

Karyological and Genetic Diversity Study Using Molecular Marker among Three Species of Oilseed *Brassica* L.

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ABSTRACT

Brassica campestris var. *Agrani*, *B. juncea* var. *BARI Sharisha-16 (BS-16)* and *B. napus* var. *BARI Sharisha-13 (BS-13)* are the three different oilseed crops belonging to the genus *Brassica*, have been investigated to amass genetic information through cytogenetic characterization by orcein staining and to unveil phylogenetic relationships among these three species by karyomorphological analyses with respect to their asymmetry indices. *B. campestris* var. *Agrani*, *B. juncea* var. *BS-16* and *B. napus* var. *BS-13* were found to possess different somatic chromosome number along with varied karyotype formulae, $2n=20=20m$, $2n=36=30m+6sm$ and $2n=38=34m+4sm$, respectively. The investigation also aimed at exploring the genetic divergence and selection of improved variety among the three *Brassica* species by protein banding pattern obtained from polyacrylamide gel electrophoresis (PAGE). According to Nei's genetic distance analysis, the highest genetic distance found between *B. juncea* var. *BS-16* was 0.4700. Contrastingly, the lowest genetic distance found between *B. campestris* var. *Agrani* and *B. napus* var. *BS-13* was 0.2877. *B. juncea* var. *BS-16* was found at distant position in the phylogenetic tree and may displayed diverse nature from other two species of *Brassica* L. The values of different karyomorphological indices also presaged that *B. juncea* var. *BS-16* exhibited some degree of asymmetry and may express with relatively advanced nature than the other two studied species in respect of evolutionary perspective. Therefore, this study regarding detailed karyomorphological data and determination of genetic relationship would be beneficial to the breeders of *Brassica* for proper characterization, designing upcoming breeding programs and conservation purposes of plant genetic resources of *Brassica* variants.

Keywords: Karyotype, Asymmetry, PAGE, *Brassica* L.

INTRODUCTION

Brassicaceae is one of the vast families of angiosperm comprising of 338 genera and

3709 species and distributed all across the world, mostly in temperate areas of the Northern Hemisphere (Kasem et al., 2011).

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Among these genera, the genus *Brassica* L. includes around 37 economically important species worldwide, along a broad range of inherited and morphological diversities (Rakow, 2004). The genus - *Brassica* is significant for producing more important agronomical and horticultural crops such as oilseed, vegetables, remedy, flavoring materials and plenty of end products utilized by humans than other genus (Akbar & Begum, 2020). In Bangladesh, the Brassicaceae family is mentioned with 12 genera and 24 species (Ahmed et al., 2008).

Identification of chromosome and karyotype construction is a stand out amongst the most vital objectives in cytogenetic investigations. Karyotyping, a helpful tool in cytogenetic examinations, which uncovers the number and attributes of chromosomes and can be utilized to illustrate the origin, ploidy and phylogenetic relationship among plants (Xiong et al., 2011; Eroğlu et al., 2013). The karyotype and its different component characteristics are utilized as a measure to decode karyoevolutionary patterns (Lavania & Srivastava, 1992). Therefore, comparative analysis of karyotype of related species has been used classically to delimitate patterns and the way of evolution of chromosomes within plant groups and to deduce the evolutionary mechanism of chromosomal changes in evolution of plants (Kamel et al., 2014).

For appropriate conservation and management of particular species, it is necessary to characterize with genetic makeup. When extensive genetic diversity and the data of these genetic resources are accessible, genetic improvement of crops can be accelerated. Molecular marker analysis helps in deciding parental forms for population mapping, marker aided choices and schemes of back cross and as a consequence different molecular markers are utilized to carry out diversity studies that help the breeders to ameliorate crop species (Begum & Alam, 2019; Paul et al., 2020). Storage proteins been employed as biochemical markers in various field such as study of genetic diversity between and within accessions, establishment

of genome relationships and as an implement for improvement of crop (Ahmed, 2012). Characterization based on proteins and choice of alluring genotypes is extraordinary significant for mustard-rapeseed breeders. Hence the electrophoresis of storage protein of *Brassica* species is considered as a feasible method to investigate genetic variation and to alling plant varieties (Turi et al., 2010).

A number of studies have been accomplished by various researchers from different parts of the world on the molecular characterization and evaluation of genetic diversity of *Brassica* germplasm with seed storage protein through SDS-PAGE (Rabbani et al., 2001; Rahman & Hirata, 2004; Sadia et al., 2009; Turi et al., 2010; Toosi et al., 2011; Choudhary et al., 2015). But no study has yet been found on the assessment of genetic divergence and molecular characterization of *Brassica* L. with only PAGE technique. In this present study, our investigation was put through with the assistance of PAGE technique from stored protein of leaf to assess genetic diversity and relationship of *Brassica* L. Olin-Faith and Heneen (1992), Olin-Faith (1994; 1996) and Hasterok and Maluszynska (2000) studied on the identification and basic cytogenetical characterization of *B. campestris* and *B. napus*, while *B. juncea* was reported by Mukherjee (1975), Kulak et al. (2002), Ma et al. (2019) and Sun et al. (2019) where all of them performed different methodological approaches with differential staining techniques. On the contrary, in our current study- cytogenetical analysis has been carried out by orcein staining with detailed karyomorphological characterization of three species of *Brassica* L. Reports of comprehensive karyological study along with the study of molecular characterization and genetic diversity through biochemical marker (PAGE) on available variants of *Brassica* L. hasn't been found from Bangladesh yet.

