

Comparative karyomorphological analysis of two varieties of *Brassica campestris* from Bangladesh

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Abstract

Two varieties of the oilseed plant viz. *Brassica campestris* var. Tori-7 and *Brassica campestris* var. BARI Sarisha-9 were karyomorphologically investigated following orcein staining. Presence of "Round Pro-chromosome Type" of interphase nuclei and "Continuous Type" of prophase chromosomes indicating that both Tori-7 and BARI Sarisha-9 were originated from common ancestor. Moreover, both the varieties were found to possess $2n=20$ chromosomes with almost similar range of length i.e.; 0.90 ± 0.04 - $2.51\pm 0.05\mu\text{m}$ for Tori-7 and 0.99 ± 0.06 - $2.60\pm 0.06\mu\text{m}$ for BARI Sharisha-9. The total length of $2n$ chromosome complement was recorded as $29.61\pm 0.76\mu\text{m}$ for Tori-7 and $29.75\pm 0.80\mu\text{m}$ for BARI Sharisha-9 where a slight gradual decrease of chromosomal length was observed in both cases. Average chromosomal length, range of individual chromosomal length, relative length and centromeric index of both varieties were more or less similar. Karyotype symmetry index and Karyotype asymmetry index indicating the symmetric nature of both varieties. All metacentric chromosomes were found in Tori-7 representing strictly symmetric karyotype. Beside metacentric, 2 sub-metacentric chromosomes were found in BARI Sharisha-9 representing almost symmetric karyotype. Thus the two varieties of *Brassica campestris* -were plants of primitive nature. However, BARI Sharisha-9 can be considered comparatively advanced than Tori-7 based on different cytogenetical features. Therefore, the compilation of these karyomorphological information will be useful for authentic identification and characterization of the two varieties of *Brassica campestris* which are very basic and important information for breeding in crop improvement programme.

Key words: Karyomorphology, Oilseed plants, Biobased economy, *Brassica*.

INTRODUCTION

It is now the age of the biobased economy, with great efforts expended in both basic and applied research to develop useful and economically important products from agriculture. Crop improvement is essential to meet the demands of this changing new world (eg, climate change, increased population, decreasing land base, resistance power etc.). Plant breeding programs are continuously striving to extend crop yield, enhance crop quality, produce genetically modified crops and improve crop tolerance to diseases and pests. Oilseed crops are such kind of crops which play an important role in human nutrition and economy. As a high energy component of food, edible oilseed is important for meeting the calorie requirement. This is also important for improving the taste of a number of food items. Oil or fat provides double the quantity of energy than the same quality of protein or carbohydrate. Mustard and rapeseed contain 42% oil and 25% protein (Khaleque, 1985). It act as carrier for fat soluble vitamins (A, D, E and K) in the body and therefore, the presence of some fats/oils in diet is essential for their absorbance. It is used to synthesize phospholipids which are important component of active tissues. It is

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served as an insulator to the human body. It is required for essential fatty acids synthesis like- Linoleic, linolenic, and arachidonic acids. *Brassica* oilseeds are collectively contribute about 15% of the world's total supply of vegetable oils and become the third-leading source of edible oil in the world (Mc Vetty *et al.*, 2016). It is used not only for edible purpose but also used in hair dressing, body massaging and different types of pickles preparation. The oil cake contains proteins of high biological value and applicable of calcium and phosphorus. It is used as very good animal feed as well as organic manure for various crops (Aktar *et al.*, 2019). Therefore, oilseed crops are very important for various purposes. Because of their importance, different oilseed crops like mustard, sesame, groundnut, linseed, niger, safflower, sunflower and soybean are being cultivated all over the world. The *Brassica* genus of plants belongs to the mustard family Brassicaceae (formerly Crucifer family) is a economically very important genus as oilseed crops. *Brassica* is the second largest oilseed crop in world oilseed production (FAO, 2010; Raymer, 2002). This genus containing 37 different species which are agriculturally important (Paul *et al.*, 2020). *B. campestris* is one of the economically important species of *Brassica* genus which is known as toria, sarson, summer turnip rape, and polish rape. *B. campestris* was distributed at least 2000 years ago from the Atlantic Island to different region in the World. It appears to possess had the widest distribution, with secondary centers of diversity in Europe, Western Russia, Central Asia and Near East (Vavilov, 1949; Mizushima & Tsunoda, 1967; Zhukovsky & Zeven, 1975).

