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Research Article

Comparative study of morphology and heterochromatin distribution in three oilseed species of *Brassica* L.

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ABSTRACT

Morphological traits and nature of heterochromatins in interphase nuclei and prophase chromosomes of three oilseed species of *Brassica* L. (*B. campestris*, *B. juncea*, and *B. napus*) have been investigated in this study for morpho-cytotaxonomical evaluation. The three *Brassica* species displayed morphologically differences in the case of plant heights, size and the shape of upper and basal leaves, types of inflorescence and color of seeds, etc. After analyzing the staining properties of heterochromatins (nature and amount) in the interphase nuclei, it was unveiled that *B. juncea* had a simple chromocenter type of interphase nuclei and might be perceived as the most evolved one among the three species of *Brassica*. Moreover, the phylogenetic relationship derived from the mean values of morphological traits also manifested the distinct position of *B. juncea* than the other two studied species of *Brassica*. Therefore, the findings obtained in this study may be utilized as a feasible implementation of biosystematics for the identification and proper characterization of the three species of *Brassica* L.

Introduction

Brassica L. is one of the most economically significant genera of the family Brassicaceae, comprising 37 different species (Akbar et al., 2020b). *Brassica* originated primarily in temperate regions of the Northern Hemisphere and then dispersed throughout the world (Al-Shehbaz, 1984). Oilseed *Brassica* is generally referred to as rapeseed (*B. campestris* L. and *B. napus* L.) and mustard (*B. juncea* (L.) Czern & Coss.) and positioned the first rank among oilseed crops in Bangladesh in addition to the second biggest oilseed crops within the world after soybean (Joya et al., 2017). In Bangladesh, 12 genera and 24 species representing the family Brassicaceae (Ahmed et al., 2008) and 154 accessions of mustard are available at the Oilseed Research Centre's gene bank of Bangladesh Agricultural Research Institute (Razzaque and Hossain, 2007).

The genus *Brassica* offers economically valuable industrial oilseed, flavor, vegetables, fodder crops and serves as a conducive source of vitamins, minerals, fibers, bioactive compounds, and phytochemical contents (Li et al., 2017). The seeds of mustard and rapeseeds are reported to possess about 42% and 25% oil and protein, respectively (Joya et al., 2017). Moreover, this genus has therapeutic esteem as anti-scurvy, diuretic, and anti-inflammatory for bladder. The mustard has antibacterial and antifungal properties because of being an excellent source of potassium, phenolics, dietary fiber, vitamins A, C and E (Zhang et al., 2006; Luciano and Holley, 2009).

Characterization with morphological features is envisaged as the initial method in classifying and describing germplasm (Smith and Smith, 1989).

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Determinations of genetic diversity of agronomically advanced accessions are beneficial for improving and developing mustard and rapeseed in Bangladesh.

The organization of interphase nuclei in plants is species-specific (Kabir and Singh, 1989). Features of chromatin in interphase nuclei and the pattern of chromosomal condensation throughout prophase are utilized in the study of karyosystematics (Ilnicki, 2014). In this way, the nature and distribution of heterochromatins in interphase nuclei and prophase chromosomes also share valuable information concerning the genome of *Brassica* species.

Several of researchers have screened a large volume of oilseed crops of *Brassica* all around the world, studying morphological, physiological, anatomical, cytogenetical, palynological, biotechnological, and genetic variability studies through different molecular markers before direct introduction or hybridization for a selected program (Mollika et al., 2011; Jan et al., 2017; Akbar and Begum, 2020; Akbar et al., 2020a,b; Saha and Begum, 2020; Singh et al., 2020).

For efficient management and proper manipulation of germplasm, in-depth research on the morphological diversity and the relationships amongst the germplasm collections are deemed necessary. Hence, the current study focuses on investigating the different morphological parameters and characterization of diverse nature and amount of heterochromatins in interphase nuclei and prophase chromosomes to evaluate these characters as preliminary biosystematics tools for identification, proper characterization, and inferring relatedness among the three species of *Brassica* (*B. campestris*, *B. juncea* and *B. napus*) before utilizing them in the breeding program, management and conservation strategies of these species.

Materials and Methods

Plant materials

Three oilseed crops of *Brassica* L. specifically, *B. campestris* var. Agrani, *B. juncea* var. BARI Sharisha-16 and *B. napus* var. BARI Sharisha-13 was analyzed to evaluate them in the present study due to their commercial values and availability in Bangladesh. Among the three *Brassica* species, the seeds of *B. campestris* var. Agrani were collected from the Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh. The seeds of *B. juncea* var. BARI Sharisha-16 and *B. napus* var. BARI Sharisha-13 was collected from the Oilseeds Research Center (ORC) of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, Bangladesh. These three species were planted in the field and earthen pots within the net house and maintained under the natural condition in the Botanical garden belonging to the Department of Botany, Jagannath University, Dhaka, Bangladesh.

