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Molecular Characterization of Thirteen Oilseed *Brassica* L. Variants from Bangladesh through Polyacrylamide Gel Electrophoresis (PAGE)

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***Brassica* L. is the most agronomical important genus of Brassicaceae family so as having economical values, an electrophoretic exploration was conveyed for proper identification of genetically diverse and agronomically superior genotypes and pursuing the extent of genetic divergence and phylogenetic relationship within the thirteen variants of *Brassica* for leaf storage protein by using Polyacrylamide Gel Electrophoresis (PAGE) as biochemical marker. A total of 19 alternative protein bands were found with highly polymorphism of 89.47%. The protein banding pattern suggested the existence of differences among the studied variants pertaining to the location, molecular weight and staining intensity of the bands which could be utilized as fingerprints for variants identification. Based on Nei's genetic distance, a wide range of genetic distance (0.0541–1.5581) offered the presence of broad genetic variability among the quested variants. A dendrogram was constructed by using UPGMA where all the analyzed *Brassica* variants grouped into two major clusters. Relied on this analysis, highest genetic variation (1.5581) was observed between BS-10 and BS-14 whilst the lowest genetic variation (0.0541) was recorded between BS-9 and BS-12, which might be furnished as a source of parental line. Consequently, it can be proposed that the protein profile of analyzed thirteen variants of *Brassica* L. by PAGE would be considered to be a contributory implement to the breeders of *Brassica* by providing sufficient information on the genetic resources of *Brassica* and improvement of new offspring in the forthcoming breeding program of *Brassica* L.**

Keywords: *Brassica* L.; Genetic diversity; Leaf protein; PAGE.

The Brassicaceae (Cruciferae) family is consisting of 338 genera and about 3709 species[1]. Among these genera, economically *Brassica* L. is the most important genus with 37 different species[2]. The genus - *Brassica* composed of six interlinked species with great morphological and genetic diversity of which three diploid species- *Brassica rapa* (A genome), *B. nigra* (B genome), and *B. oleracea* (C genome) of the genus *Brassica* were considered to be responsible for the origin of three amphidiploid species, *B. carinata* (n = 17, BC genome), *B. juncea* (n = 18, AB genome) and *B. napus* (n = 19, AC genome)[3]. *Brassica* L. seems to have derived near the Himalayan region with a large number of important vegetables to oilseed and condiment crops; a great source of bioactive compounds, minerals, phytochemical contents, vitamins and fibers[4].

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Generally, the genus *Brassica* L. has been classified into three groups particularly –rapeseed, mustard and cole. The mustard groups include species like *B. juncea*, *B. nigra* and *B. carinata*; whereas the rapeseed groups include *B. rapa* and *B. napus*[5]. Commercial production of *Brassica* has grown progressively as a vital source of oil and plant originated protein for human and animal nutrition. Currently, Rapeseed categorizes as the third source of vegetable oil (after soy and palm) and for oil meal it ranks as the third notable source (after soy and cotton)[6]. *Brassica* L. generates Indole-3-carbinol that plays significant role to reduce the growth of human breast cancer cells and the occurrence of tumors in reproductive organs[7-8].

Moreover, *Brassica* L. are not only a quality sources of potassium, dietary fiber, phenolics, vitamins A, C and E but also use as a renewable resource or biofuel in the petro-chemical industry[9]. Higher protein solubility is found in seeds of *B. napus* than *B. rapa* seeds. As a rich source of edible protein, *B. rapa*, *B. juncea*, *B. carinata*, and *B. nigra* have commercial values in food industry. In oil-extraction process, rapeseed and canola meal remain as by-product which contain up to 42.7% to 50% protein[10].

Evaluation with molecular marker facilitates in determining parental forms for mapping of population, marker assisted alternatives, line drawings of back crosses and consequently various molecular markers are applied to execute different studies which offer assistance the breeders to improve crop species[11]. Now-a-days storage proteins are widely used as biochemical markers to find genetic structure, genetic diversity and relationships within plant species. According to O'Farrell (1975)[12], polyacrylamide gel electrophoresis (PAGE) has been a well-accepted proteomic analytic method since its initiate to access protein banding patterns among different plant varieties. Hence electrophoresis of protein is considered as a method for characterization and evaluation of germplasm as well as increase the utilization of various plant genetic resources[13].

Nowadays, storage protein is one of the most significant implements to appraise genetic assortment among wild and cultivated plant species. Reviewing a number of earlier works of Turi *et al.* (2010)[13], Mukhlesur and Hirata (2004)[14], Sadia *et al.* (2009)[15], Zada *et al.* (2013)[16], Ibrahim *et al.* (2017)[17] and so on, it has been revealed that they conveyed abundant efforts to find out genetic diversity and relationship among various species of *Brassica* for improvement of crop through SDS-PAGE.

Consequently the present investigation is conducted based on leaf storage protein of thirteen different BARI (Bangladesh Agriculture Research Institution) variants of *Brassica* from Bangladesh by utilizing PAGE technique to evaluate accurate protein profile for discerning variants, extent of genetic divergence and relationship among the thirteen inquired variants of *Brassica* as well as selection of parental line for further breeding program and crop improvement.

MATERIALS AND METHODS

Plant Materials

Thirteen variants of the genus *Brassica* have been chosen for the current study reflecting a wide array of variation for diverse physio-morphological attributes (Table 1). To conduct the present investigation, all the thirteen variants of *Brassica* were collected from Oilseeds Research Center (ORC) of Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh and maintained in the Botanical garden of Jagannath University, Dhaka, Bangladesh. Analyses of leaf protein profile of the supplied variants were performed in the laboratory of Department of Botany, Jagannath University, Dhaka, Bangladesh.

Methods

Protein Isolation and Sample Preparation

Fresh and young leaves of one-month old seedlings of investigated thirteen variants of *Brassica* were collected to isolate the crude protein. Based on the methodology of Akbar *et al.* (2020)[11] the collected leaves of *Brassica* variants were gently washed with distilled water and then with ethanol to clean the microspores and other dirt from the leaves surface and then kept on flitter papers for a while to soak up the excessive amount of distilled water and ethanol from the leaves. Afterwards, 1 gm fresh leaf of each sample was grinded in icy motor-pestle and later the crude homogenates were centrifuged at 4 ºC with 13000 rpm for 15 minutes. After centrifugation, the crude protein remained

as clear supernatant and stored in refrigerator at -20 ºC as sample (isolated protein) for vertical polyacrylamide gel electrophoresis.

Estimation of Protein

According to Lowry *et al.* (1951)[18], with little modification, the protein concentration of investigated each *Brassica* L. sample was estimated using 665 nm wavelength via spectrophotometer (AUXILAB S. L. UV-VIS Spectrophotometer).

Electrophoresis

Polyacrylamide gel electrophoresis of each inquired sample was conducted by following the strait of Akbar *et al.* (2020)[11]. The entire process of electrophoresis was performed by using omniPAGE mini vertical gel electrophoresis unit. The isolated protein sample was directly resolved with 10.0% polyacrylamide as separating gel and 4.0% as stacking gel during electrophoresis. Then, 25 ìl of each sample protein was loaded with 2X diluted Bromo Phenol Blue (BPB) loading dye (20 ìl) into the well of stacking gel. To run the electrophoresis, Cleaver nano PAC – 300 constant power supply unit was employed and voltage was fixed at 90 V and current was set up at 120 amp. The protein sample with BPB loading dye was mobilized in 10X diluted running buffer solution (Tris-glycine buffer, pH 8.3) until the dye front line arrived on 2mm above to the end of the gel. Afterwards, the gel was stained by 0.25% Coomassie Brilliant Blue (CBB) R-250 for 25 minutes and distained in acetic acid - methanol - distilled water (1: 4: 5 volume ratios) until the clear bands appeared on the gel. Lastly, the distained gel was gently washed with distilled water and the photographs of the gel were taken by a DSLR (18 mega pixels Canon EOS 700D model).

