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A comparative analysis on mitotic interphase and prophase among twelve varieties of *Brassica* L. from Bangladesh: Brassicaceae

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Abstract

Brassica L. is an agronomical and economical important crop belonging to Brassicaceae family. The research was conducted to interrelate interphase nuclei and prophase chromosome of *Brassica* L. varieties. In the current analysis, the nature of interphase nuclei and prophase chromosome of twelve BARI varieties of *Brassica* L. was investigated based on orcein-staining properties. In the interphase nuclei only 'Diffuse type' and 'Simple Chromocenter type' were found in Tori-7, Dawlat, BS-11, BS-14 and SS-75, Rai-5, BS-6, BS-7, BS-8, BS-10, BS-12, BS-15, respectively. Considering prophase chromosome among these studied twelve varieties, 'Continuous type' showed in Dawlat, Rai-5, BS-7, Bs-8, BS-11, BS-14 while BS-6, BS-10, BS-12 and BS-15 observed 'Interstitial type' and 'Gradient type' showed in Tori-7, and SS-75. Therefore, the orcein staining property of interphase nuclei and prophase chromosome can provide essential information as cytological implement to discriminate the twelve analyzed *Brassica* L. varieties.

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Introduction

Brassica L. is one of the most commercially important genus of Brassicaceae family which wildly distributed throughout the world. This genus mainly originated from close regions of Himalaya and then dispersed from Asia to European-Mediterranean territory (Downey and Robelen, 1989). About contrasting 37 species includes into the genus *Brassica* L. with divergent agronomic traits as vegetable and oilseed crops (Jahan *et al.*, 2013). On the basis of divergent morphological characteristics and genetic diversity six interlinked species are found in the genus *Brassica* L., of which three amphidiploids species (*B. carinata*, *B. juncea* and *B. napus*) are evaluated from three diploid species, (*Brassica campestris*, *B. nigra*, and *B. oleracea*) (Nagahara, 1935). Among these six varieties, the four most widely cultivated species are *Brassica campestris* L., *B. juncea* (L.) Czern and Coss., *B. napus* L., and *B. carinata* Braun for oilseed and vegetables (Rakow, 2004).

Generally, the genus *Brassica* L. can be categorized into three groups: rapeseed, mustard and cole. Rapeseed-mustard is well known for edible oil and protein whereas cole is consumed as vegetables. As oil seed *Brassica* L. achieves second largest contributor role in global oil production after soybean (Raymer, 2002). Mustard oil is not only used for as cooking oil but also marinate food stuffs and salad dressings. Moreover, as an edible oil *Brassica* L. is worthier for human health due to the presence of linoleic acid (omega 3 fatty acid) and alpha linoleic acid (omega 3 fatty acid) (Mollika *et al.,* 2011).

Brassica L. is an excellent source of potassium, phenolics, dietary fiber, vitamins A, C and E as well as a renewable resource in the petro-chemical industry (Zhang *et al.,* 2006).

The oil meal of *Brassica* L. has a quality value in beef and poultry ration as a protein supplement. According to Luciano and Holley (2009), the mustard has antibacterial and antifungal properties considering the substance similar to glucosinolate. Brassinosteroids have a great influence to control both prostate and breast cancer which are highly distributed in pollen and seed of *B. campestris* and *B. napus* (Wachsman *et al.,* 2012).

Due to the commercial values of *Brassica* L., a large number of tremendous investigations have been occurred for morphological, physiological and biochemical improvement in worldwide as well as Bangladesh (Zhang, 1996; Chen *et al.,* 2001; Hu *et al.,* 2001; Khan *et al.,* 2002; Xiao *et al.,* 2004; Liu, 2007; Mollika *et al.,* 2011; Akbar and Begum, 2020; Paul *et al.,* 2020). But still it is essential to know the cytological and cytogenetical information of a species because it plays a significant role to relevant its evolution and diversification (Ropiquet *et al.*, 2008). Based on previous literature, researchers were concentrating to reveal the chromosomal information and molecular analysis of *Brassica* L. throughout the world (Takamine, 1916; Du *et al.*, 1993; Olin-Fatih 1994; Cheng *et al.*, 1995*;* Fukui *et al.*, 1998; Kulak *et al.*, 2002; Snowdon 2007; Fang *et al.*, 2014; Sun *et al.*, 2018). On account of small size chromosome, only chromosome number and classical cytogenetical information is insufficient for characterization of species or varieties (Begum and Alam, 2016). In such cases, the feature of interphase nuclei and prophase chromosome act as key cytological tool to characterization of any specimens. Therefore, the current investigation has been approached to reveal the nature of interphase nuclei and prophase chromosome of mitotic cell division using orcein staining that should provide convenient information for disguising twelve BARI varieties of *Brassica* L., because this information is not available in the contemporary scientific literatures.

