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Chromosome Characterization of Brassicaceae Family

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ABSTRACT

Indonesia is known as a rich country in various agricultural and plantation products, including vegetables such as mustard, broccoli, cabbage, and cauliflower. However, in its cultivation, the products and demand for vegetables are not offset by an increase in the production quality. One of the efforts to improve and enhance the production quality is to identify and characterize chromosomes of plants which will become the basis for plant breeding activities. The purpose of this study was to characterize the number, form and size of the chromosome in cultivars belonging to the Brassicaceae family. The study was carried out using the modified squash method. Chromosomes were prepared by fixation, maceration, and staining, then the mitotic phases were observed using a microscope and optilab, and analyzed using Image Raster 3. The results showed that mitotic time range and chromosome character of six cultivars of the Brassicaceae family were different. Broccoli ('Chief No. 2 1955' and 'Green Super') and cauliflower ('ILONA' and 'TM 126') had a mitotic time range from 04.00 to 09.00 a.m. with 2n chromosome number = 18. Green mustard ('Juwita' and 'TM Jade') and white mustard ('Sakata' and 'Shuka-shuka') had a mitotic time range from 03.00 to 08.00 a.m. with 2n chromosome number = 20. White cabbage ('CR ACE' and 'Sehati F_1') had a mitotic time range from 04.00 to 09.00 a.m and red cabbage ('Scarlet' and 'Red Globe') had a mitotic time range from 09.00 to 10.00 a.m. with 2n chromosome number = 18.

Keywords: *Brassicaceae; chromosome; squash.*

INTRODUCTION

Food crops and horticulture represents some of the main commodities for the people of Indonesia which is an agricultural country. Indonesia has a tropical climate that is highly suitable for agriculture and plantations, thus causing this country to be very rich in types and cultivars of food crops. Some examples of crop plantation commonly cultivated by Indonesian people are including those from members of the Brassicaceae family such as green mustard, chicory, broccoli, white cabbage, red cabbage, and cauliflower. Indonesian people are very fond of eating plantation products from the Brassicaceae family as food commodities. This can be seen from data published by Central Statistics Agency (BPS) from 2007 to 2012 where individual consumption of green mustard in a week is 4% (Billah, 2017).

United States Department Agriculture (USDA) in 2011 stated that the demand for broccoli was 15-20% per year. Cabbage contributes 12.05% of vegetable productions in Indonesia (BPS, 2014). According to data from the Ministry of Agriculture in 2016, exports of cauliflower, cabbage and mustard greens reached 40,240 tons. Vegetable products in Indonesia and the consumption of Indonesian people for commodities are considered high, but they are not offset by the increase in product quality. Therefore, it is necessary to carry out basic research in the field of genetics as an effort to enhance the results of innovation in the field of plant breeding. There are some previous vegetable researches in the field of genetics, but few of them rarely investigated cell division and the number of chromosomes in it.

The purpose of this study was to know and characterize chromosomes (chromosome number, form and size) of broccoli (*Brassica oleracea* L. var Italica 'Chief No.2 1955' and 'Green Super'), cauliflower (*Brassica oleracea* L. var. Botrytis 'ILONA' and 'TM 126'), green mustard cultivar (*Brassica rapa* L. 'Juwita', 'TM Jade'), chicory (*Brassica rapa* ssp. Var. pekinensis 'Sakata' and 'Shuka-shuka'), white cabbage (*Brassica oleracea* L. var. Capitata 'CR ACE' and 'Sehati F_1') and red cabbage (*Brassica oleracea* var. Capitata f. rubra 'Scarlet' and 'Red Globe').

MATERIALS AND METHOD

Samples Collection

Sampling and seed survey were performed in Banyuroto village, Sawangan sub-district, Magelang regency, Central Java province. Vegetable seeds were purchased from farm shops in Sleman area. The chromosomes were prepared and observed at Genetic and Breeding Laboratory of the Faculty of Biology UGM. Vegetables seed materials of 6 (six) cultivars of the Brassicaceae family used in this study are shown in Table 1.