Data Analysis

Evaluations of the gels were done with bare eyes on a light box. The relative mobility (R_f values) of protein subunits were calculated by measuring the migration distance from the top of the separating gel to each band and to the dye front. For each band on the gel, the R_f value was calculated using the following equation:

R_f = migration distance of the protein / migration distance of the dye front

Adduction of the discernible molecular weight of individual protein subunits was carried out using molecular weight marker proteins. Phosphorylase B, 97.2 KD; Bovine serum albumin, 66.4 KD; Ovalbumin, 44.3 KD; Carbonic anhydrase, 29.0 KD; Trypsin inhibitor, 20.1 KDa and Lysozyme, 14.3 KDa (Protein Molecular Weight Marker; Takara Bio USA) applied on the gel as marker protein. A standard curve of the log molecular weight (MW) on X axis versus relative mobility (R_f) of marker protein on Y axis was generated using computer based program Microsoft Excel. Molecular weight of individual unknown protein subunit from PAGE was determined by utilizing the equation:

$y = mx + c$, where y denotes for the molecular weight of unknown protein subunit.

The photographs of the gel were acutely reviewed on the basis of the presence (1) and absence (0) of protein bands. All the major and minor bands that apparent to eyes were considered in our current analysis and scoring of all the monomorphic and polymorphic bands was recorded. The scores acquired from PAGE analysis were then pooled for creating a single data matrix. Thereafter, the data was used to estimate proportion of polymorphic loci, Nei's (1973)[19] gene diversity (h), Shannon's Information index (Lewontin, 1972)[20] and Nei's (1972)[21] genetic distance (D) employing a computer program, POPGENE (version 1.32) (Yeh *et al*., 1999)[22]. Based on genetic distance between all pairs of individual variants, a dendrogram was prepared applying Unweighted Pair Group Method with Arithmetic averages (UPGMA).

RESULTS AND DISCUSSION

Proteins are thought about to be the forthright outcome of genes and might be used as a marker of these genes. As such protein is performed as an extra implies for characterizing systematic denomination. Hence an ample electrophoretic protein banding profile was conducted after the extraction and separation of stored leaf protein from the studied thirteen variants of *Brassica* L. through PAGE technique and represented in Fig. 1.

The electrophoretic protein banding patterns of the thirteen studied variants of *Brassica* were detected which conveyed to the marking off a total of nineteen polypeptide bands. Of the nineteen polypeptide bands were found to be present at nineteen different loci designated as **a** – **s** with molecular weight ranging from 16.36 to 97.20 KDa

Table 1. Some physio-morphological and agronomic traits of 13 *Brassica* L. variants used in the study

Species	Name of variants	Days to maturity	Silique chamber	Seed color	Agronomic traits
B. juncea	Daulot (RS-81)	90-105	2 chambered	Reddish brown	Comparatively long duration, low yielding variety. Tolerant to alternaria blight disease and environmental stresses like - drought and slightly tolerant to salinity.
B. juncea	BARI Sarisha-2 (Rai-5)	90-100	2 chambered	Reddish brown	Long duration, low yielding variety. Tolerant to drought, slightly tolerant to salinity.
B. juncea	BARI Sarisha-10 (BS-10)	90-100	2 chambered	Reddish brown	Long duration, high yielding variety. Tolerant to drought, slightly tolerant to salinity.
B. juncea	BARI Sarisha-11 (BS-11)	105-110	2 chambered	Reddish brown	Long duration, high yielding variety. Tolerant to drought and salinity.
B. napus	BARI Sarisha-7 (Napus - 3142)	90-95	2 chambered	Black	Long duration, high yielding variety, tolerant to alternaria blight disease and interim water logged condition.
B. rapa	BARI Sarisha-1 (Tori-7)	70-80	2 chambered	Black	Short duration, low yielding variety. Susceptible to pest and diseases.
B. rapa	Kollaynia (TS-72)	85-90	2 chambered	Blackish brown	Short duration, low yielding variety. Susceptible to pest and environmental stresses.
B. rapa	Sonali Sarisha (SS-75)	90-100	4 chambered	Golden yellow	Long duration, high yielding variety. Susceptible to alternaria blight disease.
B. rapa	BARI Sarisha-6 (Dholi)	90-100	2 chambered	Yellow	Long duration, high yielding variety. Susceptible to environmental stresses.
B. rapa	BARI Sarisha-9 (BS-9)	80-85	2 chambered	Reddish brown	Short duration, high yielding variety.
B. rapa	BARI Sarisha-12 (BS-12)	85-90	2 chambered	Reddish brown	Short duration, high yielding variety.
B. rapa	BARI Sarisha-14 (BS-14)	75-80	2 chambered	Yellow	Short duration, high yielding variety.
B. rapa	BARI Sarisha-15 (BS-15)	80-85	2 chambered	Yellow	Short duration, high yielding variety.

Table 2. Region, location, molecular weight, relative mobility, type of unique band and intensity values of different protein bands present in 13 *Brassica* L. variants

Region	Locus	Molecular weight (KDa)	R_f value	Unique band types	Variants												
					Daulot	Rai-5	BS-10	BS-11	BS-7	Tori-7	TS-72	SS-75	BS-6	BS-9	BS-12	BS-14	BS-15
I	a	97.20	0.028	-	++	++	-	++	++	++	++	-	++	++	++	++	++
	b	83.76	0.039	-	-	-	-	-	-	-	-	-	++	++	++	++	-
	c	74.65	0.054	Negative	++++	-	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
	d	72.95	0.081	Positive	-	-	++	-	-	-	-	-	-	-	-	-	-
	e	69.89	0.148	-	++	++	-	+++	+++	++	++	-	++	++	-	++	++
	f	55.72	0.173	-	++	-	-	-	-	++	++	++	++	++	++	++	++
II	g	52.76	0.192	Positive	++	-	-	-	-	-	-	-	-	-	-	-	-
	h	50.34	0.208	-	-	++++	++++	++++	++++	++	++	-	-	-	-	-	-
	i	49.25	0.215	-	++	-	-	++	++	++++	++++	++++	++++	++++	++++	++++	++++
	j	45.20	0.244	-	-	-	-	-	-	++	++	++	++	-	-	++	++
	k	44.30	0.262	-	++++	++	++	+++	+++	++++	-	-	-	-	-	-	-
	l	43.54	0.275	-	+++	-	-	-	-	-	+++	++	-	-	-	-	-
	m	42.16	0.295	-	++++	++++	++++	++++	++++	+++	+++	+++	+++	+++	+++	+++	+++
	n	40.20	0.325	-	-	++++	-	++++	-	-	+++	+++	+++	+++	+++	+++	+++
	o	38.46	0.353	-	-	-	-	-	-	-	+++	+++	+++	+++	+++	+++	+++
III	p	30.45	0.501	-	+	+	+	+	+	+	-	-	-	-	-	-	-
	q	27.89	0.546	-	+	+	+	+	+	+	-	-	-	-	-	-	-
	r	17.35	0.817	-	-	-	-	+	+	+	+	-	+	-	-	+	+
	s	16.36	0.854	-	+	+	+	+	+	+	+	+	+	+	+	+	+

Keys: + : very low intensity, ++ : low intensity, +++ : medium intensity, ++++ : high intensity, - : absence of band

(Table 2 and Figs. 1A–B). A close inquisition of the bands displayed that the different variants had slight differences in their protein banding patterns with respect to the presence and absence and staining intensities of the bands. Moreover, in the current study- the entire protein banding pattern of the investigated variants were vindicated into 3 different regions (I to III) based upon the manner of increasing R_f values and decreasing molecular weight of proteins (Table 2). As a consequence, region – I was found to consist of six bands having a range of molecular weight 97.20–55.72 KDa with R_f value ranging from 0.028–0.173. The six protein bands of region – I was characterized with bands of

Table 3. Inter varietal locus variation among the 13 variants of *Brassica* L.