Materials and methods

Plant materials

To conduct this current investigation, all the twelve varieties of *Brassica* L. (Table 1, Figs. 1A-W, 2A-W) were assembled from Oil Research Center (ORC) of Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur and then maintained in the Botanical garden of Jagannath University, Dhaka, Bangladesh.

Sample preparation

At first the wet filter papers were placed in a Petri plate to germinate seeds for 36 h and 15 min at room temperature in the dark and Root tips (RTs) were gently taken from germinated seeds. Then, by using 8-hydroxyquinoline (0.002 M) for the RTs were pretreated 50 min at room temperature and fixed in Carnoy's fluid (Glacial acetic acid Ethanol 1: 3) at 4°C for 24 hours and then preserved in 70% alcohol for later use. Next, a mixture of 1% aceto-orcein and 1 N HCl was used to heat the pre-treated RTs through a spirit burner. Afterwards, the prepared aceto-orcein stained RTs were mildly squashed and finally prepared slides were examined under the Optika electric microscope and microscopic images were captured by the Euromax camera (CMEX 10, DC 10000C) with 100X magnification in Auto mode.

Analytic form

For considering the behavior of interphase and prophase of inspected twelve *Brassica* L. varieties, from 40 to 50 interphase nuclei and prophase chromosome were examined and then categorized on the basis of Tanaka's (1971) classification.

Results and discussion

Depending on orcein-staining properties, the conducting investigation disclosed that noticeable darkly stained heterochromatin blocks were formed by the interphase nuclei of eight BARI *Brassica* L. varieties among studied twelve varieties in which four varieties of *B. campestris* (SS-75, BS-6, BS-12 and BS-15), two varieties of *B. juncea* (Rai-5 and BS-10) and two varieties of *B. napus* (BS-7 and BS-8) (Table 2, Figs. 3B-D, 3F, 3G, 3I, 3L-K); Tanaka (1971) classified this nature of interphase nuclei as 'Simple Chromocenter type'.

Table 1. List of twelve BARI varieties of *Brassica* L. used in present cytological analysis.

Serial No	Species	Variety
1	*Brassica campestris*	Tori-7
2	*B. campestris*	Sonali Sarisha-75 (SS-75)
3	*B. campestris*	BARI Sarisha-6 (BS-6)
4	*B. campestris*	BARI Sarisha-12 (BS-12)
5	*B. campestris*	BARI Sarisha-14 (BS-14)
6	*B. campestris*	BARI Sarisha-15 (BS-15)
7	*B. juncea*	Dawlat
8	*B. juncea*	Rai-5
9	*B. juncea*	BARI Sarisha-10 (BS-10)
10	*B. juncea*	BARI Sarisha-11 (BS-11)
11	*B. napus*	BARI Sarisha-7 (BS-7)
12	*B. napus*	BARI Sarisha-8 (BS-8)

Table 2. Types of interphase nuclei and prophase chromosomes of the genus *Brassica* L. after staining with Orcein.

Variety	Type of orcein-stained interphase nuclei	Type of orcein-stained prophase chromosomes
B. campestris var. Tori-7	Diffuse	Gradient
B. campestris var. Sonali sarisha-75	Simple chromocenter	Gradient
B. campestris var. BARI Sarisha-6	Simple chromocenter	Interstitial
B. campestris var. BARI Sarisha-12	Simple chromocenter	Interstitial
B. campestris var. BARI Sarisha-14	Diffuse	Continuous
B. campestris var. BARI Sarisha-15	Simple chromocenter	Interstitial
B. juncea var. Dawlat	Diffuse	Continuous
B. juncea var. Rai-5	Simple chromocenter	Continuous
B. juncea var. BARI Sarisha-10	Simple chromocenter	Interstitial
B. juncea var. BARI Sarisha-11	Diffuse	Continuous
B. napus var. BARI Sarisha-7	Simple chromocenter	Continuous
B. napus var. BARI Sarisha-8	Simple chromocenter	Continuous

On the other hand, the interphase nuclei of remaining four varieties were found to possess uniformly stained nucleus of which two varieties of *B. campestris* (Tori-7 and BS-14) and two varieties of *B. juncea* (Dawlat and BS-11) (Table 2, Figs. 3A, 3E, 3H, 3G, 3J), in other words heterochromatin blocks were not localized rather distributed homogenously all over the nucleus which regarded as 'Diffuse type' by Tanka (1971).