Morphological Analysis

The plant specimens were critically studied morphologically at the stage of their maturity and different qualitative traits such as size, shape and color of leaves; types of inflorescence; color, size, shape of the flower and different floral parts; size, shape, the color of the fruits and seeds were recorded. Concurrently, some of the quantitative characters *viz*. plant heights, number of branches per plant, number of leaves per plant, number of pods per plant, length of pods per plant and number of seeds per pod of examined plant materials were also recorded. The method of measurements is mentioned in Table 1. At least 25 observations of the examined plant materials from each species were made to make a table of comparative morphological traits (Table 2) and mean values of six different morphological features (Table 3). A dendrogram displaying phylogenetic relationships among the three examined plant materials was constructed using the morphological parameters mentioned in Table 3 with PAST: Paleontological Statistics Software (version 4.03) following Hammer et al. (2001). A digital camera (Canon EOS 700D model) captured the photographs of plant morphology and presented in Fig. 1.

Cytogenetical Analysis

The seeds of the three *Brassica* species were sprouted on soaked filter paper within Petri-dishes. Healthy roots were pre-treated with 8-hydroxyquinoline (0.002%) for 45-50 min at room temperature, which was then fixed in 45% acetic acid for 15 min at 4 ºC, washed with distilled water for 3-4 times, and finally preserved in 70% alcohol at 4 ºC until use. Afterward, the roots were hydrolyzed in 1 N HCl at 65 ºC for 8-10 min and stained with 1% aceto-orcein for 5-6 h. The preparations of slides were made by applying the squash method. To assess the nature and amount of heterochromatins of interphase nuclei and prophase chromosomes of *Brassica* species, 30 best-prepared interphase nuclei and prophase plates were selected and observed under Optika light microscope 100X magnification, and micrographs were taken through Euromax camera (CMEX 10, DC 10000C) in auto mode. A procedure proposed by Tanaka (1971) was followed for the nomenclature of interphase nuclei and prophase chromosomes. Heterochromatin in the interphase nuclei of the investigated materials was determined by measuring the area of the nucleus and of chromocentres by planimetry.

Results and Discussion

Morpho-taxonomic evaluation

A comparative morphological observation for the three species of *Brassica* L. *viz. B. campestris*, *B. juncea* and *B. napus* are summarized in Table 2 and Fig. 1.

Morphologically the three species of *Brassica* L. showed differences, which would provide a framework and fundamental data for the study of taxonomy and can be used as taxonomically important characters to distinguish them from each other. The height of *B. juncea* was observed as the tallest (1.02–1.25 m) species with much branching above, whereas *B. campestris* was 0.72–0.88 m tall, less branched above or often simple and *B. napus* were found with a comparatively lower height among the three examined species of *Brassica* L., which was 0.50–0.68 m, purple color at the base of the main stem (Table 2).

Table 1. List of different morphological traits and description of their measurement.

Features	Method of measurement
Plant heights	The heights were measured from the base of the mature plant to the tip of the inflorescence by a meter scale.
Number of branches and leaves	The number of branches and leaves per plant was counted.
Leaves size, shape and color	A meter scale measured the size of leaves from base to apex of the leaf, and the shape of leaves was determined as suggested by Ahmed et al. (2008); the color of leaves was determined visually.
Inflorescence	Types of inflorescence were observed, and flowers per inflorescence were counted.
Flower	Size of petal, sepal and different floral parts were measured carefully by a measuring scale, and shape of a petal, sepal, filament, anthers, stigma, etc. were determined following the procedure of Ahmed et al. (2008); the color of the flower and different floral parts were determined visually.
Fruits and number of seeds	Types of fruits were determined using the procedure of Ahmed et al. (2008); a measuring scale measured the size of fruits and their beaks, and the total number of seeds per pod of each species was counted.
Size, shape and color of seeds	Vernire calipers determined the sizes of seeds, and the shape and color of seeds were determined visually.

Table 2. Comparative morphological analysis of three species of *Brassica* L.