Though *Brassica* L. are financially imperative oilseed crop everywhere throughout the world, numerous studies have been inaugurated globally with a view to characterize and extend distinctive chemical

constitute by developing new variants with optimized bio-productivity. In these sorts of research the information on hereditary constituents is absolutely vital. Thereupon, to attain the complete success over the on-going studies of molecular genetics, the traditional cytogenetical investigations are nonetheless required due to the fact that they offer significant corroborations regarding numerical and structural attributes of chromosome complements in the light of karyotype construction. No advanced cytogenetical research has so far been led to characterize every species in Bangladesh, hence in the present research work, three oilseed crops of *Brassica* L. (*B. campestris* var. Agrani, *B. juncea* var. BS-16 and *B. napus* var. BS-13) were analyzed cytogenetically for proper identification and characterization with karyomorphological data to explain the structural alteration of karyotype within chromosome sets and at the same time to determine the possible phylogenetic relationship, genetic divergence and selection of improved variety among the three species within the genus *Brassica* L. through Polyacrylamide Gel Electrophoresis.

MATERIALS AND METHODS

Plant materials

Three oilseed crops of *Brassica* L. namely, *B. campestris* var. Agrani, *B. juncea* var. BS-16 and *B. napus* var. BS-13 were studied in this experiment. Among the three *Brassica* species, the seeds of *B. campestris* var. Agrani were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. On the other hand, the seeds of *B. juncea* var. BS-16 and *B. napus* var. BS-13 were collected from the Oilseeds Research Center (ORC) of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. These three species were planted in earthen pots in the net house and maintained in the Botanical garden of Jagannath University; whereas cytogenetical analysis and the analysis of genetic diversity from leaf storage protein by polyacrylamide gel electrophoresis was carried out in the

Cytogenetics Laboratory of department of Botany, Jagannath University, Dhaka, Bangladesh.

Cytogenetical Analysis

The seeds of studied species were germinated on filter paper moistened with distilled water on Petri dishes. Healthy roots of 31.5-32 hours old germinating seeds were collected and pre-treated for 45-50 minutes at room temperature with 8-hydroxyquinoline (0.002%). Afterwards, fixation was done in 45% acetic acid for 15 minutes at 4 °C, washed with distilled water for 3-4 times and preserved in 70% alcohol at 4 °C for further use. The pre-treated roots were then hydrolyzed for 8-10 minutes in 1N HCl at 65 °C. The root tips were squashed and stained in 1% aceto-orcein for 5-6 hours. Then these slides were observed, the best metaphase plates were photographed (100x) with a digital camera (model - 8 mega pixels Canon power shoot PC1564, 7.0X magnification, auto mode mounted on Nikon eclipse 100 microscope).

Chromosome counts were conducted on metaphase plates with well spread chromosomes. At least five metaphase plates were chosen for getting ready the karyotype of each species. Karyotypes were arranged according to the total length of homologous pair of diploid chromosomes. Karyotype formula for every species was determined from the number of somatic chromosomes as well as the centromeric position of respective chromosomes. Proposal of Levan et al. (1964) was followed for the nomenclature of chromosomes. The ideograms were prepared depend on the size of haploid chromosomes in descending manner. Individual and total haploid chromosome lengths were also determined. “Microsoft Excel” and the computer based programs “KaryoType_Win_2” (Altınordu et al., 2016), have been both used to prepare ideogram and to evaluate various karyological parameters.

Various karyomorphological parameters, indices of symmetry and asymmetry were performed to enunciate the degree of asymmetry of karyotype with such as coefficient of variation of centromeric index

and chromosome length (CVCI and CVCL) (Paszko, 2006), the total form percent (TF%) (Huziwara, 1962), the index of karyotype symmetry and chromosomal size resemblance (Syi% and Rec%) (Greilhuber and Speta, 1976), mean centromeric asymmetry (MCA) (Peruzzi and Eroğlu, 2013), the Karyotype asymmetry index (AsK%) (Arano, 1963), the intra and inter chromosomal asymmetry index (A₁ and A₂) (Zarco, 1986), the degree of asymmetry of karyotype (A) (Watanabe et al., 1999), the asymmetry index (AI) (Paszko, 2006) and Stebbins's classification (Stebbins, 1971).

Molecular Identification

Isolation and Estimation of Leaf Protein

Crude protein of three studied *Brassica* species were extracted using fresh and young leaves collected from about one month old seedlings following the modified method of Paul et al. (2020). For diverging the spores of microorganisms and other dirt, young and fresh leaves were rinsed with distilled water and ethanol, respectively and kept on clean filter paper for few minutes in order to soak the excess ethanol from the surface of leaves. 2 gm of fresh leaves of each sample was grinded with the help of ice-cold mortar pestle and the unrefined homogenates were then centrifuged at 4 °C with 13000 rpm for 15 minutes. After centrifugation of the samples, the protein recovered as clear supernatant on the top of the eppendorf tube. Then the supernatant was preserved at -20 °C as samples for the vertical polyacrylamide gel electrophoresis until its use.