In interphase, two varieties of *B. campestris* (Tori-7 and BS-14) and two varieties of *B. juncea* (Dawlat and BS-11) occupied a prominent nucleolus (Figs. 3A, 3E, 3G, 3J, arrow) but not figure out in prophase.

Fig. 1. Habit of six varieties of *Brassica* L. A-D. *B. campestris* var. Tori-7; E-H. *B. campestris* var. SS-75; I-L. *B. campestris* var. BS-6; M-P. *B. campestris* var. BS-12; Q-T. *B. campestris* var. BS-14; U-X. *B. campestris* var. BS-15; A, E, I, M, Q, U: Plant morphology; B, J, N, R, V: Flowers; C, G, K, O, S, W: Pods; D, F, L, P, T, X: Seeds.

Although, the prophase chromosome of inquired varieties was categorized into three types found on orcein-staining properties.

The gradual staining nature of prophase chromosome categorized as 'Gradient type' in the classification of Tanaka (1971) that found in two varieties of *B. campestris* (Tori-7 and SS-75) and showed one darker end to slightly stained end due to the appearance of comparative great amount of heterochromatins (Table 2, Figs. 4A-B). However, the interstitial area of prophase chromosome was observed to darkly stained meanwhile the other regions stained slightly in BS-6, BS-12, BS-15 and BS-10 (Table 2, Figs. 4C-D, 4F, 4I) and according to Tanaka (1971) theses were 'Interstitial type' of interphase nuclei.

Fig. 2. Habit of six varieties of *Brassica* L. A-D. *B. juncea* var. Dawlat; E-H. *B. juncea* var. Rai-5; I-L. *B. juncea* var. BS-10; M-P. *B. juncea* var. BS-11; Q-T. *B. napus* var. BS-7; U-X. *B. napus* var. BS-8; A, E, I, M, Q, U: Plant morphology; B, J, N, R, V: Flowers; C, G, K, O, S, W: Pods; D, F, L, P, T, X: Seeds.

In contrast, the prophase chromosomes of six varieties of inquired *Brassica* L. *viz. B. campestris* var. BS-14, three varieties of *B. juncea* (Dawlat, Rai-5 and BS-11) and two varieties of *B. napus* (BS-7 and BS-8), were considered as 'Continuous type' by following Tanaka's classification (1971) where found to possess homogenous characteristics all over the inter-length after staining (Table 2, Figs. 4G-H, 4J, 4K-L) that means uniform distribution of heterochromatins.