Features	*B. campestris*	*B. juncea*	*B. napus*
Plant*	Annual herb, 0.72-0.88 m long, glabrous.	Annual herb, 1.02-1.25 m long, pubescent.	Annual herb, 0.50-0.68 m long, glabrous.
Stem	Stem erect, simple.	Stem erect, much branching above.	Stem erect and purple colored at the base, leaves are present covering the base of the stem.
Leaf size and shape*	Basal leaves are ovate, 7.50-9.00×4.00-5.65 cm, auriculated, petioled. Upper leaves glabrous, sessile, oblong, 6.60-8.00×3.70-5.30 cm, clasping the stem, base amplexicaul, margin entire often repand.	Basal leaves are long petiolate, sparingly pubescent, oblong to lanceolate, 10.60-14.00×5.50-6.85 cm, terminal lobe ovate, repand, 1-3 deep lateral lobes on each side of mid vein, coarsely dentate. Upper leaves petiolate, oblong to oblanceolate, 7.20-9.60×5.30-6.20 cm, base attenuate, margin serrate.	Basal leaves are waxy, orbicular to oval, 6.90-8.70×5.00-6.20 cm, sometimes lobed at the base, broadly crenate, petiolate, glabrous, or sparsely pubescent. Upper leaves sessile, oblong to lanceolate, up to 7.00-9.50×5.00-6.20 cm, slightly cordate at the base, clasping the stem partially, more or less entire, glaucose.
Leaf color	Deep bluish green.	Deep green.	Pale green.
Inflorescence*	Short raceme, 15-25 flowered.	Elongated raceme, 20-35 flowered.	Flowers arranged on elongated corymbose-raceme, 20-40 flowered.
Flower	Cruciform, deep yellow colored, 8-10 mm.	Cruciform, bright yellow colored, 9-12 mm.	Cruciform, pale-bright yellow colored, 12-15 mm.
Petals	4 petals, clawed, narrowly obovate, 8.00-10.00×2.5-3.5 mm, apex emarginated.	4 petals, 9.00-11.50×3.00-5.00 mm, clawed, obovate, apex emarginated.	4 petals, clawed, broadly obovate, apex rounded, 10.00-15.00×5.00-7.50 mm.
Sepal	4 sepals, oblong, 3.00-5.00×1.50-2.00 mm.	4 sepals, oblong, 5.50-7.00×2.00-2.50 mm.	4 sepals, 5.00-7.20×1.50-2.50 mm, oblong.
Androecium	Stamens 6, tetradynamous. Filament 5.00-6.50 mm, anther oblong, dithecous basifixed 1.00-1.20 mm.	Stamens 6, tetradynamous. Filament 5.00-7.50 mm, anther dithecous, oblong, basifixed 1.10-1.50 mm.	Stamens 6, tetradynamous. Filament 6.00-8.00 mm, anther oblong, dithecous basifixed 1.70-2.50 mm.
Gynoecium	Style 1.50-2.00 mm, stigma capitates.	Style 1.50-2.50 mm, stigma capitates.	Style 1.50-2.50 mm, stigma capitates.
Fruit	Siliqua, bi-valved, 3.30-4.70×0.30-0.60 cm with 0.90-1.60 cm beak. 10-15 seeded.	Siliqua, bi-valved, 3.00-3.90×0.35-0.60 cm with 0.70-1.20 cm beak. 11-13 seeded.	Siliqua, bi-valved, 3.80-5.20×0.35-0.60 cm with 0.75-1.00 cm beak. 14-20 seeded.
Seed color, shape, and size*	Yellow colored seed, oblong to globose in shape, 1.00-1.50 mm in diameter.	Reddish brown to brown colored seed, globose in shape, 1.30-1.70 mm in diameter.	Brownish black to black colored seed, bold, globose in shape, 1.50-2.20 mm in diameter.