To estimate the concentration of leaf storage protein from the three studied species of *Brassica*, method of Lowry et al. (1951) was followed. Absorbance of each sample *Brassica* species were obtained with the help of spectrophotometer (model – SV 1600) by 660 nm wavelength.

Polyacrylamide Gel Electrophoresis (PAGE)

Vertical polyacrylamide gel electrophoresis was performed for three protein samples following the method of Majumder et al. (2012) with little modification. Protein

samples were directly analyzed by polyacrylamide gel electrophoresis using 10.0% polyacrylamide as separating gel and 4.0% as stacking gel. The gels were prepared as follows:

Preparation of separating gel:

2.5 ml of 1.5 M Tris-HCl buffer stock solution (pH 8.8), 4 ml of 30% polyacrylamide stock solution (2.9 g acrylamide and 0.1 g bis-acrylamide), 100 µl of freshly prepared 10% APS (Ammonium per sulfate), 3.4 ml of deionised doubled distilled water and 5 µl of TEMED were mixed gently within a falcon tube. The solution was then immediately poured with a micro- pipette into the gel casting plate to a height of 7 cm and left unimpeded for about 15-20 minutes at room temperature for polymerization.

Preparation of stacking gel:

After the polymerization of separating gel in the gel casting plate, stacking gel was prepared by adding 500 µl of 1.0 M Tris-HCl (pH 8.0), 2.78 ml of deionised doubled distilled water, 40 ml of 10% APS (freshly prepared), 3 µl of TEMED in a falcon tube and mixed with 670 µl of 30% polyacrylamide stock solution. The solution was then shaken mildly, and carefully poured over the separating gel. Just then, a comb was placed on the stacking gel solution and waited for few minutes at room temperature until the stacking gel settled down completely.

Loading dye preparation and sample loading:

Loading dye was prepared from a solution of 0.5 M Tris-HCl (pH 6.8), glycerol and deionised doubled distilled water in a volumetric ratio of 1: 1: 3 with a pinch of Bromo Phenol Blue (BPB), and mixed all of them thoroughly with the help of vortex machine. 25 µl of each protein sample along with 20 µl BPB loading dye were loaded into the wells of the stacking gel.

Gel Electrophoresis:

When loading of the protein samples in the wells was done, the process of electrophoresis was performed by using TETRAD 10-PS MINI PACK vertical electrophoresis unit with

multiSUB MINI Pack constant power supply unit. The total process of electrophoresis was carried out by setting the voltage at 90 V and current at 120 amp constantly in 10X diluted Tris-glycine running buffer solution (1 TrisHCl : 5 glycine in volume ratios), pH 8.3. When the BPB dye front reached to 2 mm above to the lower end of the gel, the supply of current was then switched off. Each of the gel run took 3-3.5 hours to complete.

Staining and destaining:

Immediately after the completion of electrophoresis, the gel was get off from the gel casting plate and then stained with 0.25% Coomassie Brilliant Blue (CBB) R-250 in acetic acid : methanol : distilled water (1 : 4 : 5 volume ratios) for 20 minutes and destained in acetic acid : methanol : distilled water (1 : 4 : 5 volume ratios) for 3 hours.

Photographs:

After destaining, the gel was placed on a glass plate carefully without creating any air bubbles and the glass plate was set over a thick white paper. Then photographs of the gel were taken by a digital camera (18 mega pixels Canon EOS 700 D model).

Data analysis

Amount of protein bands observed from the gel was scored. Scoring of bands was performed on the basis of the presence (1) and absence (0) of protein bands and all the monomorphic and polymorphic bands that visible to the eyes were scored. Banding patterns were scored for each studied species from at least three gel runs. From the presence and absence of bands, genetic distance matrix (Nei, 1972) was computed to estimate genetic relationship among the studied three *Brassica* species using UPGMA. Computer based program “Popgene 32” (Version 1.32) (Yeh et al., 1999) was used for the construction of the phylogenetic tree.

RESULTS AND DISCUSSION

Ample karyological observations for the three species of *Brassica* L., viz. *B. campestris* var. Agrani, *B. juncea* var. BS-16 and *B. napus* var. BS-13 were carried out in the present investigation.

Cytogenetical Analysis

Somatic chromosome numbers

An analytical observations of the three species of *Brassica* L. were made from karyomorphological features and found to possess different mitotic complements comprising of $2n=20$ in *Brassica campestris* var. Agrani, $2n=36$ in *B. juncea* var. BS-16, $2n=38$ in *B. napus* var. BS-13, respectively (Figure 1a-c, Table 1). The current report concerning $2n$ chromosome number of the three species of *Brassica* L. found consistent with the findings of antecedent researchers which are given in Table 2, and made us implied that the somatic chromosome number is a stable character for the studied species of the genus *Brassica*.