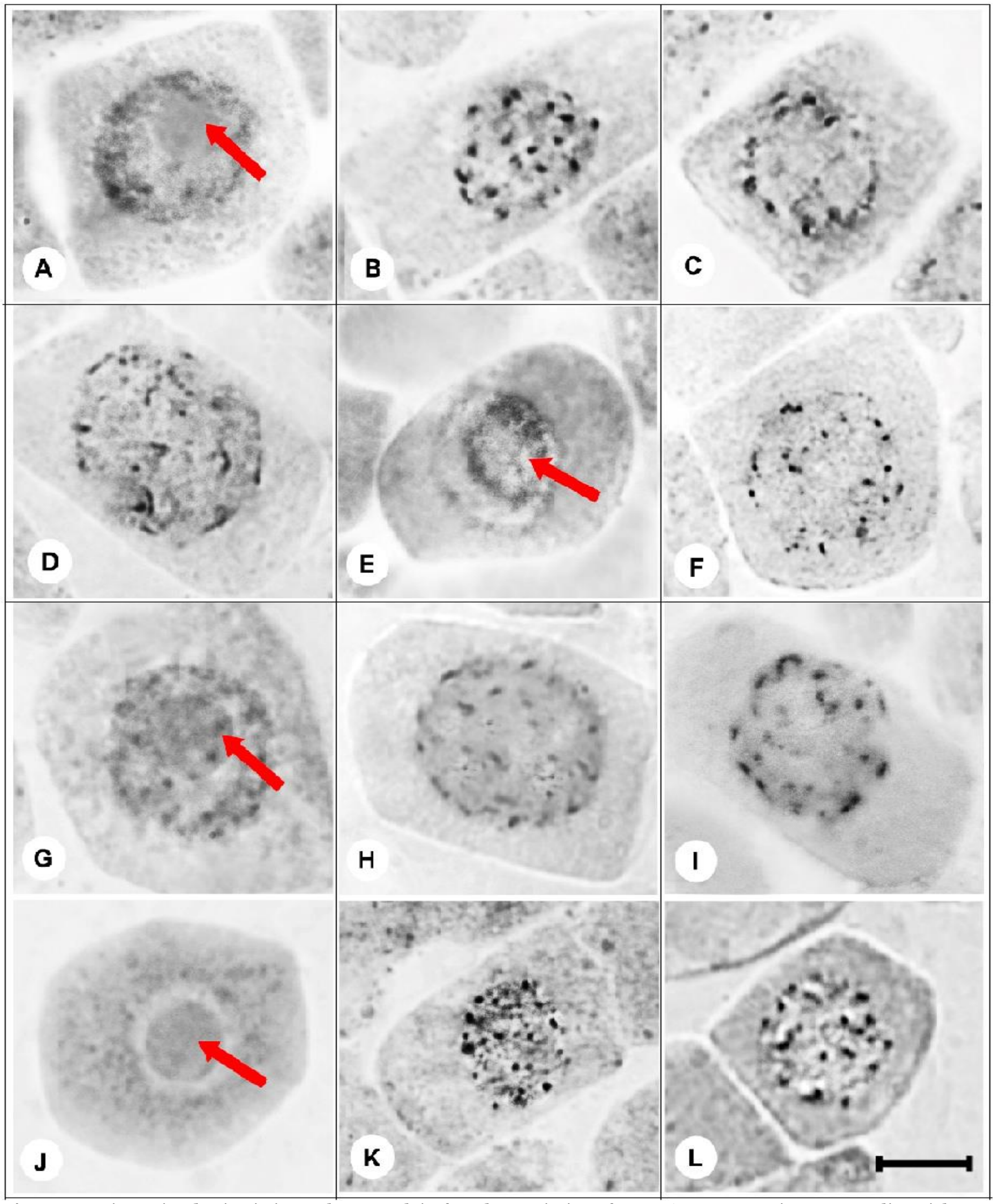

Fig. 3. Orcein-stained mitotic interphase nuclei of twelve varieties of *Brassica* L. A. Tori-7; B. Sonali Sarisha-75; C. BARI Sarisha-6; D. BARI Sarisha-12; E. BARI Sarisha-14; F. BARI Sarisha-15; G. Dawlat; H. Rai-5; I. BARI Sarisha-10; J. BARI Sarisha-11; L. BARI Sarisha-7; K. BARI Sarisha-8; Arrows indicate the presence of nucleolus; Scale Bar= 10 µm.

Considering the nature of staining properties, three types of correlation could be remained within the orcein-stained interphase nuclei and prophase chromosomes (Tanaka, 1971). Firstly, the plant type which usually showed 'Diffuse type' of interphase nuclei had of staining in 'Continuous type' prophase chromosome and if 'Simple Chromocenter type' and 'Complex Chromocenter type' of interphase nuclei were formed by any organism, they could have 'Gradient type' and 'Interstitial type' of staining in prophase chromosome, respectively. *B. campestris* var. BS-14 and two varieties of *B. juncea* (Dawlat and BS-11) supported Tanaka's (1971) proposal as their 'Diffuse type' of interphase nuclei were found to possess 'Continuous type' of prophase chromosomes (Figs. 3E, 3G, 3J, 4E, 4G, 4J).