Table-1. Vegetable seeds of 6 (six) cultivars of Brassicaceae members

No.	Cultivar Name	Cultivar Names
1.	*Brassica oleracea* L. var Italica (Brocoli)	'Chief No.2 1955' 'Green Super'
2.	*Brassica oleracea* L. var. Botrytis (Cauliflower)	'ILONA' 'TM 126'
3.	*Brassica rapa* L. (Green mustard)	'Juwita' 'TM Jade'
4.	*Brassica rapa* ssp. *pekinensis* (White mustard)	'Sakata' 'Shuka-shuka'
5.	*Brassica oleracea* L. var. *capitata* f. *rubra* (Red cabbage)	'Scarlet' 'Red Globe'
6.	*Brassica oleracea* L. var. Capitata (White cabbage)	'CR ACE' 'Sehati F_1'

Source: Privately arranged

Materials and Tools

The chemical reagents used for chromosome preparation were obtained from the Genetic and Breeding Laboratory of the Faculty of Biology, Gadjah Mada University including Glacial Acetic Acid (AAG) 45%, 1N hydrochloric acid (HCl), Aceto Orcein 1%, distilled water, glycerin, clear nail polish, and tissue. The tools used were including petri dishes, rulers, scalpel, brushes, microtubes, ovens, refrigerators, glassware, closing glasses, label paper, dropper pipettes, suction pipettes, pens, pencils, cameras (Canon Optilab), light microscope Olympus BX-41, preparatory box, petridish, Erlenmeyer, and measuring cup.

Chromosome preparation

Chromosomes were prepared using the modified squash method from Nathewet et al. (2009). The chromosomes were optimized for 24 hours with an interval of 15 minutes. Chromosome optimization was done to determine the active mitotic time range. The primary roots on the seedlings of five vegetable cultivars in both cultivars were cut at ± 0.5 cm and preparation process was done every 15-30 minutes. Samples of the root tip were put into microtube which had been filled with glacial acetic acid (AAG) 45% at 0.5 ml until the cut roots were submerged thoroughly then the bottles were placed in the refrigerator at temperature of 4°C for 24 hours. AAG reagent 45% was removed and rinsed with distilled water 3 times. Then, a solution of 1N hydrochloric acid (HCl) was dropped continually until the roots were submerged evenly. Then the bottles were incubated at 57°C for 6 minutes. Hydrochloric acid (HCl) reagents were cleaned using distilled water 3 times. Afterwards, the root samples were soaked using Aceto Orcein 1% for 2 hours. The root tips were removed using a brush and placed on top of the glass object. Then the roots were cut at ± 2-3 mm using a razor blade in

the darker area of the roots. Then glycerin was dropped and then closed using a cover glass and the squashing process were done by pressing right on the root preparations using a blunt brush tip slowly until the cells appeared to spread smoothly on the object glass. The next step was to give the nail polish on the edge of the glass cover.

Observation and Photography

Observations were made using *Olympus BX41* microscopes and Optilab cameras with 100 x 40 magnifications. Photography was focused on the prometaphase which would be used for chromosome counting and the next stage was karyotyping. The best prometaphase images were selected and then measured and cut according to observations using Image Raster 3 and CorelDraw X6. Then the data were processed using Microsoft Excel 2007 application to create an idiogram. The number of chromosomes of each cultivar was counted directly on the images taken by the microscope at prometaphase use *Image Raster 3* program. The length of short arm (p) and the length of long arm (q) of chromosomes in each cultivars were measured using analysis of chromosome number, karyotyping in the form of long arm, short arm, centromere index, determination of chromosome form, and idiogram was created manually using *Image Raster 3, Photoscape v3.6, Corel Draw X6, and Microsoft Excel* applications. Data on the length of the chromosome sizes were then used to calculate the centromere index (CI) and the ratio of chromosome's long arms to short arms of (RLK) according to Ahloowalia (1965) (Table 2).

$$CI = \frac{\text{Length of chromosome short arm (p)}}{\text{Absolute length of chromosome (p+q)}} \times 100\%$$

$$RLK = \frac{\text{Length of chromosome long arm}}{\text{Length of chromosome short arm}}$$

Table-2. The chromosome forms based on the Centromere Index (CI) and the ratio of the chromosome long arm to the short arm (RLK)