Karyological Analysis

Karyotypic formulae of three studied species of *Brassica* L. were observed diverse. *B. campestris* var. Agrani was found to have a karyotypic formula with $20m$, in *B. juncea* var. BS-16, it was $30m+6sm$ and *B. napus* var. BS-13 was observed with a karyotype formula of $34m+4sm$. Previously, Hasterok and Maluszynska (2000) reported $2n=20$ in *B. campestris* having centromeric formula $10m(SAT)+6sm+4st$ ($st=$ subterminal), of which a secondary constriction was observed on 10 no. chromosome along with a prominent satellite on the short arm. $30m+6sm$ centromeric formula was reported from *B. juncea* by Ma et al. (2019), which was found identical with our present disclosures. Sun et al. (2018) reported *B. napus* $2n=38$ with karyotypic formula $28m+10sm(2SAT)$, two satellites were discerned at the thirteen no. pair of chromosomes. Furthermore, Skarzhinskaya et al. (1998) also delineated *B. napus* with karyotype formula $2n=38=20m+10sm+8st(2SAT)$. The disparity in the karyotypic formula made us envisaged that the modification or evolution of karyotype in these species as a result of having diverse topographical and natural variables and allude them as particular lineage. In accordance with the present investigation, there was predominance of metacentric chromosomes in the karyotypes of the three species of *Brassica*

L. (Figure 1d-f, Table 1). The karyotype of *B. campestris* var. Agrani was consisted of all metacentric chromosomes ($2n=20m$), whereas 3 pairs of sub - metacentric and 15 pairs of metacentric chromosomes ($2n=30m+6sm$) were observed in *B. juncea* var. BS-16 and *B. napus* var. BS-13 was found with 4 sub - metacentric chromosomes and 34 metacentric chromosomes ($2n=34m+4sm$). Pericentric inversion, deletion, unequal translocation, dysploidy, spontaneous mutation, interspecific hybridization and so on may set about the differences in karyotypes. A significant and well established aspect in the evolutionary history of *B. juncea* and *B. napus* is - both of them are the product of natural interspecific hybridization. Interspecific gene flow may result in chromosomal evolution by rearrangement of the chromosomes. This hybridization has been proposed as an exigent invasive and stimulus, and might even contribute in the evolution of adaptations that become worthwhile towards the success of *Brassica* as oilseed crops and improve productivity.

B. campestris var. Agrani, *B. juncea* var. BS-16 and *B. napus* var. BS-13 had different total diploid chromosomal length of $46.79\pm 0.62\ \mu\text{m}$, $122.89\pm 2.05\ \mu\text{m}$ and $103.38\pm 1.06\ \mu\text{m}$, respectively (Table 1). The average chromosomal length was found higher in *B. juncea* var. BS-16 ($3.41\ \mu\text{m}$) while *B. campestris* var. Agrani and *B. napus* var. BS-13 were found with more or less close average chromosomal length of $2.34\ \mu\text{m}$ and $2.72\ \mu\text{m}$, respectively (Table 1). Percentage of relative length of individual chromosome for the studied species were 3.68–6.37 (*B. campestris* var. Agrani), 1.54–3.94 (*B. juncea* var. BS-16) and 1.28–4.18 (*B. napus* var. BS-13) and the range of individual chromosomal length was from $1.72\text{--}2.98\ \mu\text{m}$ for *B. campestris* var. Agrani, $1.89\text{--}4.84\ \mu\text{m}$ and $1.32\text{--}4.32\ \mu\text{m}$ for *B. juncea* var. BS-16 and *B. napus* var. BS-13, respectively (Table 1). The total length of haploid chromosome complement was documented as $23.43\pm 0.29\ \mu\text{m}$, $61.49\pm 1.04\ \mu\text{m}$ and $51.73\pm 0.55\ \mu\text{m}$ for *B. campestris* var. Agrani, *B. juncea* var. BS-16 and *B. napus* var.

BS-13, consecutively (Table 1). Range of haploid chromosome complements were recorded $1.76\text{--}2.94\ \mu\text{m}$ from *B. campestris* var. Agrani with no gradual decrease. Contrariwise, the range of haploid chromosome complements in *B. juncea* var. BS-16 and *B. napus* var. BS-13 were $1.98\text{--}4.78\ \mu\text{m}$ and $1.39\text{--}4.20\ \mu\text{m}$, respectively (Table 1) which presents a limpid view of gradational diminution of haploid chromosomal length. According to the statement of Stebbins (1971) concerning to the category of karyotype that based on chromosome percentage with an arm ratio within chromosome complement and the proportion of longest and shortest chromosome, the karyotype categories of the three species were belonged to 2B except that of *B. campestris* var. Agrani, which was 1A (Table 3).

Karyosystematics is considered to be one of the worth mentioning inference for defining the genetic relationship and differentiation between species or population (Guerra, 2008). Asymmetry in karyotype can be deemed as the main impetus behind speciation whereas symmetrical karyotypes are primeval in evolution (Stebbins, 1971). The CVCI and CVCL indexes are considered as reliable metrics to grasp the variations and purvey information on the heterogeneity of chromosomes. In our study the values of CVCI and CVCL include a wide variation, ranging from 0.64 to 13.81 for CVCI and 16.87–27.53 for CVCL (Table 3). Lower chromosomal heterogeneity was observed in *B. campestris* var. Agrani, with CVCI and CVCL values of 0.64 and 16.87, respectively (Table 3). In the meanwhile, *B. juncea* var. BS-16 had found with moderately higher CVCI and CVCL values of 13.81 and 22.93 which express relatively higher chromosomal heterogeneity (Table 3). As stated through Zarco (1986) and Peruzzi et al. (2009), bi-dimensional scattered plots is considered as one of the key strategies to serve karyotype asymmetry inter relationships among species, where the two asymmetry indexes are positioned on the horizontal (X) and vertical (Y) axis and point of intersection represents as each sample species. In our