Locus	Present (%)	Absent (%)	Variation (%)	Status	Genetic disagreement
Locus a	11 (84.62%)	2 (15.38%)	15.38%	Polymorphic	0.85
Locus b	4 (30.77%)	9 (69.23%)	69.23%	Polymorphic	0.31
Locus c	12 (92.31%)	1 (7.69%)	7.69%	Polymorphic	0.92
Locus d	1 (7.69%)	12 (92.31%)	92.31%	Polymorphic	0.08
Locus e	10 (76.92%)	3 (23.08%)	23.08%	Polymorphic	0.77
Locus f	9 (69.23%)	4 (30.77%)	30.77%	Polymorphic	0.69
Locus g	1 (7.69%)	12 (92.31%)	92.31%	Polymorphic	0.08
Locus h	6 (46.15%)	7 (53.85%)	53.85%	Polymorphic	0.46
Locus i	11 (84.62%)	2 (15.38%)	15.38%	Polymorphic	0.85
Locus j	6 (46.15%)	7 (53.85%)	53.85%	Polymorphic	0.46
Locus k	8 (61.54%)	5 (38.46%)	38.46%	Polymorphic	0.62
Locus l	4 (30.77%)	9 (69.23%)	69.23%	Polymorphic	0.31
Locus m	13 (100.00%)	0 (0.00%)	00.00%	Monomorphic	1.00
Locus n	9 (69.23%)	4 (30.77%)	30.77%	Polymorphic	0.69
Locus o	7 (53.85%)	6 (46.15%)	46.15%	Polymorphic	0.54
Locus p	5 (38.46%)	8 (61.54%)	61.54%	Polymorphic	0.38
Locus q	5 (38.46%)	8 (61.54%)	61.54%	Polymorphic	0.38
Locus r	7 (53.85%)	6 (46.15%)	46.15%	Polymorphic	0.54
Locus s	13 (100.00%)	0 (0.00%)	00.00%	Monomorphic	1.00
Total Polymorphism			89.47%		

Table 4. Levels of polymorphism within 13 variants of *Brassica* L.

Variants of *Brassica*	No. of polymorphic bands	% of polymorphism exists in *Brassica* variants	Average % of polymorphism exists in each *Brassica* species	Average % of polymorphism exists in 13 *Brassica* variants
Brassica juncea var. Daulot	8	42.11	47.37	
B. juncea var. Rai-5	10	52.63		
B. juncea var. BS-10	11	57.89		
B. juncea var. BS-11	7	36.84		
B. napus var. BS-7	8	42.11	42.11	44.13
B. rapa var. Tori-7	8	42.11	42.76	
B. rapa var. TS-72	7	36.84		
B. rapa var. SS-75	10	52.63		
B. rapa var. BS-6	7	36.84		
B. rapa var.BS-9	9	47.37		
B. rapa var.BS-12	10	52.63		
B. rapa var.BS-14	6	31.58		
B. rapa var. BS-15	8	42.11		

mostly low intensity at loci – **a**, **b**, **d** and **f**, whereas in locus – **e**, moderate and low level intensity protein bands were found, protein bands of high intensity in all the variants were observed at locus – **c**, except in Rai-5 (negative unique band). The only low intensity protein band with molecular weight and R_f value of 72.95 KDa and 0.081 respectively, was detected from BS-10 at locus – **d**, which can be considered as a unique band (positive unique band)

Table 5. Estimation of genetic variability among 13 variants of *Brassica* L.

Locus	Nei's (1973) gene diversity (h)	Shannon's information index (i)
Locus a	0.3550	0.5402
Locus b	0.4260	0.6172
Locus c	0.1420	0.2712
Locus d	0.1420	0.2712
Locus e	0.3550	0.5402
Locus f	0.4260	0.6172
Locus g	0.1420	0.2712
Locus h	0.4970	0.6902
Locus i	0.4970	0.5402
Locus j	0.3550	0.6902
Locus k	0.4970	0.6902
Locus l	0.4260	0.6172
Locus m	0.0000	0.0000
Locus n	0.4260	0.6172
Locus o	0.4970	0.6902
Locus p	0.4794	0.6663
Locus q	0.4794	0.6663
Locus r	0.4970	0.6902
Locus s	0.0000	0.0000
Mean	0.3488	0.5098
St. Dev	0.1726	0.2299

and may be used as an implement for particular varietal characterization of *Brassica* L. (Table 2 and Figs. 1A–B). All the bands in region – I was found polymorphic. Region – II was observed with nine protein bands having molecular weight and R_f value ranging from 52.76–38.46 KDa and 0.192–0.353, respectively. The protein bands revealed in this region were pre-eminently discerned with medium to high intensity of proteins at loci – **m**, **n** and **o** whose R_f value lie between 0.295–0.353. Two bands with low and high intensities of protein were present at loci – **h** and **i**. A single band with all low intensities of protein (at locus – **j**) and low and moderate intensities of protein were appeared at locus – **l**. At locus – **g**, a single band of low intensity protein with R_f value of 0.192 and molecular weight 52.76 KDa was apparent in Daulot whereas no bands of protein was observed in rest of the twelve variants of *Brassica* at this locus, which made the band to be envisaged as an unique band (positive unique band) and assist in distinguishing the variant – Daulot from the other examined variants of *Brassica* (Table 2 and Figs. 1A–B). The region – III was characterized with very low intensities of protein bands found to be present at loci – **p** to **s** with molecular weight and R_f values ranging from 30.45–16.36 KDa and 0.501–0.854 (Table 2 and Figs. 1A–B). By taking into account the intensity of protein bands in different loci of the entire electrophoretic profile, it was observed that the region – II was more diverse with an average of 3 bands as compared to region – I and III, where the average numbers of bands were 2 and 1.33, respectively.

The change-over in the staining intensity and number of the polypeptide bands might be

Table 6. Summary of Nei's genetic distances of 13 variants of *Brassica* L.