Centromere Index	RLK	Chromosome Forms
37.50 - 50.00	1.00 - 1.68	Metacentric
25.00 - 37.49	1.68 - 3.00	Submetacentric
12.50 - 24.99	3.01 - 7.00	Acrocentric
0 - 12.49	≥ 7.00	Telocentric

Source: Ahloowalia (1965)

RESULTS AND DISCUSSION

Brassicaceae family consists of broccoli (*Brassica oleracea* L. var Italica 'Chief No.2 1955' and 'Green Super'), cauliflower (*Brassica oleracea* L. var. Botrytis 'ILONA' and 'TM 126'), green mustard cultivar (*Brassica rapa* L. 'Juwita', 'TM Jade'),

chicory (*Brassica rapa* ssp. var. pekinensis 'Sakata' and 'Shuka-shuka'), white cabbage (*Brassica oleracea* L. var. Capitata 'CR ACE 'and' Sehati F_1') and red cabbage (*Brassica oleracea* var. Capitata f. *rubra* 'Scarlet' and 'Red Globe'). The active mitotic time range of 6 (six) cultivars of the Brassicaceae family was starting from 06.00 to 10.00 a.m. Meanwhile, the numbers of chromosomes acquired from each Brassicaceae member cultivars are shown in Figure 1. The figure 1 shows that the chromosome numbers of 6 (six) cultivars of Brassicaceae were different. Green mustard (Juwita and Jade cultivars) and white mustard (Sakata and Shuka-shuka cultivars) have 20 chromosomes, while white cabbage (CR ACE and Sehati F1cultivars), red cabbage (Scarlet and Red Globe cultivars), broccoli (Chief No.2 1955 and Green Super), and cauliflower (kulitvar ILONA and TM 126) have 18 chromosomes.

The difference in chromosome number of cultivars belonging to the Brassicaceae family may be caused by the fact that each cultivar and have different genomic content from one another which will change or influence the form, size, structure and gene sequence of chromosome. Although the same genus has the same basic chromosome number, but the genomic content of such chromosome would be different where it is influenced by a cross breeding system by plant breeders or can also be influenced by environmental factors. As a result of the differences in the cross-breeding method which may affect the meiosis process in the cultivars, the pattern and arrangement of genes that are spread on the plant chromosomes will also be affected. Environmental factors can affect the activation time and location of related genes in cell cycle activity in these plants, so the plants that had been adapted to different environments will certainly have different mitotic time range. The same cultivar not only have different mitotic time range, they can also have distinct phenotypes.

The number of chromosomes can be known and counted through the prometaphase stage in mitotic division. In this phase, chromosomes moved jerkily which caused the chromosomes to be condensed and spread to the cytoplasm, making it easier to count the number of chromosomes without observing the overlapping chromosomes. Plants belonging to the same cultivar have the same basic chromosome number, this is because one of the characters expressing an organism in the same cultivar besides the morphological character of the flower as a reproductive organ is the number of basic chromosomes.