Few oilseed crops are being cultivated in Bangladesh. Mustard and rapeseed (*Brassica spp.*) are considered as the major oil crops in our country. The advantageous chemical composition and its relatively low price offer wide possibilities for uses of this valuable seed. But it is general opinion of the farmers of Bangladesh that oil crops are not profitable. For that reason, the farmers do not cultivate oil crops as profitable cash crops and not using any imputes like fertilizer, irrigation etc. Beside this other some major constrains such as-low yield of traditional varieties, non-availability of high yield varieties and good quality seeds, lack of pest and disease resistant varieties, narrow seedling time and growing period, extreme land competition in winter season etc. discourage the farmer to cultivate this crop. As a result their harvest is very low and only one-third of the total requirement can be met up from our internal production. The shortfall is met up by import. But our neighboring country like-Pakistan, Sri Lanka etc. is more than double than that of ours. To meet the demand, it is an argent need to take immediate actions for increasing the internal production to reduce the import to some extent. It is possible by develop special oil yielding crops to increase the production of oil to satisfy the ever increasing demands in these sectors. Under these circumstances only improved germplasm would be needed to extend the production.

However, the crop has received little research attention. Consequently, the genetic improvement of this crop has been reported to be limited since it is a virgin research field in Bangladesh. Improvement of different oilseed crops is possible through proper breeding programme. For such a programme, the genomic information is very essential. Unfortunately very limited information is available in Bangladesh. On the other hand, Bangladesh Agricultural Research Institute and other organization (like-BINA, BINA, BAU, BADC etc.) have a good number of oilseed germplasm. These germplasm were

only characterized on the basis of their morphological features. This type of characterization sometimes creates problem since phenotypic features are not always reproducible. For this purpose, an authentic characterization and genetic information of each germplasm is needed to choose the most useful parents for breeding programme and also for germplasm conservation. The complete knowledge and understanding of the genetic diversity within and among genetic resources of the available germplasm is essential for successful breeding program. This will enable plant breeders to settle on parental sources that generate diverse populations for selection (Esmail *et al.*, 2008). The extent of genetic diversity in species is important in determining the selection to be performed and for the ability to adapt to variable environmental conditions. At the onset of a breeding program, information concerning genetic relationships is often used to improve the breeding population. Stable and reliable method must be followed for genetic characterization of different germplasm. Karyotype analysis is such kind of method since specific for each specimen.

Orcein staining properties of interphase nuclei and prophase chromosomes are karyomorphological parameters since it depends upon the nature of heterochromatin condensation. Tanaka (1971) was the pioneer to classify interphase nuclei and prophase chromosomes on the basis of heterochromatin nature with orcein staining. Later different workers tried to characterize interphase nuclei and prophase chromosomes by differential staining (Alam & Kondo, 1995; Fawzia & Alam, 2011; Shahla & Alam, 2011; Warasy, 2013). The outcome of this study showed that various species even members of a species, varieties, cultivar and morphological form could be distinguished by their staining properties of interphase nuclei and prophase chromosomes.

It is very difficult to characterize any specimen without any detailed karyotypic features and DNA content analysis which are very basic and important information for breeding as well as molecular biological work in crop improvement programme. Cytogenetical information has been used by plant breeders to manipulate sets of chromosomes, individual chromosomes or chromosomal segments to solve particular problems. Karyotyping is a useful tool for cytogenetical studies which can be used to elucidate the origin, polidy, and phylogenetic relationships among plants (Vanzela *et al.*, 2003; Ali *et al.*, 2005; Feitoza *et al.*, 2010; Felix *et al.*, 2011; Xiong & Pires, 2011).

In the present study, a karyomorphological analysis was carried out for the first time in Bangladesh to characterize two varieties of *Brassica campestris* as oilseed crop. Therefore, the aim of this study was to: i. compare the staining property of the interphase nuclei and prophase chromosomes after staining with orcein. ii. count the 2n chromosome number of both the varieties. iii. make a full strength orcein-stained karyotype for each variety. iv. characterize each species with cytogenetical markers.