*Important Taxonomic Features

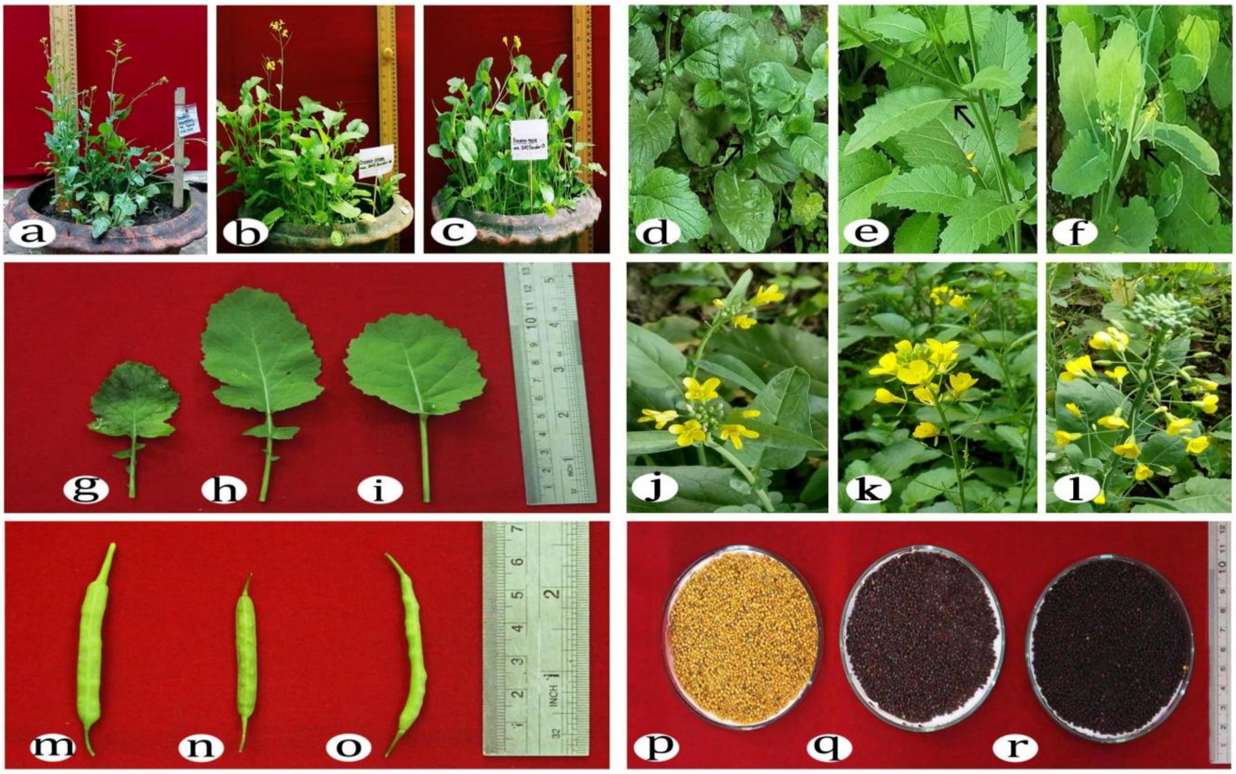

Fig. 1. Comparative morphological traits- plant habit, upper leaves, basal leaves, inflorescence, fruits and seeds (1a–r) of three species of *Brassica* L. (1a, 1d, 1g, 1j, 1m and 1p) *B. campestris*; (1b, 1e, 1h, 1k, 1n and 1q) *B. juncea* and (1c, 1f, 1i, 1l, 1o and 1r) *B. napus* (arrow indicates the arrangement of upper leaf on the stem).

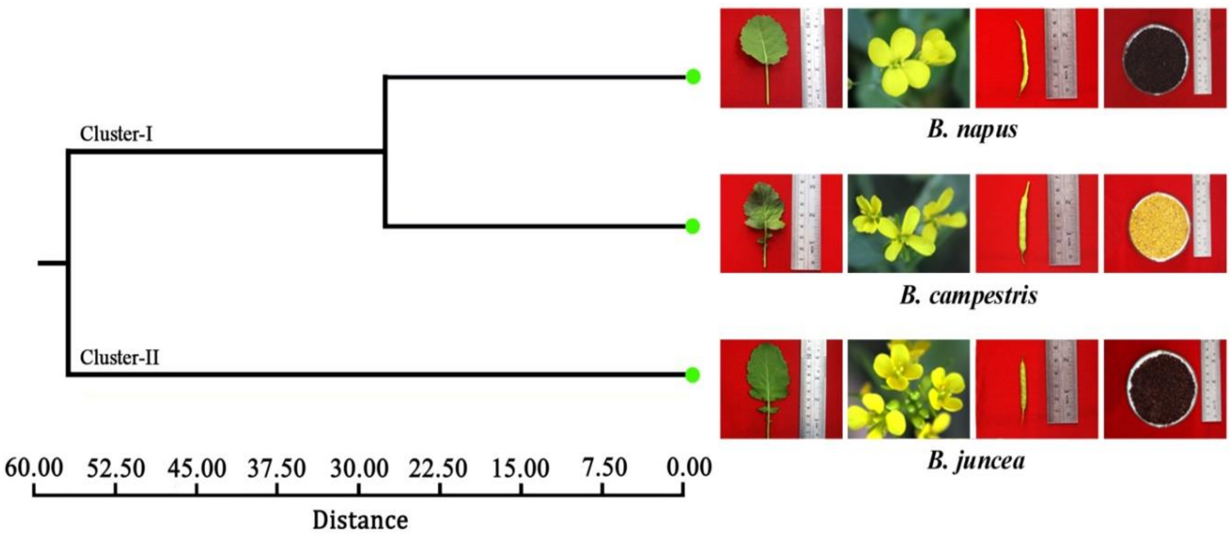

Fig. 2. Dendrogram presenting the phylogenetic relationship among three species of *Brassica* L. based on the mean values of six different morphological features of plant: (a) plant height; (b) the number of branches per plant; (c) the number of leaves per plant; (d) the number of pods per plant; (e) length of pods per plant and (f) the number of seeds per pod.