Brassica variants	Daulot	Rai-5	BS-10	BS-11	BS-7	Tori-7	TS-72	SS-75	BS-6	BS-9	BS-12	BS-14	BS-15
Daulot	0												
Rai-5	0.3795	0											
BS-10	0.4595	0.3054	0										
BS-11	0.4595	0.1719	0.3795	0									
BS-7	0.3795	0.2364	0.3054	0.0541	0								
Tori-7	0.5465	0.5465	0.6419	0.3054	0.2364	0							
TS-72	0.8650	0.8650	0.9985	0.5465	0.6419	0.3054	0						
SS-75	0.7472	0.9985	0.8650	0.8650	0.9985	0.5465	0.1719	0					
BS-6	0.8650	0.8650	1.3350	0.5465	0.6419	0.3054	0.2364	0.3054	0				
BS-9	0.6419	0.6419	0.9985	0.5465	0.6419	0.4595	0.3795	0.3054	0.1112	0			
BS-12	0.7472	0.7472	0.8650	0.6419	0.7472	0.5465	0.4595	0.2364	0.1719	0.0541	0		
BS-14	0.7472	0.9985	1.5581	0.6419	0.7472	0.3795	0.1719	0.2364	0.0541	0.1719	0.2364	0	
BS-15	0.7472	0.7472	1.1527	0.4595	0.5465	0.2364	0.1719	0.2364	0.0541	0.1719	0.2364	0.1112	0

by virtue of differential extraction or disparity in solubility of protein or inadequacy of separation of varied sorts of proteins having identical migration rates[23]. They also suggested that the qualities of bands (i.e., the difference in the number, position and intensity of bands) in varieties even in accessions of the same species are governance through the quantitative gene system.

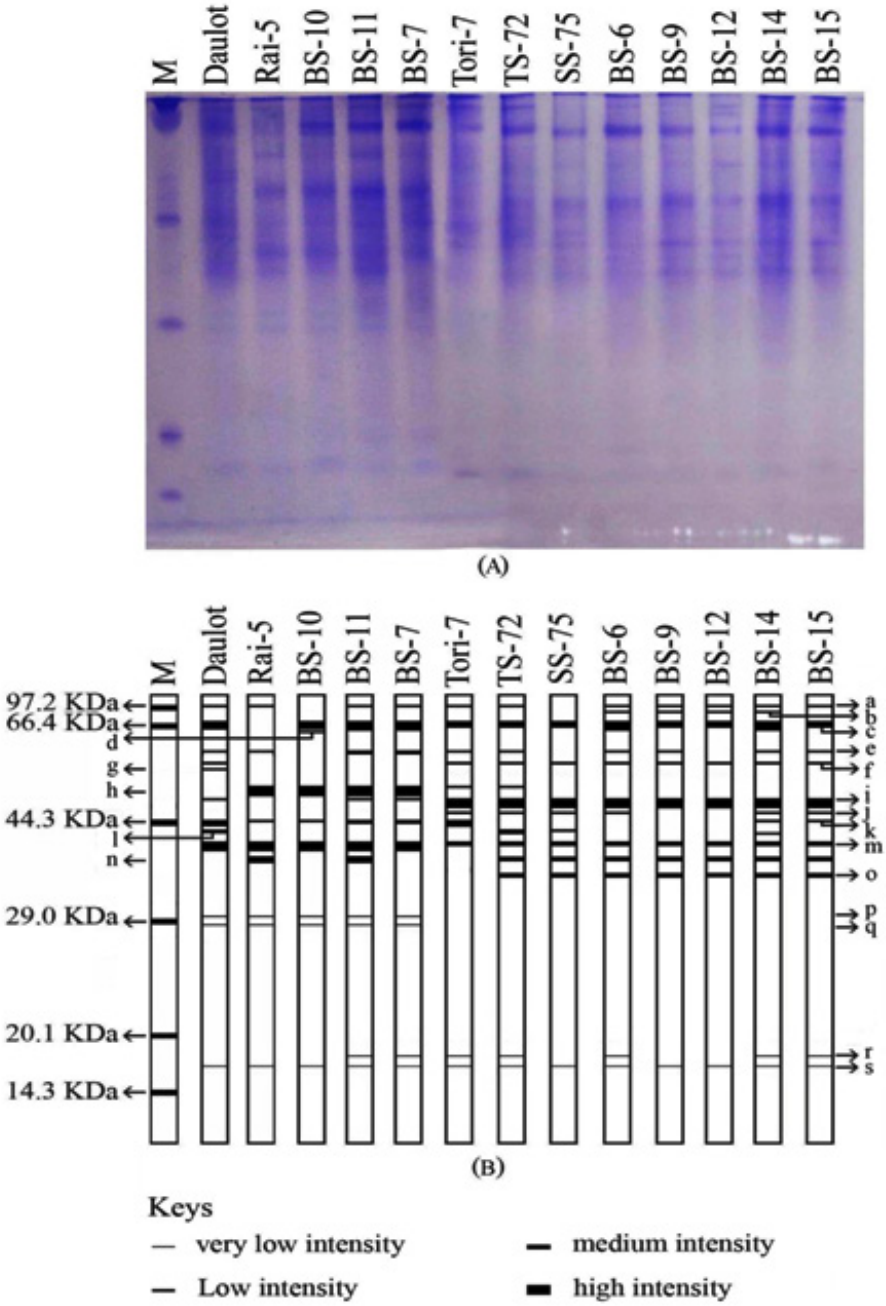

Fig. 1. Banding pattern of leaf storage protein of thirteen variants of *Brassica* through PAGE, (A) stained with Coomassie Brilliant Blue (CBB; R-250) and (B) diagrammatic representation of protein bands on polyacrylamide gel. Lane M- Molecular weight protein marker, KDa- Kilo Dalton (arrows indicate different loci produced during polyacrylamide gel electrophoresis).

Observations based on intensity of protein bands from different varieties of *Brassica* species were reported by many investigators[13, 15, 24-25]. Likewise, delineations on divergent plant species regarding to the intensities of protein bands were debriefed by Odeigah *et al.* (1999)[26] in Nigerian varieties of pepper, Devi (2000)[27] in sunflower, Varma *et al.* (2005)[28] in maize genotypes, Vijayan (2005)[29] in rice, Paul and Datta (2006)[30] in celery and ajowan, Nisha (2007)[31] in wheat, Sumathi (2007)[32] in oats, Abdulrahaman *et al.* (2015)[33] in lady's finger and Begum and Alam (2019)[34] in chick-pea.

Divergence within the loci concerning the position, staining intensity and values of molecular weight were observed in the electrophorogram (Table 2 and Figs. 1A–B). Two bands with

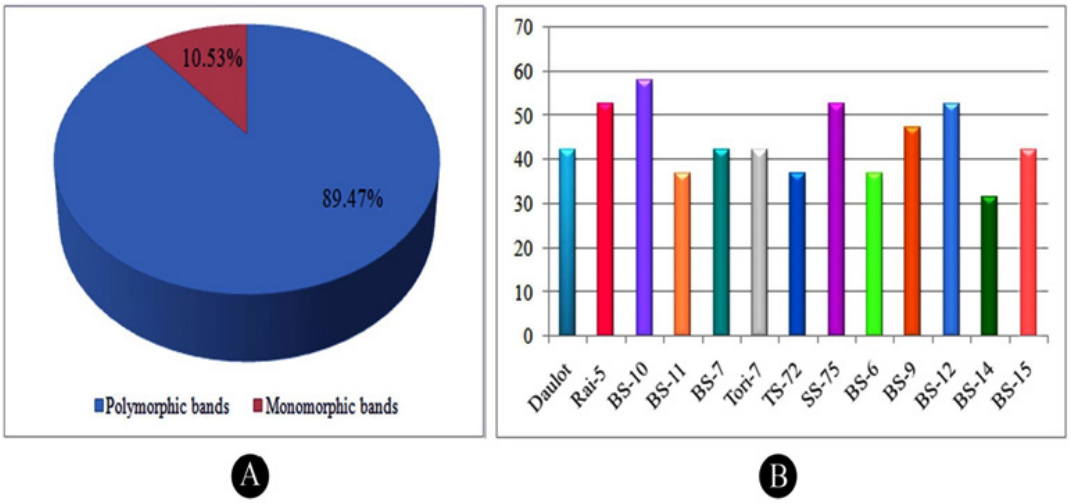

Fig. 2. Percentage of polymorphism (A) total percentage of polymorphic and monomorphic protein bands, (B) percentage of polymorphism obtained from thirteen different variants of *Brassica* L.