MATERIALS AND METHODS

For the determination of karyomorphological features, two oilseed varieties viz. *Brassica campestris* var. Tori-7 and *Brassica campestris* var. BARI Sarisha-9 were collected from Bangladesh Agricultural Research Institute and maintained in the Botanic garden, Department of Botany, Jahangirnagar University, Bangladesh.

A comparative morphological analysis among these two varieties is given below as a tabular form (Table 1).

Table 1. Morphological features of two varieties of *Brassica campestris*

Features	(Tori-7)	(BARI Sharisha-9)
Types	Annual herb	Annual herb
Height	60-70cm	80-85cm
Primary branch	3-5	4-6
Planting time	Mid October-Mid November	Mid October-Mid November
Inflorescence	Racemose	Racemose
Pod	2 chamber	2 chamber
Seed	Round and black in colour	Round and black in colour
Seed weight (gm)	2.5-2.7 (1000 seeds)	2.50-2.65 (1000 seeds)
Ripening time (days)	70-75	80-85
Yield (Kg/per hectore)	900-1000	1250-1450
Oil content(%)	40-41	42-43

Healthy roots were collected from petriplate, as the seed were kept in water soaked petriplate for germination, in the department of Botany, Jahangirnagar University. The optimum time of root collection for obtaining maximum number of dividing cells was 2.45-3.00 pm for Tori-7 and 1.30 pm for BARI Sharisha-9. The collected root was pretreated with cold water at room temperature (28-30⁰C) followed by 15 min fixation in 45% acetic acid at 4⁰C. These were then hydrolyzed in a mixture of 1 N HCl and 45% acetic acid (2:1) at 60⁰C for 8-9 sec. Then the hydrolyzed root was soaked on a filter paper and taken in a clean slide. The meristematic region was cut with fine blade. A drop of 1% aceto-orcein was added to the material. A clean cover glass was placed on the material. Then the materials were tapped gently by a tooth pick and then squashed by placing thumbs. Finally, the slides were observed under microscope (Olympus-DP72, Japan) with a digital camera. Final measurement was calculated by dividing the magnification of the objective 100x.

RESULTS AND DISCUSSION

Orcein stained interphase nuclei and prophase chromosomes: The staining properties of interphase nuclei and prophase chromosomes provide karyomorphological features that help to characterize different germplasm. In this study, the nature of orcein staining of interphase nuclei of two varieties of *Brassica campestris* was grouped into same categories i.e. "Round Pro-chromosome Type" of interphase nuclei where the number of darkly stained round heterochromatic regions corresponding to its 2n chromosome number were present (Figs. 1, 2, Table 2). The prophase chromosomes of both varieties of *Brassica campestris* were found to possess "Continuous Type" where most of the chromosomes stained homogenously along the entire length (Figs. 3, 4, Table 2).

Tanaka (1971) found that the nature of staining properties of heterochromatins presents in the interphase nuclei and prophase chromosomes were different in different species. He was the pioneer of proposing these criteria for karyomorphological features. Tanaka (1971) classified interphase nuclei and prophase chromosomes in five different categories

in each case on the basis of the staining property. Later different scientist applied these criteria in characterizing different plant materials (Begum & Alam 2004, Akther & Warasy 2016, Bonna *et al.*, 2018).

Generally, the localized heterochromatin (as observed in the interphase nuclei) is occupying different locations in the prophase chromosomes. But in the present study homogeneously distributed prophase chromosomes was observed, rather than occupy different locations. The present findings did not support the usual regulation regarding the distribution of heterochromatin in prophase chromosomes. The comparative staining property of interphase nuclei and prophase chromosomes indicated the presence of facultative heterochromatin which firmly aggregated in the interphase nuclei and then somehow had been homogeneously distributed in prophase chromosomes. The reasons for this disagreement might be presence of facultative heterochromatin. Whatever the reason is, the two varieties could be characterized on the basis of these karyomorphological characters.

In the available literatures and internet sources, reports on the nature of mitotic interphase and prophase chromosomes after orcein staining were not found. Therefore, characterization of two varieties of *Brassica campestris* by the above parameters was the pioneer attempt.