experiment, CVCI and AI are ascertained as two preferable indices to exhibit the correlation for assuming asymmetry of karyotype. Higher AI and CVCI values represent asymmetric karyotype whereas lower AI and CVCI values show primeval nature of karyotype. A scattered diagram of the degree of karyotype asymmetry of three *Brassica* species was made with the coefficient of variation of centromeric index (CVCI) as the longitudinal coordinate and the karyotype asymmetry (AI) as the transverse coordinate, which was demonstrated in Figure 2a. *B. juncea* var. BS-16 was located in the upper right corner, indicating that it was the most evolved whereas *B. campestris* var. Agrani was located at the bottom left of *B. juncea* var. BS-16, which alludes it as relatively primitive in nature. Mean centromeric asymmetry (MCA) also envisaged as a useful matrix to determine karyotype asymmetry of species. A scattered diagram between MCA (X axis) and CVCI (Y axis) expressed karyotype symmetry in *B. campestris* var. Agrani as it depicted with lowest values of MCA (1.72) and CVCI (0.64) (Table 3) and present at the lower bottom position of the diagram (Figure 2b), at the meantime *B. juncea* var. BS-16 displayed karyotype asymmetry with higher MCA (8.76) and CVCI (13.81) values (Table 3) and located at the upper top position of the scattered diagram (Figure 2b). The other karyomorphological indexes, viz. TF% serves a statistical significant inverse correlation with AsK%, A₁, A₂, AI and A; contrariwise it possessed a high positive correlation with the Syi% index (Table 3). Regarding TF% and AsK% values (Table 3) the most symmetrical karyotype was found in *B. campestris* var. Agrani with higher TF% (49.13%) value and lower AsK% (50.87%) among the three species and the most asymmetrical one was found in *B. juncea* var. BS-16 with lower TF% (44.71%) and higher AsK% (55.29%) values. By taking into account in relation to karyotypic nature, symmetric karyotype is well conceived of as primitive and asymmetric karyotype offered with advance characters (Stebbins 1971). Based on the above all observations it can be inferred that *B.*

juncea var. BS-16 was relatively advanced in characters than the remaining two studied species of *Brassica* L. in evolutionary point of view.

Molecular Analysis

A total of 20 protein bands were found to be generated consequent upon the destaining of the gel, of which six bands of protein were observed in *B. juncea* var. BS-16 and seven protein bands were sighted in both *B. campestris* var. Agrani and *B. napus* var. BS-13, (Figure 3a-b, Table 4). Of these protein bands appeared at eight different loci designated as A, B, C, D, E, F, G and H, respectively (Figure 3a-b). From the verdict of existing study 14.29% polymorphism was documented from both *Brassica campestris* var. Agrani and *B. napus* var. BS-13 and *B. juncea* var. BS-16 was recorded with 33.33% polymorphism (Table 4). 20.64% average amount of polymorphism was found from the three species of *Brassica* L. (Table 4), which endorsed the presence of low genetic variability as a result of low polymorphism among the studied species of *Brassica* L. The finding of our current study is further strengthened by the early report of Rahman and Hirata (2004), they concluded that 21.2% of polymorphism was recorded in the varieties of *B. campestris* followed by 6.3% in *B. napus* and 3.2% in *B. juncea* after appraising various varieties of different *Brassica* species. Rabbani et al. (2001) also acquiesced to the presence of narrow genetics distance in Indian mustard (*B. juncea*) having different geographic origin. Turi et al. (2010) were also reported similar observations of minimum genetic diversity in 234 accessions of locally collected *Brassica* species for total seed storage protein content. However, Sadia et al. (2009) revealed the existence of extensive genetic diversity in *Brassica* germplasm collection with regard to their total seed protein profiles.

In our current study, 20.64% of polymorphism has been found from three species of *Brassica* by conducting polyacrylamide gel electrophoresis (Table 4). Besides, a variant level of polymorphisms have also been debriefed by many other researchers in diverse plant species employing

gel electrophoresis technique such as – in Egyptian soybean 30.43% (Rayan & Osman, 2019), in European cultivars of common wheat 63.2% (Hložáková et al., 2016), in broad bean 65.12% (El-Nahas et al., 2015), in Tunisian castor bean 82.00% (Vivodík et al., 2018) of polymorphism have been reported.

Having knowledge on genetic similarity (distance) between germplasm and among individuals or populations is advantageous to a breeding program as it allows germplasm organization and provides more efficacious sampling of germplasm to be crossed for the population development. Considering protein banding pattern for all the three *Brassica* species, genetic distance matrix was determined according to Nei (1972). Genetic variance among the three species of *Brassica* ranged in between 0.2877 and 0.4700 (Figure 4). In the present inquest, the lowest genetic distance was found between *B. campestris* var. Agrani and *B. napus* var. BS-13 (0.2877) (Figure 4). On the other hand, the highest genetic distance came into view from *B. juncea* var. BS-16 which was 0.4700 (Figure 4). In the study, a dendrogram prepared from UPGMA analysis and distance matrix coefficients displayed desirable relationships among the species studied (Figure 4).