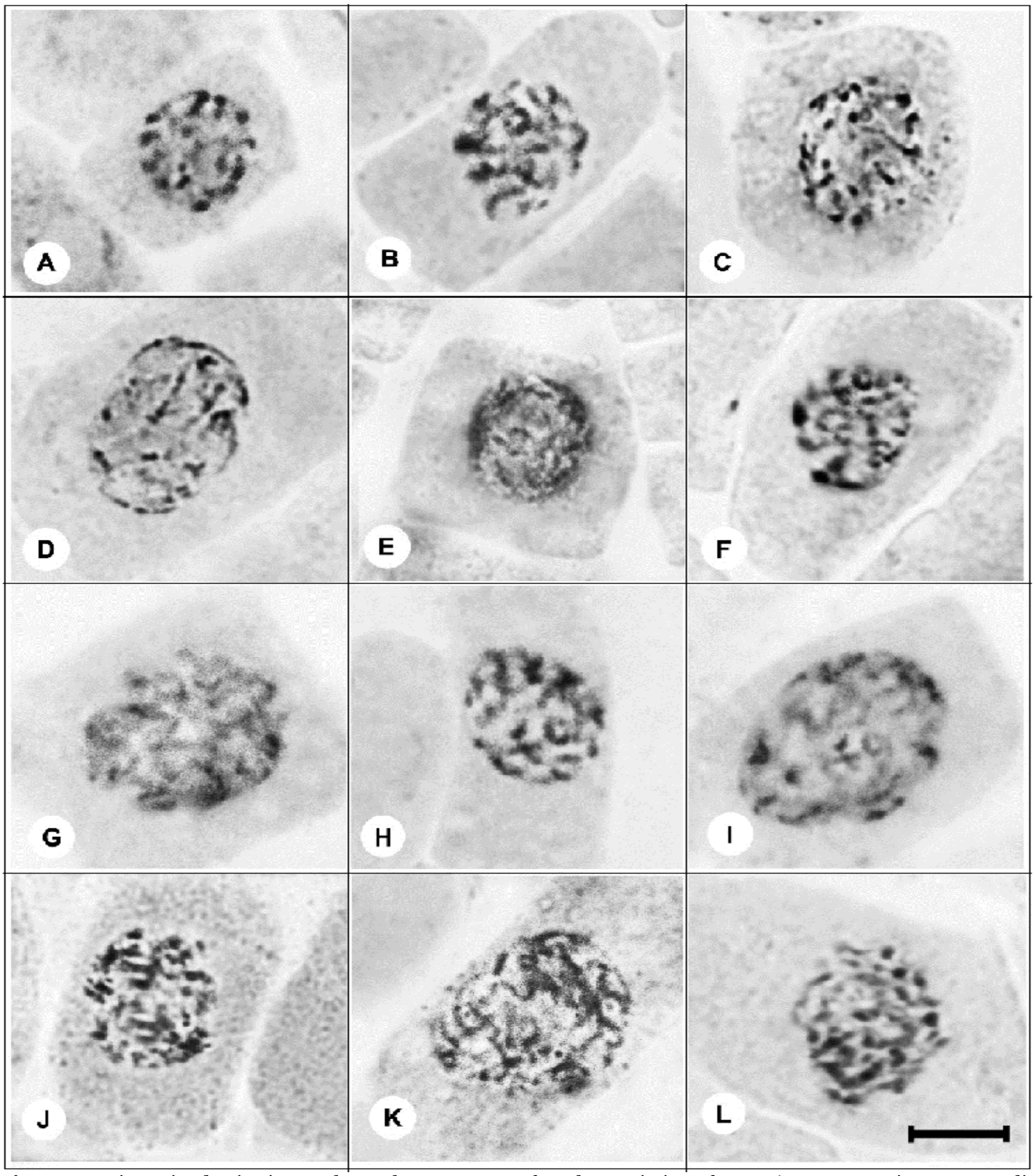

Fig. 4. Orcein-stained mitotic prophase chromosomes of twelve varieties of *Brassica* L. A. Tori-7; B. Sonali Sarisha-75; C. BARI Sarisha-6; D. BARI Sarisha-12; E. BARI Sarisha-14; F. BARI Sarisha-15; G. Dawlat; H. Rai-5; I. BARI Sarisha-10; J. BARI Sarisha-11; L. BARI Sarisha-7; K. BARI Sarisha-8; Scale Bar= 10 µm.

In case of *B. campestris* var. SS-75, the current findings assisted that abundance of localized heterochromatins in interphase nuclei and afterward occupied gradual and homogenous distribution in prophase chromosome which was as per assumption (Figs. 3B, 4B).

The homogenously distributed facultative heterochromatins of interphase nuclei further gradually distribution in the prophase chromosome of *B. campestris* var. Tori-7 (Figs. 3A, 4A). In the interphase nuclei of four varieties of *Brassica* L. *viz.* BS-6, BS-12, BS-15 and BS-10, vigorously arranged localized heterochromatins were found but in some way aggregated in interstitial area of the prophase chromosomes (Figs. 3C-D, 3F, 3I, 4C-D, 4F, 4I). Besides, the confined heterochromatins were showed in the interphase nuclei in *B. juncea* var. Rai-5 and two varieties of *B. napus* (BS-7 and BS-8) but the prophase chromosomes were homogeneously distributed (Figs. 3H, 3K-L, 4H, 4K-L). According to Sultana and Alam 2016, heterochromatin of interphase nuclei remained as condense status rather localized in a specific region that possibly diffused in the prophase chromosomes. So, the current detections did not assist the general directive concerning the distribution of heterochromatin in prophase chromosomes by the reason of facultative heterochromatins appearance.

Conclusion

It may be concluded that the present investigation correlated the nature of mitotic interphase nuclei and prophase chromosome of twelve BARI varieties of *Brassica* L. Moreover, this investigation might be a contribution to count the nature of mitotic interphase nuclei and prophase chromosome as a cytological tool and provided sufficient information to categorize as well as further investigation of investigated twelve varieties of *Brassica* L. from Bangladesh.

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