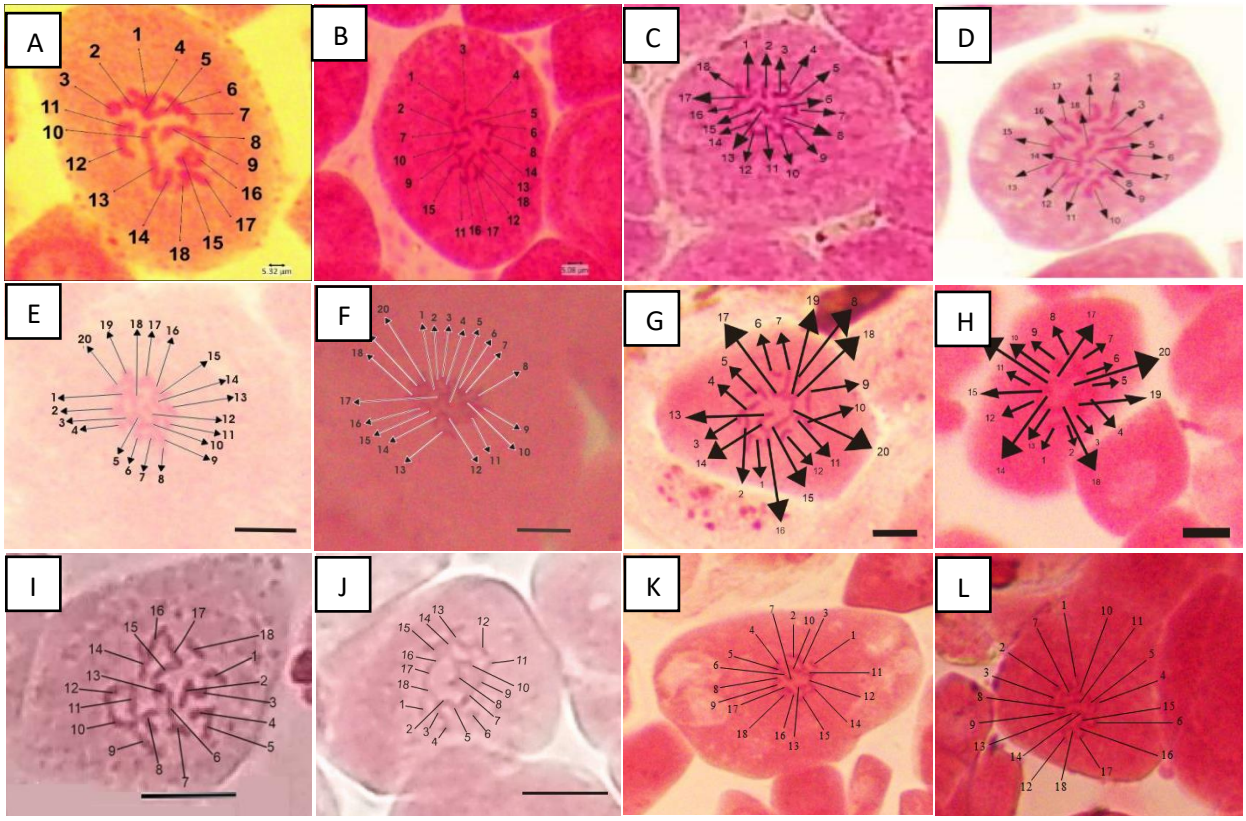

Figure-1: Number of chromosomes of six cultivars belonging to Brassicaceae family. Broccoli (*Brassica oleracea* L.'Chief No.2 1955' (A) and 'Green Super' (B)); Cauliflower (*Brassica oleracea* L.'ILONA' (C) and 'TM 126' (D)); Green Mustard (*Brassica rapa* L. 'Juwita' (E) and 'TM Jade' (F)); White Mustard (*Brassica rapa* ssp. Pekinensis 'Sakata' (G) and 'Shuka-shuka' (H)), White Cabbage (*Brassica oleracea* L.'CR ACE' (I) and 'Sehati F_1' (J)); and Red Cabbage (*Brassica oleracea* var. capitata f. *rubra* 'Scarlet'(K) and 'Red Globe' (L)). Source: Private results.

Plants belonging to the same cultivar can pollinate one another and can produce fertile progeny thereby some plants can be produced under cultivar categories such as subcultivar, varieties and cultivars that can be crossbred and produce new fertile plants. Besides being caused by the suitability of the reproductive organ, it is also due to the same basic chromosome number. Thus, in the meiosis process each chromosome can have a homolog, because the chromosome numbers of both parents are the same. However, in one family, there are cultivar which have varying numbers of basic chromosomes. This is caused by the fact that when the level of the taxon is higher, there will be more differences, including difference in molecular level. The difference in the number of basic chromosomes represents a reproductive isolation mechanism to prevent interbreeding occurrence between different cultivar, genus, families or other categories. If breeding occurred between two different cultivars, then it will not produce new progeny due to differences in the basic chromosome number. However, some plants can naturally or artificially produce fertile or sterile new progeny.