Dendrogram developed by using UPGMA analysis was distributed among three species of *Brassica* (Figure 4). Based on protein banding pattern, two clusters were visualised in the dendrogram (Figure 4). *Brassica juncea* var. BS-16 (also called Indian

mustard) formed a separate cluster, which was totally different from other two species of *Brassica* L. *B. campestris* var. Agrani and *B. napus* var. BS-13 clustered in another cluster. The cluster result indicated that *B. campestris* var. Agrani and *B. napus* var. BS-13 were closely related whereas *B. juncea* BS-16 distantly related with the remaining two species of *Brassica* L. The result of cluster analysis suggested that *Brassica juncea* var. BS-16 may have high genetic diversity among the three studied *Brassica* species. Regarding inter specific variation, it also supposed the presence of variation among the three species of *Brassica* L. So it seems to presage that polyacrylamide gel electrophoresis technique may utilize to be a feasible tool in supporting classical taxonomy studies.

Finally the in all view of clustering, pertaining to the protein banding pattern found congruent with our current study based on cytogenetical analysis which also implied that *B. juncea* var. BS-16 was genetically diverse from the other two studied species in this investigation. Thus, *B. juncea* var. BS-16 can be presumed as a vital resource of genetic diversity to be conserved and utilized in oncoming programmes of breeding to flourish the plant attributes and yield of *Brassica* varieties in Bangladesh. Furthermore, it is suggested that the inclusion of more variants may put in superfluous tools would throw more light on better understanding of biosystematics relationships within the genus *Brassica*.

Table 1: Karyological features of the studied *Brassica* L. species

Taxa	2n	DCL (µm)		ACL (µm)	TDCL (µm)	HCL (µm)		THCL (µm)	Arm ratio		RL (%)		KF
		Min	Max			Min	Max		Min	Max	Min	Max	
<i>B. campestris</i> var. Agrani	20	1.72	2.98	2.34	46.79 ± 0.62	1.76	2.94	23.43 ± 0.29	1.02	1.06	3.68	6.37	20m
<i>B. juncea</i> var. BS-16	36	1.89	4.84	3.41	122.89 ± 2.05	1.98	4.78	61.49 ± 1.04	1.01	2.86	1.54	3.94	30m + 6sm
<i>B. napus</i> var. BS-13	38	1.32	4.32	2.72	103.38 ± 1.06	1.39	4.20	51.73 ± 0.55	1.02	2.19	1.28	4.18	34m + 4sm

[Abbreviations: 2n= somatic chromosome number, DCL= Diploid Chromosomal Length, ACL= Average Chromosomal Length, TDCL=Total Diploid Chromosomal Length, HCL= Haploid Chromosomal Length, THCL= Total Haploid

Chromosomal Length, RL= Relative Length, KF= Karyotype formula, m= metacentric chromosome, sm= sub-metacentric chromosome]

Table 2: Previous chromosome number reports on the three species of *Brassica* L.

Species	Chromosome number (2n)	Authors
<i>B. campestris</i>	20	Viinikka and Sovero, 1988; Olin-Faith and Heneen, 1992; Olin-Faith, 1994, 1996; Cheng et al., 1995; Fukui et al., 1998; Hasterok and Maluszynska, 2000; Snowdon, 2007
<i>B. juncea</i>	36	Mukherjee, 1975; Du et al., 1993; Kulak et al., 2002; Fang et al., 2014; Liu et al., 2019; Ma et al., 2019; Sun et al., 2019
<i>B. napus</i>	38	Olin-Faith and Heneen, 1992; Olin-Faith, 1994, 1996; Skarzhinskaya et al., 1998; Hasterok and Maluszynska, 2000; Kulak et al., 2002; Snowdon, 2007; Sun et al., 2018

Table 3: Values of asymmetry indices of the three studied species of *Brassica* L.

Taxa	CVCI	CVCL	MCA	AsK%	TF%	Syi%	Rec%	A1	A2	A	AI	Sc
<i>B. campestris</i> var. Agrani	0.64	16.87	1.72	50.87	49.13	96.60	78.51	0.03	0.17	0.02	0.11	1A
<i>B. juncea</i> var. BS-16	13.81	22.93	8.76	55.29	44.71	80.88	70.53	0.14	0.23	0.09	3.17	2B
<i>B. napus</i> var. BS-13	10.40	27.53	6.55	54.02	45.98	85.10	62.98	0.11	0.21	0.07	2.86	2B

[**Abbreviations:** CVCI= Coefficient of variation of centromeric index, CVCL= Coefficient of variation of chromosome length, MCA= Mean centromeric asymmetry, AsK%= Karyotype asymmetry index (%), TF%= Total form percent (%), Syi%= Index of karyotype symmetry (%), Rec%= Index of chromosomal size resemblance (%), A1= Intra chromosomal asymmetry index, A2= Inter chromosomal asymmetry index, A= Degree of asymmetry of karyotype, AI= Asymmetry index and Sc= Stebbins's classification]

Table 4: Levels of polymorphism within *Brassica* L. species

Taxa	No. of total bands	No. of polymorphic bands	% of polymorphism exists in each <i>Brassica</i> species	Average % of polymorphism exists in <i>Brassica</i> species
<i>B. campestris</i> var. Agrani	7	1	14.29	
<i>B. juncea</i> var. BS-16	6	2	33.33	20.64
<i>B. napus</i> var. BS-13	7	1	14.29	

