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Karyotype comparison and characterization of two cauliflower cultivars from Bangladesh

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Abstract

Two cauliflower cultivars were karyotyped for correct characterization after orcein staining. Both cultivars have a simple chromocenter type of interphase nuclei and a gradient type of prophase chromosomes, indicating that they descended from a common ancestor. In addition, both strains were found to have 2n=18 chromosomes with an almost identical range of chromosome lengths. In terms of overall length, the white snow cultivar is almost 7 m shorter than the fresh market cultivar, indicating the presence of chromatin length diversification between specimens. In none of the cases was there a noticeable gradual decrease in chromosome length. The metacentric and submetacentric chromosomes were observed in both samples examined. Apart from other karyotypic features, TF, Syi and AsK % indicate the moderately symmetrical nature of the karyotype. As a result, the two cultivars of cauliflower were moderately primitive in nature. The collection of this karyotypic data will aid in the authentic characterization of the two cauliflower cultivars, which is essential information for the breeding program.

Key words: Karyotype, vegetable plants, characterization, cauliflower.

INTRODUCTION

Bangladesh is one of the most densely populated countries in the world. As a result, Bangladesh needs to increase food production to meet the demands of a growing population and an ever-changing new world. Apart from the main crops, i.e. rice, wheat, legumes, jute and so on, the government is trying to manage vegetable production initiatives as it is important for increasing food diversification and farmers' income. Vegetables have also played a crucial role in the growth and stability of Bangladesh's national economy (Sharmin *et al*., 2018). Aside from these benefits, it also helps create jobs, increase financial gain, generate higher returns and better earnings, and reduce poverty in developing countries like Bangladesh (Weinberger & Genova, 2005; Sharmin, 2015 and Mitra & Yonus, 2018).

For this reason, plant breeders in Bangladesh are constantly working to improve crop yield and quality, produce genetically modified crops and disease-resistant crops. As a result, vegetable production in Bangladesh has increased dramatically over the past forty years. Cauliflower, a slightly modified form of cabbage within the family Brassicaceae, so quite vegetable that it is grown for its edible masses of partially developed flower structures and fleshy stalks. The Mediterranean region gave the plant

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Risr. Nowadays it has become common in Bangladesh, Chaina, India, Poland, Italy, France and many alternative countries of the world. Cauliflower is one of the most important winter annual vegetables in Bangladesh, which plays a crucial role in human nutrition and economy. It's low-fat, low-carb, but a decent supply of lots of fiber and other essential elements. A high fiber intake has been linked to a significantly lower risk of developing coronary artery disease, stroke, high blood pressure, diabetes and obesity, a trusted source. It is commonly served cooked or raw in salads and relishes, cauliflower crust pizza, cauliflower and cheese soup. Its ingredients may strengthen bones, improve cardiovascular health, and prevent cancer (Beecher, 1994).

Therefore, these vegetable crops are critical for a variety of purposes. Due to their importance, they are grown all over the world. There are several (more than a hundred) historic and current commercial varieties in use around the world. Commercially, white cauliflower is most commonly grown in Bangladesh, although orange, green, purple, and brown varieties also exist. Due to its beneficial chemical composition, nutritional value and low price, this valuable vegetable has a wide range of uses. However, farmers in Bangladesh believe that some important limitations such as low yield, high quality seed and short seedling and growing time etc. are preventing farmers from growing this vegetable. As a result, these vegetables are not grown in our country like other profitable cash crops and our internal production can only cover a third of the total needs. But our neighboring country China is the largest cauliflower producer in the world. Many other countries such as India, Poland, Italy, France, etc. collectively produce significant amounts of cauliflower. Therefore, in order to meet the demand, it is very important to take measures to increase domestic production. With the development of special cauliflowers (available all year round), it is possible to expand the composition to meet the ever-increasing demands in these sectors through the development of special cauliflowers (available all year round). To increase production under these conditions, all that would be required is improved germplasm.