Leaf characters such as the size and shape of upper and basal leaves, arrangement of upper leaves on the stem of the three species were employed in distinguishing the three investigated species of *Brassica* L. Leaves of *B. napus* were waxy with glabrous underside but no layer of wax was observed on the underside of the leaves of *B. campestris* and *B. juncea*, respectively. The basal leaves size of *B. juncea* was larger than *B. campestris* and *B. napus* (Fig. 1g–i and Table 2). Basal leaves were petiolate, glabrous (*B. campestris*, *B. napus*) or sparsely pubescent (*B. juncea*), ovate (*B. campestris*), orbicular to oval (*B. napus*) or oblong or lanceolate in shape with 1–3 deep lobes at the base in *B. juncea* (Figs. 1g–i and Table 2). The upper leaves of *B. campestris* and *B. napus* were sessile, oblong to lanceolate, base amplexicaul, margin undulate, slightly lobed at the base, clasping the stem partially (*B. napus*) or completely (*B. campestris*), which was found similar with the report of Gulden et al. (2008). On the other hand, the upper leaves of *B. juncea* were found long petiolate, oblong to oblanceolate, base crenate-attenuate with serrate margin. Types and position of inflorescence also provided much information in the differentiation of the three inquired species of *Brassica* L. Inflorescence of elongated raceme and elongated corymbose-receme were noticed in the main and axillary branches of *B. juncea* and on the main branch of *B. napus* respectively, with open flowers appeared below the flower buds (Figs. 1k–l), but an alternate pattern of flowering was observed in *B. campestris*, where flowers bloomed above the flower buds and inflorescence was short raceme on the main branch (Fig. 1j and Table 2). This characteristic feature was also found to correspond with the report of Gulden et al. (2008). Morphology of flowers in the studied species was almost similar except for the slight differences observed in the size of flowers and various floral parts (Table 2). The size and shape of pods, the size, shape, and color of seeds (Figs. 1m–r and Table 2) also presented distinct differences in the three species, which may help to differentiate them into different species. The fruits of *B. juncea* were comparatively dwarf with flattened cylindrical siliqua (Fig. 1n), siliqua of *B. campestris* were long, cylindrical and fleshy, with long beak (Fig. 1); on the contrary, *B. napus* were observed with comparatively longer cylindrical silique with slight constrictions at regular intervals (Fig. 1o). Oblong to minutely globose, yellow-colored seeds were observed in *B. campestris* (Fig. 1p), whereas globose, reddish-brown to brown seeds in *B. juncea* and bold, globose, brownish-black to black seeds were observed in *B. napus* (Figs. 1q–r, Table 2).

According to Khalid et al. (2010), the scientific classification of plants still relies upon morphological traits. However, this technique might seem like a low-grade but effective taxonomic tool. It could be applied for the preliminary categorization of the inquired species before their characterization with more specific marker technologies.

Assessment of phylogenetic relationship with morphological features

The mean value of six diverse morphological features of the three examined *Brassica* L. are documented in Table 3. A dendrogram was prepared from these mean values and presented in Fig. 2, which exhibited phylogenetic relationship among the investigated *Brassica* species.

Dendrogram reveals two clusters. Of the two clusters, *B. campestris* and *B. napus* were closely related (cluster-I) with more or less close morphological features in plant height, number of leaves per plant, number of pods per plant, and length of pods per plant. On the contrary, *B. juncea* was found to be present distantly and placed in cluster-II, forming a monophyletic group which showed superiority in case of plant height, number of branches per plant, number of leaves per plant and number of pods per plant over the other two species (*B. campestris* and *B. napus*) of *Brassica* L. Since morphological traits are deemed as a traditional approach, the above mentioned morphometric analysis would be conducive to assessing genetic divergence and to classifying existing species of *Brassica* oilseed crops.

Table 3. Mean values of six different morphological features among three species of *Brassica* L.

Species	Plant height (cm)	No. of branches/plant	No. of leaves/plant	No. of pods/plant	Length of pods/plant (cm)	No. of seeds/pod
B. campestris	83.10 ± 3.64	3.00 ± 1.00	21.67 ± 2.52	43.67 ± 5.51	6.67 ± 0.38	14.20 ± 2.52
B. juncea	109.17 ± 4.75	5.33 ± 1.91	52.33 ± 5.13	86.00 ± 6 .66	5.61 ± 0.57	13.00 ± 1.73
B. napus	61.83 ± 3.25	4.25 ± 1.58	27.00 ± 3.61	59.87 ± 6.00	7.47 ± 0.67	16.33 ± 3.21

Values are in Mean ± SD.

The determination of phylogenetic relationship from morphometric data in the current investigation was found to correspond with the outcome of genetic divergence analysis of *Brassica* species through biochemical (protein) marker by Akbar et al. (2020a); where *B. juncea* was offered with genetically diverse nature than *B. campestris* and *B. napus*. Therefore, it could be envisaged that *B. juncea* was not only phylogenetically distinct in position by morphological features but also a genetically different and agronomically advanced accession compared to *B. campestris* and *B. napus* and would be utilized as a source of parental line in the improvement of mustard-rapeseed oilseed crops in Bangladesh.

Nature and amount of heterochromatins in orcein stained interphase nuclei and prophase chromosomes

At times, the staining properties of interphase nuclei and prophase chromosomes provide karyomorphological highlights and offer assistance in the characterization of distinctive species. Tanaka (1971) was the pioneer of featuring these standards for karyomorphological properties. He disclosed that the staining natures of heterochromatins present within the interphase nuclei and prophase chromosomes varied in different species. Later on, different workers applied these criteria in characterizing diverse plant materials.