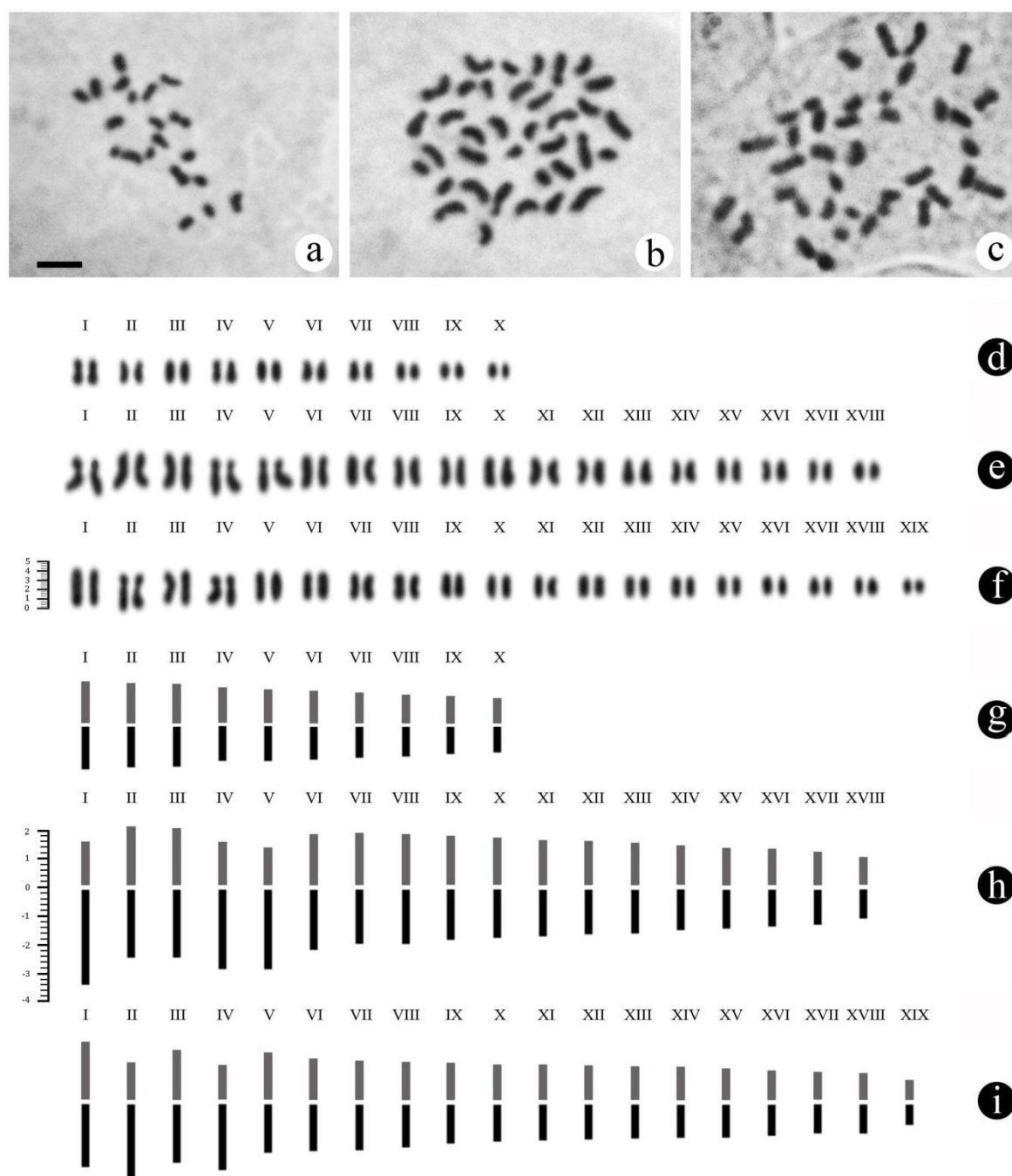


Fig. 1: Mitotic metaphase chromosomes (a-c), karyotype (d-f) and haploid ideogram (g-i) prepared from orcein-stained mitotic metaphase chromosomes of three species of *Brassica* L. (a, d and g) *B. campestris* var. Agrani, (b, e and h) *B. juncea* var. BS-16 and (c, f and i) *B. napus* var. BS-13, Scale bar = 5 μm for metaphase chromosomes and karyotype and scale bar = 6 μm for haploid ideogram.

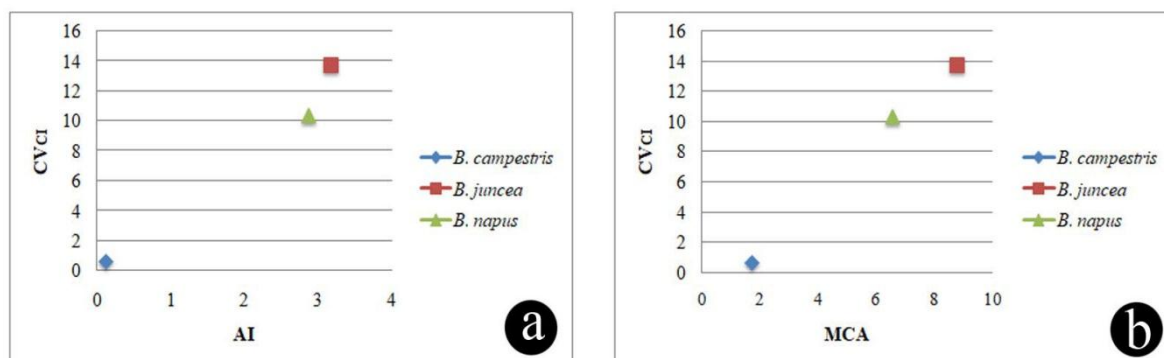


Fig. 2: A scattered diagram presenting karyotype asymmetry inter relationship among the three species of *Brassica* L. (a) AI versus CV_{ci} parameter and (b) MCA versus CV_{ci} parameter (CV_{ci} = Coefficient of variation of centromeric index, AI= Asymmetry index, MCA= Mean centromeric asymmetry).