Table 2. Types of interphase nuclei and prophase chromosomes of two varieties of *Brassica campestris* after staining with orcein

Varieties of <i>Brassica campestris</i>	Type of Orcein-stained interphase nuclei	Type of Orcein-stained prophase chromosomes
(Tori-7)	Round prochromosome type	Continuous
(BARI Sharisha-9)	Round prochromosome type	Continuous

2n chromosome number: In the present investigation, both the varieties of *Brassica campestris* viz. Tori-7 and BARI Sarisha-9 were found to possess 2n=20 chromosomes (Figs. 5,6,7,8, Table 3). No chromosomal information of this species is available in the internet sources and relevant literature in case of Bangladesh. Therefore, the chromosome count of *Brassica campestris* was the pioneer attempt in Bangladesh. Same chromosome count 2n=20 for *Brassica campestris* was reported in abroad by different scientist (Morinagana 1934_{a,b}). So the present finding was correlate to the earlier findings.

Total chromosomal length: In the present study, the total length of 2n chromosome complement was recorded as 29.61±0.76 µm for Tori-7 and 29.75±0.80 µm for BARI Sharisha-9. The total length of 2n chromosome compliments was almost equal. Therefore, the present results clearly indicated the absence of diversification of chromatin length among the specimens studied in this research.

Average and Range of individual chromosomal length: Between the two varieties of *Brassica campestris* the range of chromosomal length was 0.90±0.04-2.51±0.05µm for Tori-7 and 0.99±0.06-2.60±0.06µm for BARI Sharisha-9 where no prominent gradual decrease of chromosomal length was observed in both cases (Figs. 7,8,9,10; Table 3). Average chromosomal length of Tori-7 and BARI Sharisha-9 was 1.48 µm and 1.49 µm,

respectively. Average chromosomal length and range of individual chromosomal length of both the varieties was more or less similar.

Relative length: The range of relative length was 0.03-0.08 and 0.03-0.09 for Tori-7 and BARI Sharisha-9, respectively. Difference between relative lengths was 0.05 for Tori-7 and 0.06 for BARI Sharisha-9 (Table 3). In respect of this karyotypic parameter more or less similarity also observed among the studied two varieties.

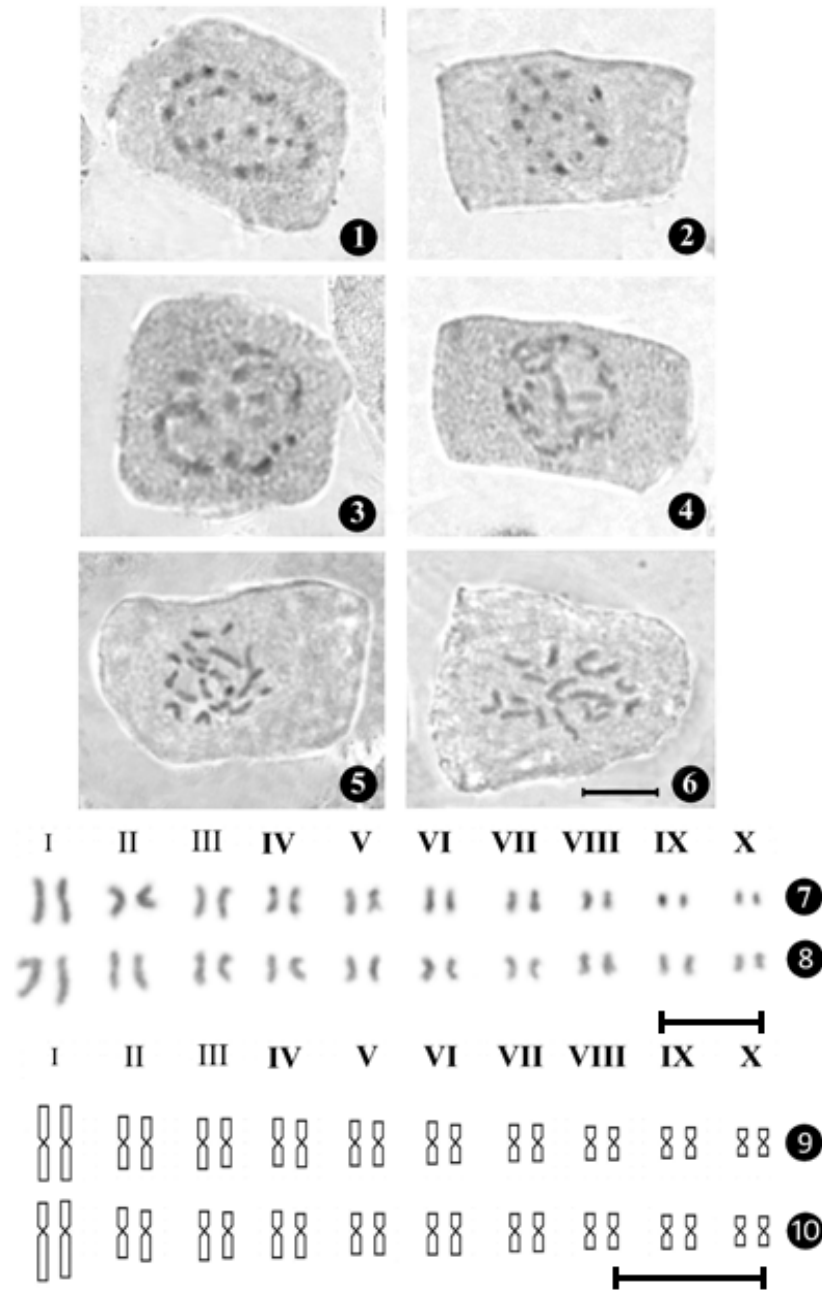
Centromeric feature: Two varieties of *Brassica campestris* were found to possess more or less similar centromeric index range that was 48.61-50.00 for Tori-7 and 36.54-50.00 for BARI Sharisha-9. In case of centromeric formula, all metacentric chromosomes were found in Tori-7 representing strictly symmetric karyotype. Whereas BARI Sharisha-9 was found to possess 18 metacentric and 2 sub-metacentric chromosomes representing almost symmetric karyotype. The sub-metacentric chromosomes might have originated from metacentric chromosomes by some chromosomal aberration viz. terminal deletion, pericentric inversion, non-reciprocal or unequal translocation. Stebbins (1971) mentioned that the symmetric karyotype indicates primitive character and from that point of view two varieties of *Brassica campestris* are plants of primitive nature. But BARI Sharisha-9 was comparatively advanced than Tori-7 considering of centromeric formula.