However, the crop has received very little analytical attention. Therefore, although it is a new area of research in Bangladesh, it has been reported that the genetic improvement of this crop is limited. Various vegetable crops can be improved through an appropriate breeding program. The genomic data is crucial for this type of program. But for vegetables, especially cauliflower, terribly limited data are available in Bangladesh, relating only to production, yield, vegetative growth and area, etc. (Monayem *et al*., 1998; Singh & Singh, 2000; Shara *et al*., 2002; Vagen *et al*., 2004; Haque *et al*., 2007; Khan *et al*., 2009; Khayer, 2009; Mamun *et al*., 2010; Hoq *et al*., 2012; Hasan *et al*., 2014; and Sharmin *et al*., 2018). Even for Bangladesh, no karyotypic information of this kind is available on the internet or in the relevant literature. Various scientists abroad reported a chromosome number of 2n=18 (Mukherjee, 1974; Murin, 1978; Snogerup et al., 1990; Chen et al., 2003 and Hasterok et al., 2006) and 2n=20, 36 (Datta & Deb, 1976) for *Brassica oleracea*. On the other hand, various organizations in Bangladesh (such as BARI, BAU, BINA and BADC) have germplasm of various vegetables.

These were characterized solely by their morphological features. This type of characterization is generally problematic because phenotypic traits are not always reliable. To this end, accurate characterization and genetic data of each germplasm are required to select the beneficial parents for the plant improvement program and additionally for their conservation. A thorough understanding of the genetic diversity within and between the genetic resources of the available germplasm is essential to a successful breeding program. Plant breeders will be able to select parental sources, resulting in different populations for selection (Esmail *et al*., 2008). A stable and reliable methodology should be used for the genetic characterization of different germplasm. Karyotype analysis is a unique method specific to each sample. Another karyomorphological parameter is the orcein staining properties of interphase nuclei and prophase chromosomes, which depend on the nature of heterochromatin condensation. Tanaka (1971) was the first to use orcein staining to classify them.

Several researchers later attempted to characterize interphase nuclei and prophase chromosomes using differential staining (Begum & Alam, 2004; Fawzia & Alam, 2011; Shahla & Alam, 2011; Warasy, 2013; Akther & Warasy, 2016; Sultana & Alam, 2016; Bonna *et al*., 2018 and Warasy, 2021). This study found that these traits can distinguish numerous species, including members of a species, varieties, cultivars, and morphological forms indicating different taxonomic status. Plant breeders have used cytogenetic studies, particularly karyotypic information, to control sets of chromosomes or individual chromosomes to solve various specific problems. For cytogenetic studies, karyotyping is an excellent tool to characterize unknown samples and elucidate their origin (Vanzela *et al*., 2003; Ali *et al*., 2005; Feitoza *et al*., 2010 and Xiong & Pires, 2011). In this study, karyotype analysis was used for the first time in Bangladesh to characterize two cauliflower cultivars as a vegetable crop. Consequently, the purpose of this study was: i. Compare the orcein staining properties of interphase nuclei and prophase chromosomes. ii. Determine the diploid chromosome number of each cultivar. iii. Prepare and analyze a full strength karyotype. iv. Use cytogenetic markers to characterize each species.

MATERIALS AND METHODS

To determine karyotypic characteristics, two germplasm of a vegetable plant, viz. white snow cultivar and fresh market cultivar of cauliflower (*Brassica oleracea* var. *botrytis* L.) were collected from Mim Seed Center, Nama bazar, Savar Dhaka and preserved in Botanical Garden, Department of Botany, Jahangirnagar University, Savar, Dhaka, Bangladesh. Roots (healthy) were collected from the Petri dish while seeds were kept in a water soaked petri dish for germination. The best root collection time for most dividing cells was 11:00-11:30 am. The roots were pretreated using cold water at room temperature (28-30°C), followed by fixation in acetic acid (45%) at 40°C for 15 minutes. These were then hydrolyzed for 8-10 seconds at 60°C in a 2:1 mixture of 1N HCl and 45% acetic acid. The hydrolyzed roots were then placed on a filter paper to soak and placed on a clean glass slide. A fine blade was used to cut the meristematic area. The material was treated with 1% acetoorcein. A coverslip was placed on the material. The materials were then gently tapped with a toothpick and

crushed with the thumbs. Finally, the slides were photographed with a digital camera and an Olympus DP72 microscope (Japan). The final measurement was obtained by dividing the objective magnification by 100x.