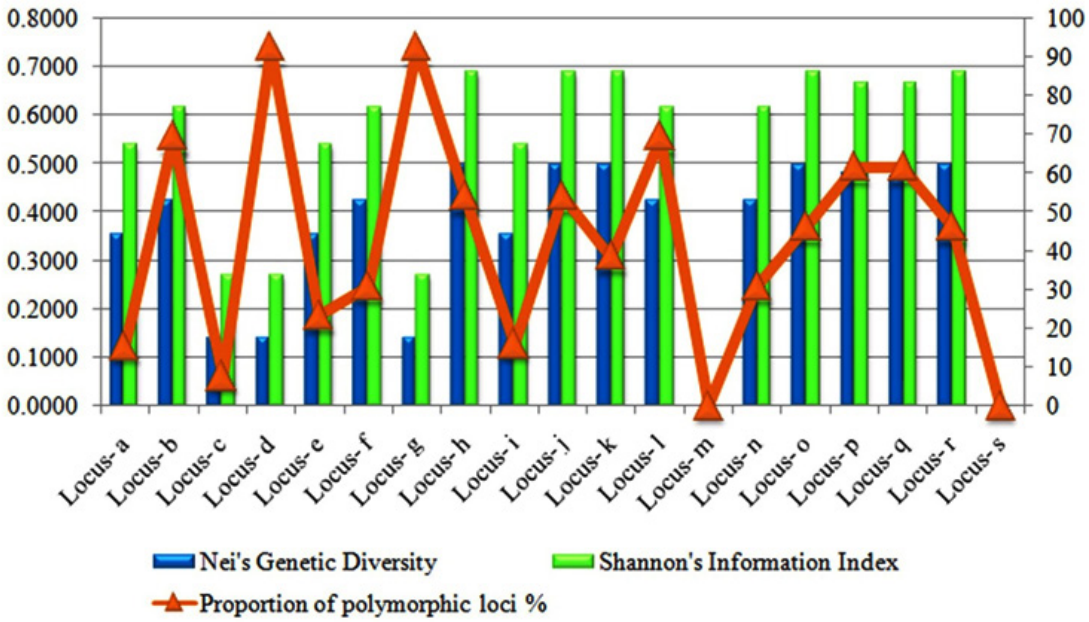

Fig. 3. Estimated genetic diversity (Nei's genetic diversity, Shannon's information index and proportion of polymorphic loci found at different loci) of studied *Brassica* variants

molecular weight 55.72 and 49.25 KDa (one low and another one was high in intensity) were obtained at loci – **f** and **i,** respectively which were found to be present in all the inquired variants of *B*. *rapa* (Tori-7, TS-72, SS-75, BS-6, BS-9, BS-12, BS-14 and BS-15) whereas two consecutive bands of very low intensity with molecular weight of 30.45 and 27.89 KDa at loci – **p** and **q** were spotted out from the variants of *B*. *juncea* and *B*. *napus* (Daulot, Rai-5, BS-10, BS-11 and BS-7). The presence of bands with particular molecular weight at definite locus within particular species made us to imply that the species *B*. *rapa* exhibited species specific bands at loci **f** and **i** of molecular weight 55.72 and 49.25 KDa. Concurrently, *B*. *juncea* and *B*. *napus* displayed species specificity for molecular weight 30.45 and 27.89 KDa at **p** and **q** loci, discretely. Thereupon, in the light of diversity regarding to the position, intensity and values of molecular weight for each of the locus can be aided as feasible tool for proper distinguishing of species within the observed variants of *Brassica* by the electrophoresis of the total soluble protein from leaves.

In our present inquest, scrutiny of soluble protein banding pattern from leaves of thirteen tested variants of *Brassica* L. by PAGE technique presented three distinct different profiles and could be an excellent genre of biochemical fingerprint for discerning different variants of *Brassica*. The presence of a negative (-ve) unique band at locus- **c** in Rai-5 and a positive(+ve) unique band in each of the variety of BS-10 and Daulot at locus – **d** and **g** singly, could be esteemed as fingerprints for discerning these respective variants (Figs. 1A–B and Table 2).

Inter varietal locus variation can be deemed of to be a mainspring for estimation of different degree of genetic divergence within diverse species, where non - appearances of a few protein polypeptides in few variants express variation and consequently taken into considered

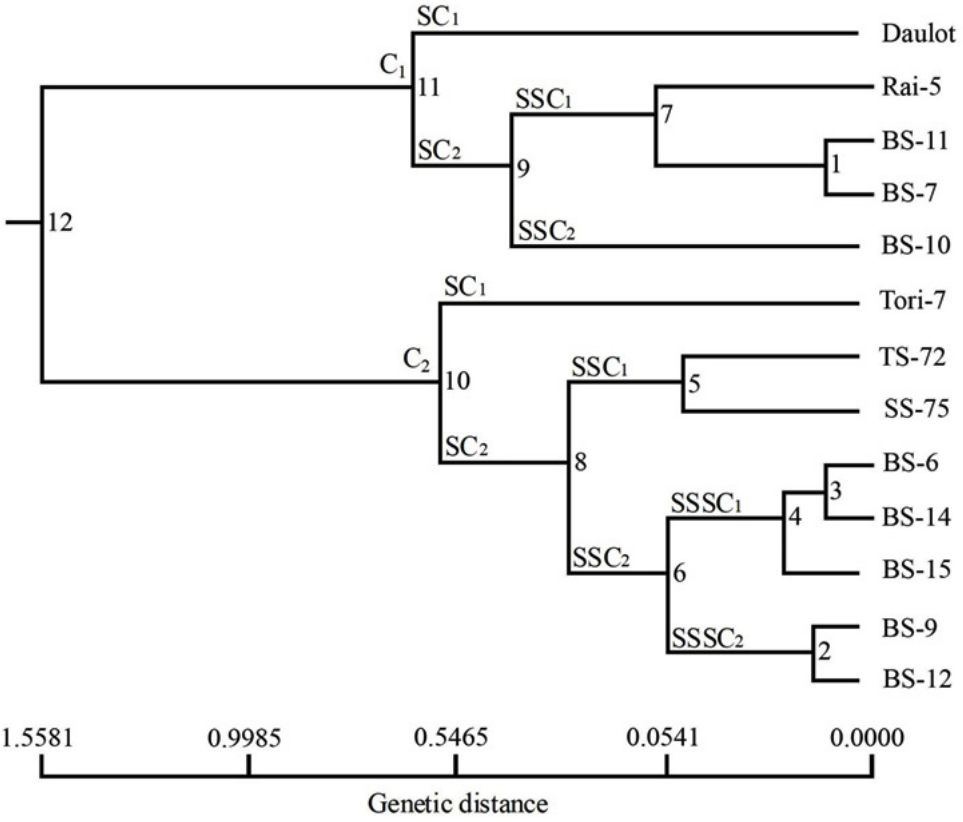

Fig. 4. Dendrogram of thirteen *Brassica* variants produced by UPGMA clustering method based on Nei's (1972) genetic distance