In the present investigation, a number of darkly stained small heterochromatic blocks were found to be present sparsely around the nuclei of *B. juncea* and no nucleolus was noticed following orcein staining in the interphase (Fig. 3b). Such sort of staining nature of interphase nuclei was categorized as ‘Simple Chromocenter Type’ by Tanaka (1971). Tanaka moreover expressed that in simple chromocenter type of interphase nuclei, the numbers of heterochromatic blocks were more or less equivalent to the number of somatic chromosomes of the particular species. However, the number of heterochromatic blocks (22–27) was found less than the expected number of somatic chromosome complements of *B. juncea* 2n=36 in the present investigation. The reduction in the number of heterochromatic blocks in *B. juncea* was probably because of fusion or overlapping of heterochromatins suggesting the somatic affiliation of chromosomes. Dayal and Prasad (1983) stated that the numbers of chromocentres are considered to be regulated genetically, and it is, therefore, a species-specific character. A similar type of interphase nuclei following differential staining technique has been reported in two species of *Orchid* namely *Oberonia iridifolia* (Roxb). Lindl., *Peristylus constrictus* (Lindl.) by Begum and Alam (2005), and in eight different varieties of *Gossypium hirsutum* by Sultana and Alam (2016), in four different floral variants of *Impatiens balsamina* L. by Dash et al. (2019).

Table 4. Types and amount of heterochromatins in interphase nuclei and prophase chromosomes of three species of *Brassica* L.

Species	Types of interphase nuclei	Types of prophase chromosomes	Amount of heterochromatins in interphase nuclei area (μm^2)
B. campestris	Diffuse	Continuous	262.21 ± 25.71
B. juncea	Simple chromocenter	Continuous	60.36 ± 14.99
B. napus	Diffuse	Continuous	254.26 ± 20.63

Values are in Mean ± SD

The nuclei of *B. campestris* and *B. napus* were found to be stained homogeneously. In accord with Tanaka (1971), this type of nucleus was regarded as “Diffuse Type” in interphase nuclei. An enormous and conspicuous nucleolus was noticed in nearly each interphase stage of both *B. campestris* and *B. napus* (Figs. 3a and 3c). In the majority of the cases, nucleolus manifested as translucent entities, generally orbicular in form. They ranged in size from small structures which were barely detectable to big conspicuous ones. These recommended late transcription of rDNA to rRNA and late transportation of rRNA from the nucleus to the cytoplasm at the mitotic interphase stage (Sultana and Alam, 2016). This nucleolus was found with the tendency of disappearance at the prophase stages of mitosis in these two species.

Heterochomatins were observed to be homogeneously stained in the interphase nuclei of *B. campestris* and *B. napus*. Being in concurrence with the enunciation of Lavania and Sharma (1983), there might be a propensity to shed heterochromatic segments during the evolutionary pathway of a plant. Thus, the primeval species may have more heterochromatins than the recent one, and based on of this concept; *B. campestris* (262.21±25.71 μm^2) and *B. napus* (254.26±20.63 μm^2) may be regarded as the primitive; while *B. juncea* may be perceived as the evolved one among the three species of *Brassica*, when small heterochromatic regions (60.36±14.99 μm^2) were found in the interphase nuclei in the present study.

The prophase chromosomes of all the three species of *Brassica* L. were stained uniformly along the entire length of chromosomes (Figs. 3d-f) and detected to be “Continuous Type” of prophase chromosomes (Tanaka, 1971). From the above observation, two distinctive correlations have been revealed from the staining properties of interphase nuclei and prophase chromosomes of the three species of *Brassica* L. Some darkly stained heterochromatic blocks were found (as observed in the interphase nuclei of *B. juncea*), (Fig. 3b), which were then noticed with heterochromatins distributed homogeneously all through the prophase chromosomes (Fig. 3e). The localized heterochromatins (as observed in the interphase nuclei of *B. juncea*) are not typically expected to be distributed homogeneously in the prophase chromosomes, but rather to be occupied specific locations of prophase chromosomes (Tanaka, 1971). The present findings do not support the usual feature concerning the distribution of heterochromatins in prophase chromosomes as stated by Tanaka (1971). One of the reasons for this discrepancy might be the presence of facultative heterochromatins. The above finding probably indicates the existence of facultative heterochromatin, which were firmly aggregated in the interphase nuclei and then somehow dispersed in the prophase chromosomes homogenously. In another finding of *B. campestris* and *B. napus*, it was supposed to be the presence of constitutive heterochromatins in the interphase nuclei of the respective species where the heterochromatins were found evenly distributed in the nuclei of interphase (Figs. 3d and 3f) and then