RESULTS AND DISCUSSION

Orcein stained interphase nuclei and prophase chromosomes: The staining properties of interphase nuclei and prophase chromosomes provide karyomorphological features that aid in the identification of distinct germplasm. *Brassica oleracea* var. *botrytis* cultivars were classified as a single chromocenter type in this study (Figures 1, 2, Table 1). The chromosomes of both strains were found to be gradient type in prophase, with the chromosomes being darkly stained at one end and gradually weakening at the other (Figures 3, 4, Table 1). As a result, one end of these chromosomes was significantly darker than the other.

Tanaka (1971) discovered that the heterochromatin nature in interphase nuclei and prophase chromosomes differed between species. He was the first to propose these criteria for karyomorphological characters. Tanaka (1971) classified interphase nuclei and prophase chromosomes into five groups based on the characteristics of heterochromatin staining. These criteria were later used by other scientists to characterize different plant materials (Begum & Alam, 2004; Akther & Warasy, 2016; Sultana & Alam, 2016; Bonna *et al*., 2018 and Warasy, 2021). Localized heterochromatin (as seen in interphase nuclei) can be found in a variety of locations on prophase chromosomes. As a result, the current results provide indications of the frequently observed regulation of heterochromatin distribution in prophase chromosomes. Staining characteristics of interphase nuclei and prophase chromosomes revealed the presence of facultative heterochromatin, which aggregates tightly in the interphase nuclei before occupying different locations on the prophase chromosomes. This finding suggests that constitutive heterochromatin is present in interphase nuclei and prophase chromosomes. There have been no reports on the nature of the interphase and prophase mitotic chromosomes after orcein staining in the available literature or internet sources. As a result, the pioneering attempt was to characterize two cultivars of *Brassica oleracea* var. *botrytis* L. using the parameters above.

Table 1. Orcein staining interphase nuclei and prophase chromosomes of two cultivars of cauliflower

Cultivars	Type of interphase nuclei	Type of prophase chromosomes
White snow cultivar	Simple chromocenter	Gradient
Fresh market cultivar	Simple chromocenter	Gradient

2n chromosome number: Both the cultivars of *Brassica oleracea* var. *botrytis* L. were found in the present study to have 2n=18 chromosomes (Figures 5, 6, 7, 8, 9, 10; Tables 2, 3, 4). In the case of Bangladesh, no chromosomal information about this species is available on the internet or in the relevant literature. As a result of this