as polymorphic loci. Throughout the course of the prevailing look at, inter varietal variation of loci among the thirteen variants of *Brassica* L. were also disclosed and presented in Table 3. Out of add up to nineteen loci; the loci – **m** and **s** are vitally monomorphic because of the prevalence of 100% protein bands. The remaining loci of the entire electrophorogram exhibited variation within themselves. The highest amount of variation was observed from loci – **d** and **g** with 92.31% of variability and 0.08 genetic disagreement, whereas the lowest amount of divergence was noticed from locus – **c** with 7.69% variability that existing with a high value of genetic disagreement (0.92) (Table 3). Besides, the showing up of different level of variableness in the loci of the studied variants like 15.38% (at loci – **a** and **i**), 23.08% (at locus – **e**), 30.77% (at loci – **f** and **n**), 38.46% (at locus – **k**), 46.15% (at loci – **o** and **r**), 53.85% (at loci – **h** and **j**), 61.54% (at loci – **p** and **q**) and 69.23% (at loci – **b** and **l**) along with respective genetic disagreement of 0.85, 0.77, 0.69, 0.62, 0.54, 0.46, 0.38 and 0.31 made it pondered to have worth mentionable genetic diversity among the quested varieties of *Brassica* L.

The proficiency of molecular marker approaches rely on the level of polymorphism within a group of variants inquired. During the study of PAGE, a complete of nineteen bands of polypeptide were amplified of that seventeen (89.47%) were found to be polymorphic and the remaining two bands (10.53%) were monomorphic in nature (Fig. 2A). An average of 44.13% of polymorphism was observed from the tested variants of *Brassica* L. (Table 4). As a consequence of presence of high amount of polymorphism among the variants expressed by the proportion of polymorphic loci (89.47%), it can be suggested that a broad genetic variation may be present among the studied variants of *Brassica* L. From the verdict of current study, the highest amount of polymorphism was documented from BS-10, which was 57.89%. Contrastingly, BS-14 was offered with 31.58% of polymorphism which was found as the lowest value of polymorphism among the examined variants of *Brassica* L. The studied variants of *Brassica* L. exhibited different degree of polymorphism that was displayed in the Table 4 and Fig. 2B. Of the three investigated species of *Brassica* L., 42.11% and 42.76% of average polymorphism was revealed from *B. napus* and *B. rapa*, respectively whereas *B. juncea* was observed with 47.37% of average polymorphism (Table 4).

As stated through Majumder *et al.* (2012)[35] assessment of genetic diversity aided with protein markers have been ascertained as a sterling tool in characterization of many crops species at gene level. The values of Nei's (1973)[19] gene diversity and Shannon's information index (Lewontin, 1972)[20] for the inquired *Brassica* variants across all the loci are provided in Table 5 and Fig. 3. The estimation of Nei's (1973)[19] gene diversity for all the variants was 0.3488 ± 0.1726 and Shannon's information index was 0.5098 ± 0.2299. Estimation of Nei's (1973)[19] gene diversity (0.3488) and Shannon's information index (0.5098) across all loci (Table 5) also assisted with the subsistence of high level of genetic variation in all studied materials of *Brassica* L.

The only disclosure on PAGE for leaf stored protein of *Brassica* L. was reported by Akbar *et al.* (2020)[11], in which an average of 20.64% polymorphism was documented from three different varieties of the species – *B. rapa*, *B. juncea* and *B. napus*. Hence it can be suggested that more number of varieties and species are required to assess the degree of polymorphism of *Brassica* L. The determination of our existing perusal has been then assimilated with the findings of Mukhlesur and Hirata (2004)[14], where the cultivars of *B. rapa*, *B. juncea*, *B. napus*, *B. carinata*, *B. oleracea* and hexaploid *Brassica* from various geographical origins were examined for leaf protein analysis by SDS-PAGE and the consequence found incongruous with the outcome of ours one as no significant difference was observed within the cultivars even between different species by them. The possible reasons for such type of inconsistency may be due to – (i) difference in morphology, ploidy level and constituents of genome, (ii) for different geographical distribution of the respective variants, (iii) difference in cultural practices, (iv) difference in methodological approaches.

Mentionable amount of studies have been performed early by many workers concerning the degree of polymorphism for total seed storage protein of *Brassica* as well as other species of cereal, pulses and oilseed crops with SDS-PAGE technique throughout the world. 21.2% of polymorphism was recorded within the varieties

of *B. campestris* imitated by 6.3% in *B. napus* and 3.2% in *B. juncea* after evaluating varied varieties of distinctive *Brassica* species (Mukhlesur and Hirata, 2004)[14]. Ibrahim *et al.* (2017)[17] apprised 58% of polymorphism from 53 genotypes of Indian mustard (*B. juncea* L.) germplasm. 94.44% polymorphism was reported from the diverse genotypes of *Eruca sativa* by Shinwari *et al.* (2013)[36]. From six cultivars of Egyptian soybean, a total of 30.43% polymorphism was assayed by Rayan and Osman (2019)[37], 63.2% polymorphism was conveyed by Hlozáková *et al.* (2016)[38] in four European cultivars of common wheat, in hundred-five accessions of Pakistani sesame 70% polymorphism was debriefed by Akbar *et al.* (2008)[39], 82.00% of polymorphism have been evaluated by Vivodík *et al.* (2018)[40] from fifty-six genotypes of Tunisian castor bean. Bhargav *et al.* (2016)[41] assessed 91% of polymorphism from twenty Indian local genotype of common bean.

Knowledge on genetic similarity (distance) between germplasm and among individuals or populations is beneficial in an exceedingly breeding application since it lets incorporation of germplasm and offers greater effective sampling of germplasm to go for the improvement of populations. In the current research, the dendrogram constructed from the UPGMA analysis and coefficients of distance matrix unconcealed great connections between a numbers of variants (Fig. 4 and Table 6). By taking into account the banding pattern of leaf protein, genetic distance matrix for all the thirteen quested samples of *Brassica* variants were determined concurring to Nei's genetic distance (1972)[21] (Table 6). Genetic variation among the variants typically screen via the way of means of genetic distance matrix. In our existing disclosure, the values of pair-wise comparison of Nei's (1972)[21] genetic distance among thirteen *Brassica* variants ranged from 0.0541 to 1.5581 (Table 6 and Fig. 4). The highest genetic distance was observed between BS-10 and BS-14 (1.5581) among the variants (Table 6) that indubitably demonstrated the presences of greater genetic distance between these two populations, notably the previous one representing the variant of *B. juncea* whilst the last mentioned one represented the variant of *B. rapa*. Likewise, pair wise genetic distance with relatively high values was detected between BS-10 and BS-6 (1.3350), BS-10 and BS-15 (1.1527), BS-10 and TS-72 (0.9985), BS-10 and BS-9 (0.9985), BS-7 and SS-75 (0.9985), Rai-5 and SS-75 (0.9985), Rai-5 and BS-14 (0.9985). Contrastingly, the lowest genetic distance was found between BS-9 and BS-12 (0.0541), both were variants from *B. rapa* (Table 6). The difference between the highest (1.5581) and the lowest value of genetic distance (0.0541) revealed the wide range of genetic variability persisting among the thirteen rapeseed-mustard variants. High genetic distance values between variants pair may be found because of difference in hereditary constituents. Taking account the genetic distance values, the findings revealed that variants were genetically distinctive from each other and which may well be utilized in breeding program to achieve potential hereditary picks up.