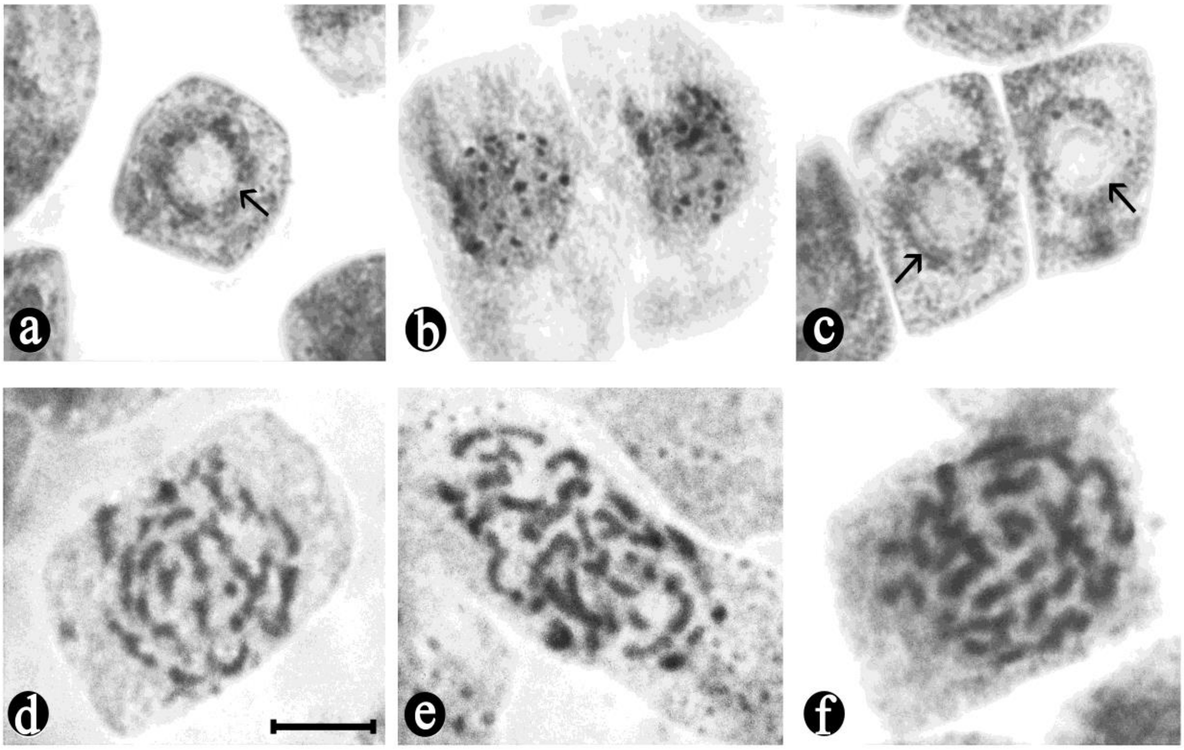

Fig. 3. Mitotic interphase nuclei and prophase chromosomes (3a–f) of the three species of *Brassica* L. (3a and 3d) interphase nuclei and prophase chromosomes of *B. campestris*; (3b and 3e) interphase nuclei and prophase chromosomes of *B. juncea* and (3c and 3f) interphase nuclei and prophase chromosomes of *B. napus* (arrows indicate nucleolus). Scale Bar = 10 µm.

distributed uniformly within the chromosomes of mitotic prophase stage. This finding fully endorses the usual regulation pertaining to the distribution of heterochromatins in prophase chromosomes. Therefore, in the present study, the staining nature and correlation of heterochromatins at interphase nuclei and prophase chromosomes served as a conducive cytogenetical implement, by which the three studied species of *Brassica* L. could be categorized on the basis of these features.

Conclusions

The present findings revealed that the three *Brassica* species could be differentiated with the comparative morphological traits, morphometric analysis and the staining property (nature and amount) of heterochromatins at interphase nuclei and prophase chromosomes. These findings might lead to a critical biosystematics tool for characterization and identification of existing germplasm of *Brassica* L. for achieving significant success in breeding technologies and improving of oilseed production in Bangladesh.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

References

Ahmed ZU, Hassan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmad M, Ahmed ATA, Rahman AKA and Haque EU, eds., *Encyclopedia of Flora and Fauna of Bangladesh. Angiosperms: Dicotyledons (Balsaminaceae - Euphorbiaceae), Volume **7***, Dhaka: Asiatic Society of Bangladesh, 2008; p. 52-58.

Akbar F and Begum KN. A comparative anatomical investigation of three taxa of *Brassica* L. from Bangladesh. *Bangladesh J. Plant Taxon.* 2020; 27(1): 15-26.

Akbar F, Paul M and Begum KN. Karyological and genetic diversity study using molecular marker among three species of oilseed *Brassica* L. *Indian J. Pure Appl. Biosci.* 2020a; 8(4): 267-281.