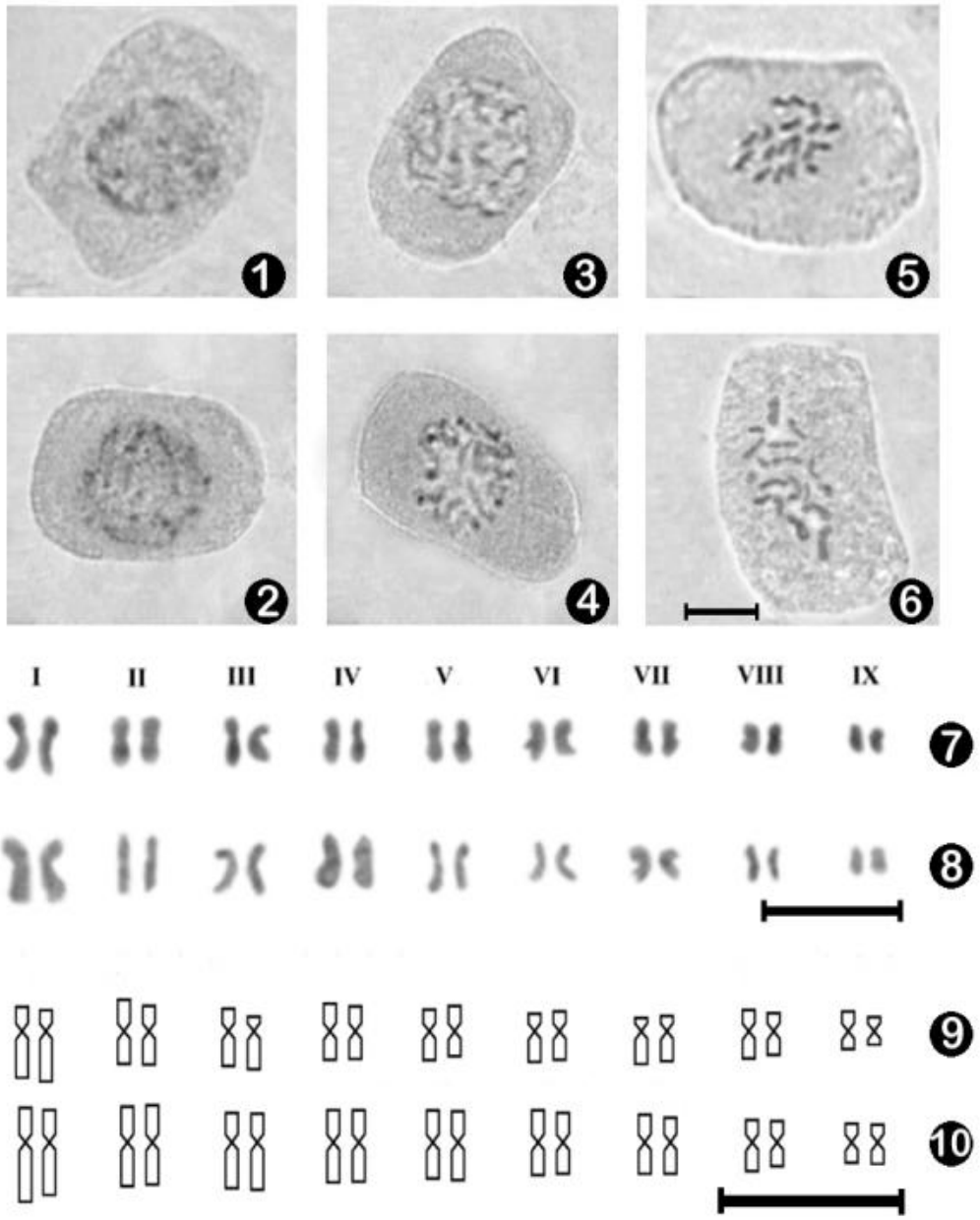
Brassica oleracea var. *botrytis* L. chromosome count was the first attempt in Bangladesh. However, the same chromosome number 2n=18 has been reported abroad by different scientists (Mukherjee, 1974; Murin, 1978; Snogerup *et al*., 1990; Chen *et al*., 2003 and Hasterok *et al*., 2006). The current finding was thus linked to the previous findings. In addition, 2n=20, 36 (Datta & Deb, 1976) was also reported for *Brassica oleracea* abroad.

Total chromosomal length: The total length of the 2n chromosome complement was measured in the present study to be 26.60±0.84 µm for the white snow cultivar and 33.33±2.75 µm for the fresh market cultivar (Tables 2, 3, 4). The overall length of diploid chromosomal complements was not similar. The white snow cultivar is almost 7 µm shorter than the overall length of the fresh market cultivar. Therefore, the current results clearly demonstrated the presence of chromatin length diversification between the samples examined in this study.