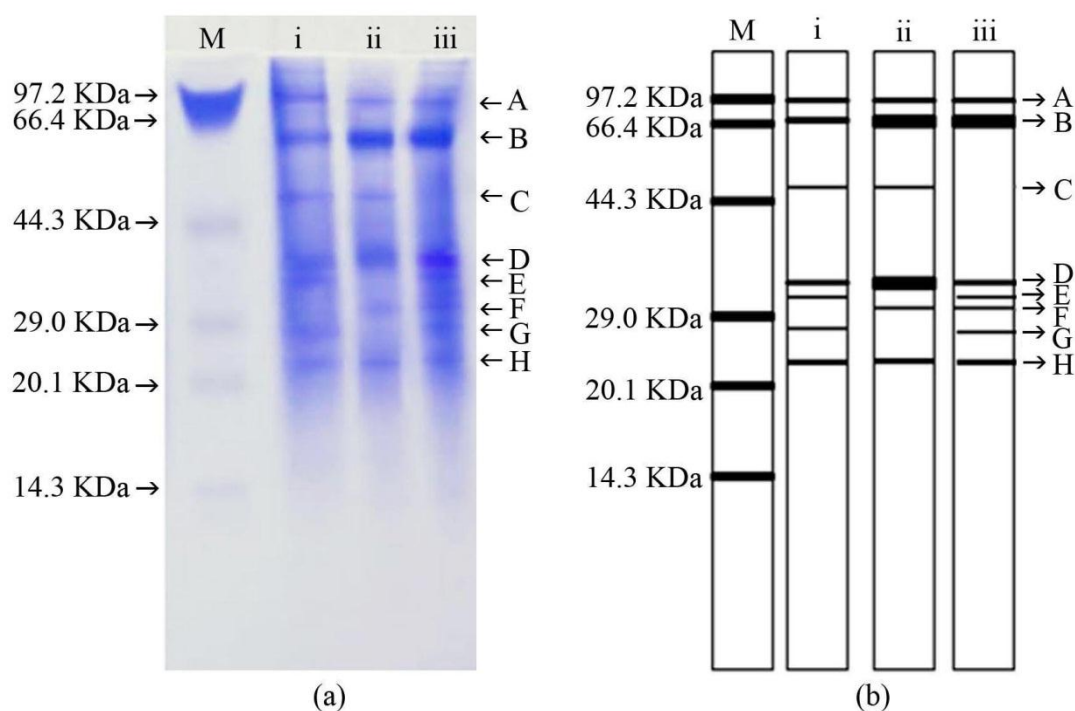


Fig. 3: Banding pattern of leaf storage protein (a) stained with Coomassie Brilliant Blue (CBB; R-250) and (b) diagrammatic representation of protein bands on polyacrylamide gel. (Lane M) - Molecular weight protein marker, (Lane i) - *B. campestris* var. Agrani, (Lane ii) - *B. juncea* var. BS-16 and (Lane iii) - *B. napus* var. BS-13 (arrows indicate different loci produced during polyacrylamide gel electrophoresis), KDa- Kilo Dalton.

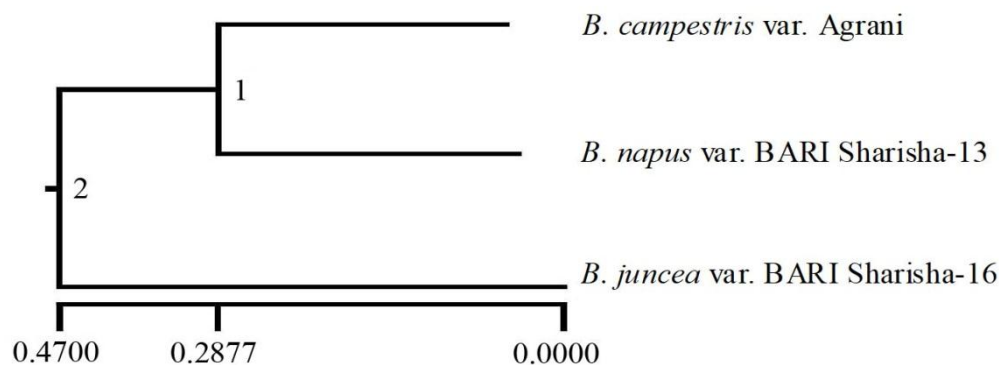


Fig. 4: UPGMA constructed based on Nei's genetic distance summarizing the data on differentiation among *Brassica* L. by Polyacrylamide Gel Electrophoresis (PAGE) analysis.

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