Akbar F, Saha S, Pinky MS and Begum KN. Molecular characterization of thirteen oilseed *Brassica* L. variants from Bangladesh through Polyacrylamide Gel Electrophoresis (PAGE). *Biosci. Biotechnol. Res. Asia;* 2020b; 17(4): 741-755.

Al-Shehbaz IA. The tribes of Cruciferae (Brassicaceae) in the southeastern United States. *J. Arnold Arbor.* 1984; 65: 343-373.

Begum R and Alam SkS. Karyotype analysis of seven Orchid species from Bangladesh. *Bangladesh J. Bot.* 2005; 34(1): 31-36.

Dash CK, Rashid MH, Sultana SS and Rahman MO. Cytotaxonomy of four floral variants of *Impatiens balsamina* L. *J. Bangladesh Acad. Sci.* 2019; 43(1): 1-9.

Dayal N and Prasad C. Genetic regulation of chromocentres in radish, *Raphanus sativus* L. *Cytologia;* 1983; 48(2): 245-252.

Gulden RH, Warwick SI and Thomas AG. The biology of Canadian weeds. 137. *Brassica napus* L. and *B. rapa* L. *Can. J. Plant Sci.* 2008; 88(5): 951-996.

Hammer Ø, Harper DAT and Ryan PD. PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 2001; 4(1): 1-9.

Ilnicki T. Plant biosystematics with the help of cytology and cytogenetics. *Caryologia;* 2014; 67(3): 199-208.

Jan SA, Shinwari ZK, Rabbani MA, Niaz IA and Shah SH. Assessment of quantitative agro-morphological variations among *Brassica rapa* diverse populations. *Pak. J. Bot.* 2017; 49(2): 561-567.

Joya SD, Shamsuddin KM and Nath UK. Genetic diversity of rapeseed (*Brassica napus* L.) genotypes in Bangladesh. *Bangladesh J. Plant Breed. Genet.* 2017; 30(2): 29-34.

Kabir G and Singh RM. Interphase nuclear structure and heterochromatin in *Cicer* Species. *Cytologia;* 1989; 54: 27-32.

Khalid M, Farhatullah, Khan NU, Din R, Khan MY, Akmal M and Ali N. Linkage of morphological markers in *Brassica*. *Pak. J. Bot.* 2010; 42: 2995-3000.

Lavania UC and Sharma AK. Chromosome banding in evolutionary plant cytogenetics. *Proc. Indian Acad. Sci. (Plant Sci.);* 1983; 92(1): 51-79.

Li P, Zhang S, Li F, Zhang S, Zhang H, Wang X, Sun R, Bonnema G and Borm TJA. A phylogenetic analysis of chloroplast genomes elucidates the relationships of the six economically important *Brassica* species comprising the triangle of U. *Front. Plant Sci.* 2017.

Luciano FB and Holley RA. Enzymatic inhibition by Allyl-isothiocyanate and factors affecting its antimicrobial action against *Escherichia coli* O157: H7. *Int. J. Food Microbiol.* 2009; 131(2-3): 240-245.

Mollika SR, Sarkar RH and Hoque MI. *In vitro* plant regeneration in *Brassica* spp. *Plant Tissue Cult. Biotechnol*. 2011; 21(2): 127-134.

Razzaque MA and Hossain MG. *The second report on Plant Genetic Resources for Food and Agriculture of Bangladesh: The state of Activities*: A Cooperative Programme of Bangladesh Agricultural Research Council and Food and Agricultural Organization of the United Nations: Dhaka; 2007. 74 p. Report No: 2.

Smith JSC and Smith OS. The description and assessment of distance between lines of maize. 2: The utility of morphological-biochemical and genetic descriptors and a scheme for the testing of distinctiveness between inbred lines. *Maydica*, 1989; 34: 151-161.

Saha S and Begum KN. A comparative analysis on pollen morphology of the genus *Brassica* L. (Brassicaceae) in Bangladesh. *Int. J. Bot. Stud.* 2020; 5(5): 191-194.

Singh L, Sharma D, Parmar M, Singh KH, Jain R, Rai PK, Wani SH and Thakur AK. Genetic diversity studies in Indian mustard (*Brassica juncea* (L.) Czern & Coss.) using molecular marker. In: *Brassica Improvement-Molecular, Genetics and Genomic perspectives.* Wani SH, Thakur AK and Khan YJ, eds., New York: Spinger, 2020; p. 215-244.

Sultana SS and Alam SkS. Karyomorphology of eleven varieties of *Gossypium hirsutum* L. *Bangladesh J. Bot.* 2016; 45(1): 251-259.

Tanaka R. Type of resting nuclei in Orchidaceae. *Bot. Mag. Tokyo;* 1971; 84: 118-122.

Zhang S, Chiang CY, Xie YF, Park SJ, Lu Y, Hu JW, Dostrovsky JO and Sessle BJ. Central sensitization in thalamic nociceptive neurons included by mustard oil application to rat molar tooth pulp. *Neuroscience*, 2006; 142: 833-842.