Karyotype analysis and comparison of six cultivar of Brasicaceae family (Figure 2) showed differences in chromosome formulas, form, number, and sizes. Figures 2A and 2B show a comparison of chromosomes karyotype in broccoli plants of 'Chief No. 2 1955' and 'Green Super' cultivars where such chromosomes are different, which are 2n=18=14m+4sm with absolute lengths of 0.72 to 1.14 µm and 2n=18=16m+2sm with absolute lengths of 1.42 to 3.55 µm. Broccoli of 'Chief No. 2 1955' and 'Super Green' cultivar have asymmetrical karyotype with various formulas as they consist of metacentric and submetacentric chromosomes. In the 'Chief No. 2 1955' cultivar, the submetacentric chromosomes are located on pairs of chromosomes number 1 and 2, while in cultivar 'Green Super', they are located on pairs of chromosomes number 3.

Figure 2C and 2D show the comparison of chromosome karyotype in cauliflower of 'ILONA' and 'TM 126' cultivars, respectively. Both have the same form and arrangement of metacentric chromosomes. This is known from the measurement of each chromosome by measuring the ratio of the length of the chromosome's long arm short arms. The data showed that the two cultivars are belonging to the same cultivar and the arrangement of genes in the chromosomes also showed a high degree of similarity.

Figures 2E and 2F show a comparison of chromosome karyotype on green mustard of 'Juwita' cultivar and green mustard of 'TM Jade' cultivar that have the same chromosome formula, but have difference in absolute lengths of 2n=20=18m+2sm with absolute lengths of 0.86 to 2.25 µm and Juwita cultivar has absolute lengths of 1.23 to 2.18 µm. Green mustard of TM Jade and Juwita cultivars has asymmetrical karyotype with various formulas, because they consist of metacentric and sub-metricric chromosomes. This plant can be considered as a result of new cultivation and is more advanced (Singh, 1999). Both cultivars have the same chromosome formula, but also have differences in sub-metacentric chromosomes location. In TM Jade cultivars, sub-metricric chromosomes are located on the number 4 chromosome pair, and number 1 in Juwita cultivars.

Figures 2G and 2H show the comparison of chromosomes karyotype in chicory plants of 'Sakata' and 'Shuka-Shuka' cultivars, having different chromosomes of 2n=20=20m with absolute lengths of 1.5 to 3.3 µm and 2n=20=16m+4sm with absolute lengths of 1.4 to 3.8 µm. White mustard of 'Shuka-shuka' cultivar has metacentric chromosomes, while 'Sakata' cultivar has submetacentric chromosome pairs on number 2 and 6.

Figures 2I and 2J show the comparison of the chromosome karyotype in cabbage plants of 'CR ACE' and 'Sehati F1' cultivars having different chromosomes of 2n=18=8m+10sm with absolute chromosome lengths of 0.19 to 0.93m and

2n=18=10m+8sm with the absolute chromosome length of 0.19 to 1.08 μm. Both cultivars have metasentric and submetacentric chromosomes. The morphology of the habitus shows difference. The 'CR ACE' cultivar has light green leaves, while 'Sehati F1' cultivar has 'dark green' leaves.

Figures 2K and 2L show the comparison of the chromosome karyotypes in the red cabbage plants of 'Scarlet' and 'Red Globe' cultivars having different chromosomes of 2n=18=12m+6sm with absolute arm lengths of 1,285 to 3.11 μm and 2n=18=14m+4sm with absolute arm length of 1,495 up to 3,065μm. Both cultivars have metasentric and submetacentric chromosomes. There are differences in the habitus morphology where 'Red Globe' cultivar has a lighter weight of crop around 1.5 kg while the 'Scarlet' cultivar has a crop weight about 2-2.5 kg. "Scarlet" cultivar has faster sprout growth than "Red Globe".