Based on Nei's (1972)[21] genetic distance obtained from protein banding pattern, a dendrogram was drawn up employing UPGMA in which the thirteen variants of *Brassica* Figure: 4 were differentiated into two main clusters or groups C1 and C2 (Fig. 4). The first cluster C1 consisted of five variants of *Brassica* of which four variants from *B. juncea* (Daulot, Rai-5, BS-10 and BS-11) and one variant from *B. napus*, BS-7 were present. The explanation for two species had a place to same cluster is probable that the nearly introduced genotype might be shared some genes from the AA genomic base of other variants which have been utilized in this consider. It might also possibly that the alleles of CC genome which had original gene base of the species near to that of the other two elemental species. The cluster or group C1 was divided into two sub-clusters. Daulot was found to be present in sub-cluster I (SC1) of cluster C1, and sub-cluster II (SC2) was further divided into two sub-sub cluster where sub-sub cluster II (SSC2) was found with only BS-10. Concurrently, sub-sub cluster I (SSC1) was consisted of three closely related variants of Rai-5 with BS-11 and BS-7. On the other hand, the major cluster C2 included eight variants of *B. rapa* (Tori-7, TS-72, SS-75, BS-6, BS-9, BS-12, BS-14 and BS-15) and divided into two sub-clusters (SC1) and (SC2). The sub-cluster (SC1) of cluster C2 was presented with Tori-7 alone; however, the second sub-clusters (SC2) included the rest of the variants of *B. rapa*. The second sub-clusters (SC2) further segregated into two sub-sub clusters; SSC1 and SSC2. In sub-sub cluster I (SSC1), there was TS-72 and SS-75

whereas sub-sub cluster II (SSC2), was further divided in two sub-sub-sub clusters. Sub-sub-sub cluster I was present with three closely related variants of BS-6, BS-14 and BS-15 whereas, in sub-sub-sub cluster II there was BS-9 and BS-12 with minimal genetic distance of 0.0541. It was observed that the five variants of *B. rapa* found in sub-sub-sub cluster II showed low genetic distances among them ranging from 0.0541–0.2364. In this way, there was a limpid clustering pattern of geographically closer variants within the present ponder showing that the affiliation between genetic relatedness and geological distance has significance.

Presently, after the comparison between the come out of physio-morphological and agronomic traits and genetic distance matrix (Table 1 and Fig. 4) of the inquired variants of *Brassica*, it was revealed that in the first cluster C1, Daulot, Rai-5, BS-10, BS-11 and BS-7 had almost the similar seed color (reddish brown to black seed) and other agronomic characteristics (such as- tolerance to biotic and abiotic stresses of environment). Similarly, in the major cluster C2 – the morphological traits which include seed coloring were alike in BS-6, BS-14 and BS-15 (yellow seeded variants), whereas in BS-9 and BS-12 (reddish brown seeded variants). It was also observed that SS-75 and TS-72 grouped in a distant pair based on agronomic aspects (susceptibility to pest and environmental factors) and deviated from black seeded variety Tori-7 via cluster analysis (Fig. 4 and Table 1). The report of Saha *et al.* (2008)[42] on genetic assessment of four *Brassica* species through RAPD marker found more or less congruent with our current disclosure that yellow seeded *Brassica* variants could be separated from the brown seeded variants by cluster analysis. Therefore, it looks through that cluster analysis would play a noteworthy implement in ascertaining genetic diversity regarding the diverse physio-morphological characters and other agronomic traits of plant species.

CONCLUSION

Genetic variation alludes to the differences within the constitutions of heredity in an individual of a species and it is imperative in keeping up the developmental steadiness and biological latent of plant species. More genetic variability within variants and noteworthy differentiation between variants states plenty of genetic resources of a species. The outcome of our existing quest exhibited differences in the position, number and staining intensity of protein bands among the studied variants which manifests the application of PAGE for differentiation of inquired variants of *Brassica* L. High level of polymorphisms (89.47%) along with wide range of genetic distance (0.0541–1.5581) was viewed from the thirteen variants of *Brassica*. Broad range of polymorphism and genetic distance brought to light the presence of wide variability within *Brassica* spp. The variants of BS-10 and BS-14 contain the highest genetic variation, whereas BS-9 and BS-12 contain the lowest genetic variation among the variants employed in this analysis. Variants having near vicinity in their origin, morphological traits and stratagem of breeding are possibly to have less genetic distance from each other. Hence, the results of this inquisition put forward for consideration that the variants of BS-10, BS-9, BS-12 and BS-14 could furnish the amenities for selection as parental source in coming breeding program to ameliorate *Brassica* variants in Bangladesh. Howsoever, it is far recommended that exceeding molecular information is required to own better evaluation of genetic variability of *Brassica* germplasm in Bangladesh as well as currently launched varieties/ lines and therefore more efficacious utilization of existing variability for advancement of *Brassica* crop in Bangladesh.

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Conflict of interest

The authors pertaining to this research work declared no conflict of interest.