Karyotype symmetry and asymmetry index: Karyotype symmetry index (Syi %) is nearer to 100% that was 93.53% was found in case of Tori-7 and comparatively small karyotype symmetry index value that was 77.20 % was found in BARI Sharisha-9 (Table 3). Karyotype symmetry index values decreased with increasing asymmetry, indication the karyotype of both the varieties was symmetry. On the other hand, karyotype asymmetry index (AsK %) was 51.67% for Tori-7 and 56.47% for BARI Sharisha-9 was observed (Table 3). The value of AsK% increases with the increasing asymmetry. Thus the above findings indicating the symmetric nature of both the varieties of *Brassica campestris*.

Table 3. Comparative orcein stained karyotype analysis in two varieties of *Brassica campestris* after staining with orcein

Cytologgenetical Parameters	(Tori-7)	(BARI Sharisha-9)
2n	20	20
Total length of chromosome complement (μm)	29.61 \pm 0.76	29.75 \pm 0.80
Range of individual chromosome length (μm)	0.90 \pm 0.04-2.51 \pm 0.05	0.99 \pm 0.06-2.60 \pm 0.06
Average chromosomal length (μm)	1.48	1.49
Range of relative length	0.03-0.08	0.03-0.09
Difference of relative length (DRL)	0.05	0.06
Centromeric index range (CI)	48.61-50.00	36.54-50.00
Centromeric formula	20m	18m+2sm
Karyotype symmetry index (Syi %)	93.53	77.20
Karyotype Asymmetry index (AsK %)	51.67	56.47

m = metacentric, sm = sub-metacentric chromosome.



Figs. 1-10. Orcein-stained mitotic interphase, prophase, metaphase, karyotype and Idiogram of two varieties of *Brassica campestris*. 1. Interphase nuclei of Tori-7, 2. Interphase nuclei of BARI Sharisha-9. 3. Prophase chromosome of Tori-7 4. Prophase chromosome of BARI Sharisha-9. 5. Mitotic metaphase of Tori-7. 6. Mitotic metaphase of BARI Sharisha-9. 7. Karyotype prepared from mitotic metaphase chromosomes of Tori-7. 8. Karyotype prepared from mitotic metaphase chromosomes of BARI Sharisha-9. 9. Idiogram of Tori-7. 10. Idiogram of BARI Sharisha-9. Bar=5 μ

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