Table 2. Orcein-stained karyotypical features of cauliflower (White snow cultivar)

Chrom-osome pair	Long arm (l) ($\bar{x}$± SD)	Short arm(s) ($\bar{x}$ ± SD)	Total length (T) ($\bar{x}$ ± SD)	Arm ratio (l/s)	Relative length (RL)	Centro meric index (CI)	Centro meric type (CT)
I	1.33±0.04	0.68±0.01	2.01±0.05	1.95	0.08	33.89	sm
	1.42±0.03	0.58±0.02	2.00±0.04	2.47	0.08	28.83	sm
II	0.94±0.03	0.88±0.02	1.83±0.04	1.07	0.07	48.36	m
	0.95±0.03	0.72±0.01	1.66±0.03	1.32	0.06	43.09	m
III	1.00±0.01	0.63±0.02	1.63±0.02	1.57	0.06	38.85	sm
	1.18±0.01	0.42±0.04	1.60±0.05	2.79	0.06	26.40	sm
IV	0.84±0.02	0.76±0.03	1.60±0.05	1.11	0.06	47.40	m
	0.81±0.01	0.70±0.03	1.51±0.04	1.15	0.06	46.48	m
V	0.85±0.03	0.61±0.01	1.46±0.04	1.40	0.05	41.65	m
	0.74±0.03	0.72±0.01	1.46±0.04	1.03	0.05	49.20	m
VI	0.89±0.02	0.50±0.05	1.40±0.06	1.77	0.05	36.12	sm
	0.83±0.02	0.55±0.05	1.38±0.06	1.51	0.05	39.90	sm
VII	0.94±0.04	0.38±0.04	1.32±0.08	2.46	0.05	28.86	sm
	0.86±0.04	0.44±0.03	1.29±0.06	1.96	0.05	33.76	sm
VIII	0.70±0.05	0.59±0.03	1.28±0.07	1.19	0.05	45.71	m
	0.70±0.04	0.52±0.02	1.22±0.06	1.35	0.05	42.47	m
IX	0.54±0.04	0.55±0.01	1.12±0.08	0.99	0.04	50.30	m
	0.42±0.04	0.42±0.01	0.83±0.04	1.00	0.03	50.00	m
		Total	26.60±0.84				

Individual chromosomal length: In the two cultivars, the chromosomal length range was 0.83±0.04-2.01±0.05 µm for the white snow cultivar and 1.13±0.23-2.50±0.26 µm for the fresh market cultivar, in which no significant gradual decrease occurred (Figures 7, 8, 9, 10; Tables 2, 3, 4). The individual chromosome length range and mean chromosome length of the white snow cultivar and fresh market cultivar was almost similar.



Figs. 1-10. Mitotic interphase nuclei, prophase chromosome, metaphase chromosome, karyotype and Idiogram of two cultivars of *Brassica oleracea* var. *botrytis* L. by orcein-staining 1. Interphase nuclei of white snow cultivar. 2. Interphase nuclei of fresh market cultivar. 3. Prophase chromosome of white snow cultivar. 4. Prophase chromosome of fresh market cultivar. 5. Mitotic metaphase of white snow cultivar. 6. Mitotic metaphase of fresh market cultivar. 7. Karyotype of white snow cultivar. 8. Karyotype of fresh market cultivar. 9. Idiogram of white snow cultivar. 10. Idiogram of fresh market cultivar. Bar=5µ.

Relative length: The difference between relative length and range was the same and was 0.05 and 0.03-0.08, respectively (Table 4). With regard to this karyotypic parameter, a strict similarity was found between the two cultivars studied.

Centromeric feature: Both cultivars were found to have a slight difference in the centromeric index range, which was 26.40-50.30 for the white snow cultivar and 37.56-49.09 for the fresh market cultivar (Tables 2, 3). As a centromeric formula, 10 metacentric and 8 submetacentric chromosomes were found in the white snow cultivar. On the other hand, 14 metacentric and 4 submetacentric chromosomes were observed in fresh market cultivars. Therefore, both the metacentric and submetacentric chromosomes were observed in both samples examined, representing a moderately symmetrical karyotypic nature. According to Stebbins (1971), the symmetric karyotype indicates primitive character and the asymmetric karyotype indicates advanced character. From this point of view, two cultivars studied are plants of moderately primitive nature.