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REFERENCES

1. Kasem W. T., Ghareeb A., Marwa E. Seed morphology and seed coat sculpturing of 32 taxa of family Brassicaceae. *J. Am. Sci.* 2011; 7(2): 166–178.
2. Gomez – Campo C. Studies on Cruciferae V. Chromosome numbers for twenty-five taxa. *Anales del Instituto Botanica A. J. Cavanilles*. 1980; **35**: 177–182.
3. Nagahara U. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *Jpn. J. Bot.* 1935; **7**: 389–452.
4. Hedge I. C.: A systematic and geographical survey of old world Cruciferae. In: *The biology and chemistry of the Cruciferae* (Vaughan JG, MacLeod AJ, Jones BMG, eds). New York: Academic Press. 1976; pp 1–46.
5. Yarnell S. H. Cytogenetics of the vegetable crops. II. Crucifers. *Bot. Rev.* 1956; **22**: 81–166.
6. Thiyam U., Kuhlmann A., Stockmann H., Schwarz K. Prospects of rapeseed oil by-products with respect to antioxidative potential. *C. R. Chim.* 2004; **7**(6): 611–616.
7. Cover C. M., Hsieh S. J., Tran S. H., Hallden G., Kim G. S., Bjeldanes L. F., Firestone G. L. Indole-3-carbonyl inhibits the expression of cyclin-dependent kinase-6 and induces a G1-cell cycle arrest of human breast cancer cells independent of estrogen receptor signaling. *J. Biol. Chem.* 1998; **273**(7): 3838–3847.
8. Staub R. E., Feng C., Onisko B., Bailey G. S., Firestone G. L., Bjeldanes, L. F. Fate of indole-3-carbinol in cultured human breast tumor cells. *Chem. Res. Toxicol.* 2002; **15**(2): 101–109.
9. Zhang J., Yuan Q., Meng Y., Li X., Nan Z., Wang Y., Zhang W. A genetic diversity analysis of wild *Lespedeza* populations based on morphological characters, allozyme and RAPD methods. *Plant Breed*. 2007; **126**: 89–94.
10. Ghodsvali A., Khodaparast M. H. H., Vosoughi M., Diosady L. L. Preparation of canola protein materials using membrane technology and evaluation of meals functional properties. *Food Res. Int.* 2005; **38**(2): 223–231.
11. Akbar F., Paul M., Begum K. N. Karyological and genetic diversity study using molecular marker among three species of oilseed *Brassica* L. *Ind. J. Pure App. Biosci.* 2020; **8**(4): 267–281.
12. O'Farrell P. H. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 1975; **250**(10): 4007–4021.
13. Turi N. A., Farhatullah, Rabbani M. A., Khan N. U., Akmal M., Pervaiz Z. H., Aslam, M. U. Study of total seed storage protein in indigenous *Brassica* species based on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). *Afr. J. Biotechnol.* 2010; **9**(45): 7595–7602.
14. Mukhlesur R. Md., Hirata Y. Genetic diversity in *Brassica* species using SDS-PAGE analysis. *J. Biol. Sci.* 2004; **4**(2): 234–238.
15. Sadia M., Malik S. A., Rabbani M. A., Pearce S. R. Electrophoretic characterization and the relationship between some *Brassica* Species. *Electron. J. Biol.* 2009; **5**(1): 1–4.
16. Zada M., Zakir N., Rabbani Z., Shinwari Z. K. Assessment of genetic variation in Ethopian mustard (*Brassica carinata* A. Braun) germplasm using multivariate techniques. *Pak. J. Bot.* 2013; **45**: 583–593.
17. Ibrahim M. I., Abbasi F. M., Rabbani M. A., Inamullah, Jan S. A., Ilyas M., Khurshid H. Evaluation of genetic variation among Indian Mustard (*Brassica juncea* L.) genotypes by SDS-PAGE method. *Proc. Pakistan Acad. Sci. (B. Life and Environmental Sciences)* 2017; **54**(4): 333–339.
18. Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951; **193**(1): 265–275.
19. Nei M. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci.* 1973; **70**(12): 3321–3323.
20. Lewontin, R. C.: The apportionment of human diversity. In: *Evolutionary Biology* (Dobzhansky T, Hecht MK, Steere WC, eds). Heidelberg: Springer. 1972; pp 381–398.
21. Nei M. Genetic distance between populations. *Am. Nat.* 1972; **106**(949): 283–292.
22. Yeh F. C., Yang R. C., Boyle T. B. J., Ye Z. H., Mao J. X. POPGENE, the user-friendly software for population genetic analysis. *Mol. Biol. Biotechnol. Cent*. 1999; University of Alberta, Canada.
23. Ladizinsky G., Hymowitz D. Seed protein electrophoresis in taxonomic and evolutionary studies. *Theor. Appl. Genet.* 1976; **54**: 145–151.
24. Geetha V. V., Balamurugan P. SDS PAGE electrophoresis in mustard cultivars. *Int. J. Agric. Res.* 2011; **6**(5): 437–443.
25. Kakaei M., Kahrizi D. Study of seed proteins pattern of *Brassica napus* varieties via sodium dodecyl sulfate polyacrylamid gel electrophoresis. *Int. Res. J. Biotechnol.* 2011; **2**(1): 26–28.
26. Odeigah P. G. C., Oboh B., Aghalokpe I. O. The characterization of Nigerian varieties of pepper, *Capsicum annuum* and *Capsicum frutescens* by SDS-polyacrylamide gel electrophoresis of seed

proteins. *Genet. Resour. Crop Evol.* 1999; **46**: 127–131.

27. Devi S. G. Varietal identification through electrophoresis in sunflower (*Helianthus annus* L.) 2000; Master's thesis, Tamil Nadu Agricultural University, Coimbatore.
28. Varma V. L. K., Reddy N. M., Keshavulu K., Ankaiah R. Characterization of maize (*Zea mays* L.) genotypes through seed protein electrophoresis (SDS-PAGE). *Crop. Res*. 2005; **30**: 124–127.
29. Vijayan R. Organic seed production in rice cv. ADT 43. 2005; Doctoral dissertation, Tamil Nadu Agricultural University, Coimbatore.
30. Paul R., Datta A. K. Seed protein profiles in celery (*Apium graveolens* L.) and Ajowan (*Trachyospermum amni* L.) plant types. *Int. J. Plant. Sci.* 2006; **1**(1): 36–38.
31. Nisha C. Assessment of seed quality in wheat *(Triticum aestivum L.)* varieties as influenced by different alien rust resistant genes under varying production conditions. 2007; Doctoral dissertation, Tamil Nadu Agricultural University, Coimbatore.
32. Sumathi S. Varietal identification in oats (*Avena sativa* L.). 2007; Master's thesis, Tamil Nadu Agricultural University, Coimbatore.
33. Abdulrahaman A. A, Kolawole O. S., Onile O. G., Oladele F. Seed electrophorentic characterization and taxonomic implications of some accessions of *Abelmoschus esculentus* L. (Moench) in Nigeria. *Jewel J. Sci. Res.* 2015; **3**(1): 136–145.
34. Begum K. N., Alam Sk. S. Genetic diversity in nine chick-pea (*Cicer arietinum* L.) varieties based on different molecular markers. *Bangladesh J. Bot.* 2019; **48**(1): 195–203.
35. Majumder D. A. N., Hassan L., Rahim M. A., Kabir M. A. Analysis of genetic diversity in mango (*Mangifera indica* L.) using isozymetic polymorphism. *Afr. J. Biotechnol.* 2012; **11**(87): 15310–15323.
36. Shinwari S., Akbar F., Rabbani M. A., Mumtaz A. S., Shinwari Z. K. Evaluation of genetic diversity in different genotypes of *Eruca sativa* from Pakistan by SDS-PGE analysis. *Pak. J. Bot.* 2013; **45**(4): 1235–1240.
37. Rayan W. A., Osman S. A. (2019). Phylogenetic relationships of some Egyptian soybean cultivars (*Glycine max* L.) using SCoT marker and protein pattern. *Bull. Natl. Res. Cent.* 2019; **43**(161).
38. Hlozáková T. K., Gregová E., Vivodík M., Gálová, Z. Genetic diversity of European cultivars of common wheat (*Triticum aestivum* L.) based on RAPD and protein markers. *J. Cent. Eur. Agric.* 2016; **17**(4): 957–969.
39. Akbar F., Yousaf N., Rabbani M. A., Shinwari Z. K., Masood M. S. Study of total seed proteins pattern of sesame (*Sesamum indicum* L.) Landraces via sodium dodecyl sulfate polyacrylamide gel elecrophoresis (SDS-PAGE). *Pak. J. Bot.* 2008; **44**(6): 2009–2014.
40. Vivodík M., Saadaoui E., Balážová Ž., Gálová Z., Petrovièová L. Characterization of Tunisian castor bean genotypes using SDS-PAGE of total seed storage proteins. *Potr. S. J. F. Sci.* 2018; **12**(1): 701–706.
41. Bhargav S., Rai G. K., Mallick S. A., Rai S. K., Kumar R. R., Singh M., Parveen A., Jamwal D., Saikia S. L., Bagati S. Assessment of genetic diversity in local genotypes of common bean (*Phaseolus vulgaris*) using biochemical markers. *The Bioscan* 2016; **11**(3): 1955–1961.
42. Saha S., Molla Md. R., Chandra D., Rahman, L. Assessment of genetic variation and relationships within the varieties of four *Brassica* species by RAPD markers. *Aust. J. Crop Sci.* 2008; **2**(3): 105–114.