan were examined in this study, which showed similar banding types with respective species of Bangladesh origin. However, due to the limited number of cultivars, we can't draw any definite conclusion of the mentioned cultivars.

The greatest genetic diversity was found in the cultivars of Bangladeshi yellow sarson and other brown seeded *B. rapa,* where yellow sarson showed four unique protein types. The yellow sarsons are special ecotypes from India, belongs to ssp. *trilocularis* and are self-compatible, whereas other brown seeded *B. rapa* belongs to ssp. *oleifera* and are self-incompatible. In case of brown seeded Bangladeshi cultivars of *B. rapa*, different seeds of same cultivars exhibited different protein types at four proteins subunits. The possible explanations for the distribution of protein types in yellow sarson and brown seeded *B. rapa* are as follows: (I) The four unique protein types were originated in yellow sarson and these types have been introduced to brown seeded cultivars. (ii) Due to self-compatibility of yellow sarson, they did not show any protein variation pattern in the same cultivars of different seeds, whereas the brown seeded types are self-incompatible and after introgression of the characters from yellow sarson, showed different protein types. The F_1 of the cross of yellow sarson and brown seeded Japanese cultivars confirmed the dominant nature of yellow sarson specific polymorphic bands. (iii) The brown seeded Japanese and Chinese cultivars of *B. rapa* uniquely had 'G' band, where different seeds of same cultivars of Bangladeshi brown seeded *B. rapa* showed presence-or-absence type of the same band. This may be due to cultural practice of yellow sarson in Bangladesh, where the character (lack of band) introduced to Bangladeshi brown seeded cultivars and, not in Japanese and Chinese cultivars. The F_1 of yellow sarson and Japanese and Chinese cultivars of *B. rapa* showed lack of 'G' band.

The result of differentiation of yellow sarson and brown seeded types of B. *rapa,* shows a similar agreement with the report of Das *et al.*[10], where the result clearly separated the yellow seeded, self-compatible cultivars from the brown seeded, self-incompatible cultivars by using RAPD and AFLP analysis.

Highest Shannon's index of diversity (Ho) followed the same trend as genetic diversity (H_{EP}) and sum of the effective number of alleles (SENA). The highest value of genetic diversity was found in trigenomic hexaploid *Brassica* (AABBCC), followed by other amphidiploids (*B. napus* AACC, *B. juncea* AABB, *B. carinata* BBCC) and diploid (*B. rapa* AA, *B. oleracea* CC) species. The result indicated that higher level of genome constitution donated higher number of polymorphic bands, resulting higher diversity value. In the case of yellow sarson and brown seeded *B. rapa,* the yellow types exhibited more number of bands showing higher diversity value. No report has not been available in this connection.

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