Table 3. Orcein-stained karyotypical features of cauliflower (Fresh market cultivar)

Chrom-osome pair	Long arm (l) ($\bar{x}$± SD)	Short arm (s) ($\bar{x}$ ± SD)	Total length (T) ($\bar{x}$ ± SD)	Arm ratio (l/s)	Relative lengt (RL)	Centro meric index (CI)	Centro meric type (CT)
I	1.53±0.12	0.97±0.38	2.50±0.26	1.59	0.08	38.67	sm
	1.47±0.06	0.90±0.36	2.37±0.31	1.63	0.07	38.03	sm
II	1.23±0.15	1.00±0.00	2.23±0.15	1.23	0.07	44.78	m
	1.17±0.21	1.03±0.06	2.20±0.20	1.13	0.07	46.97	m
III	1.30±0.10	0.83±0.15	2.13±0.21	1.57	0.06	38.97	sm
	1.33±0.15	0.80±0.17	2.13±0.21	1.67	0.06	37.56	sm
IV	1.13±0.15	0.90±0.10	2.03±0.12	1.26	0.06	44.26	m
	1.10±0.17	0.90±0.10	2.00±0.10	1.22	0.06	45.00	m
V	1.03±0.06	0.90±0.10	1.93±0.12	1.15	0.06	46.55	m
	1.07±0.12	0.87±0.12	1.93±0.12	1.23	0.06	44.83	m
VI	0.93±0.12	0.90±0.10	1.83±0.21	1.04	0.06	49.09	m
	0.90±0.10	0.83±0.06	1.73±0.12	1.08	0.05	48.08	m
VII	0.90±0.10	0.77±0.06	1.67±0.06	1.17	0.05	46.00	m
	0.90±0.17	0.70±0.10	1.60±0.10	1.29	0.05	43.75	m
VIII	0.83±0.15	0.63±0.06	1.47±0.12	1.32	0.04	43.18	m
	0.70±0.10	0.60±0.00	1.30±0.10	1.17	0.04	46.15	m
IX	0.60±0.17	0.53±0.06	1.13±0.23	1.13	0.03	47.06	m
	0.62±0.16	0.52±0.08	1.13±0.23	1.19	0.03	45.59	m
			Total	33.33±2.75			

Karyotype symmetry (Syi %) and asymmetry index (AsK %): The karyotype symmetry index was 66.29% in the case of the white snow cultivar and 77.88% in the fresh market cultivar (Table 4). Karyotype symmetry index values decreased with increasing asymmetry, indicating that the karyotype of both cultivars was moderately symmetric. In contrast, the karyotype asymmetry index was 59.89% for the white snow cultivar and 56.26% for the fresh market cultivar (Table 4). The value of AsK%

increased with increasing asymmetry. Total form percentage of the white snow cultivar and the fresh market cultivar were 40.00% and 43.74%, respectively. As a result, the above findings indicate that both cauliflower cultivas are moderately symmetrical.

Therefore, the two vegetables, viz. white snow cultivar and fresh market cultivar of cauliflower (*Brassica oleracea* var. *botrytis* L.) were karyotyped and characterized with the above traits, which are very important data and would be an asset to the crop improvement program.

Table 4. Comparative karyotype analysis in two cauliflower cultivars

Cytogenetical Parameters	White snow cultivar	Fresh market cultivar
2n	18	18
Total length of chromosome complement (μm)	26.60±0.84	33.33±2.75
Range of individual chromosome length (μm)	0.83±0.04-2.01±0.05	1.13±0.23-2.50±0.26
Average chromosomal length (μm)	1.48	1.49
Relative length range	0.03-0.08	0.03-0.08
Difference of relative length	0.05	0.05
Centromeric formula	10m+8sm	14m+4sm
Total form percentage	40.00	43.74
Karyotype symmetry index	66.29	77.88
Karyotype Asymmetry index	59.89	56.26

m = metacentric, sm = sub-metacentric chromosome.

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