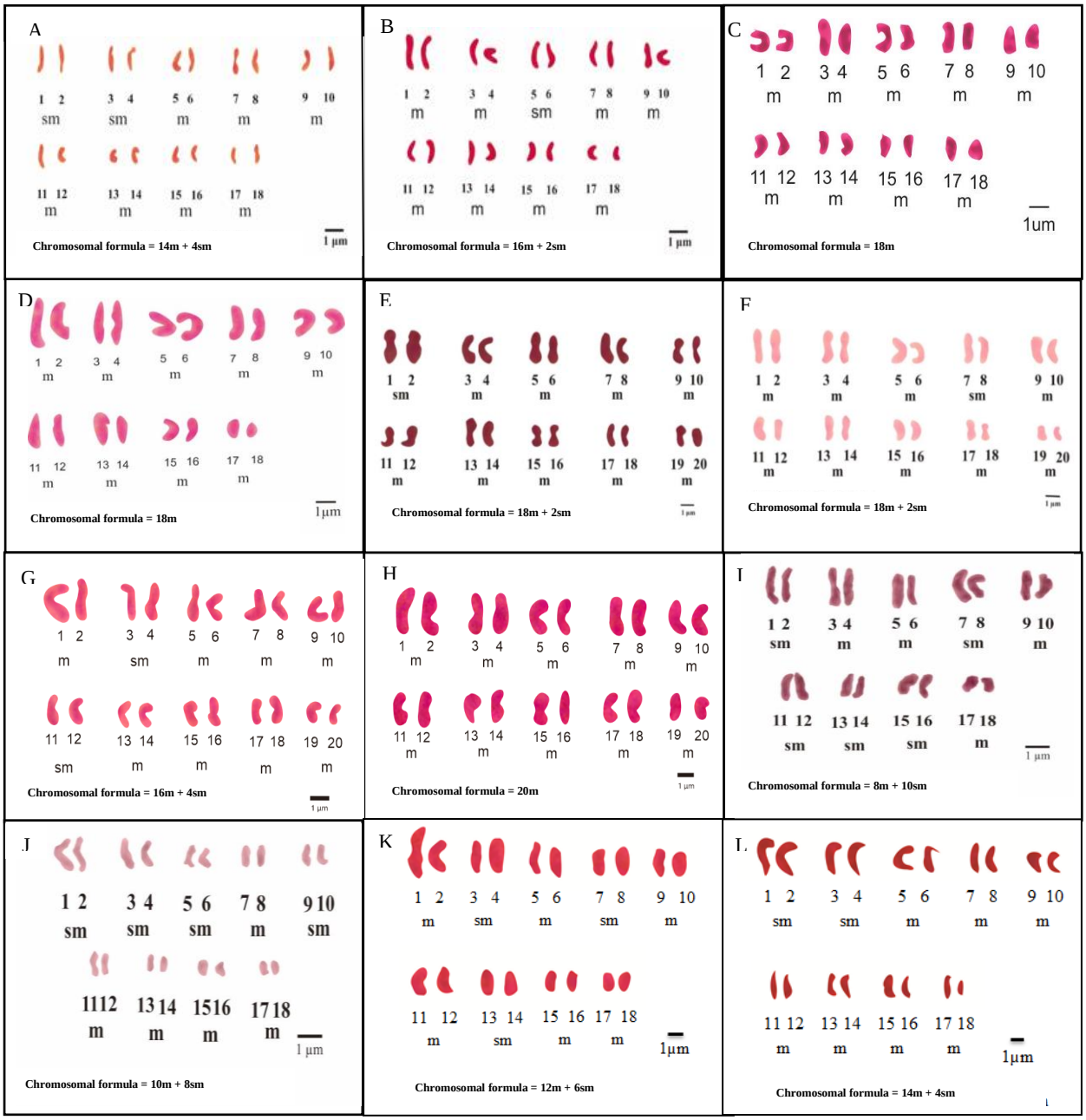

Figure-2. Chromosome Karyotype of several vegetable cultivars belonging to Brassicaceae. Broccoli (*Brassica oleracea* L. 'Chief No.2 1955' (A) and 'Green Super' (B)); Cauliflower (*Brassica oleracea* L. 'ILONA' (C) and 'TM 126' (D); Green Mustard (*Brassica rapa* L. 'Juwita' (E) and 'TM Jade' (F); White Mustard (*Brassica rapa* ssp. Pekinensis 'Sakata' (G) and 'Shuka-shuka' (H), White Cabbage (*Brassica oleracea* L. 'CR ACE' (I) and 'Sehati F_1' (J); and Red Cabbage (*Brassica oleracea* var. Capitata. f. *rubra* 'Scarlet' (K) and 'Red Globe' (L))

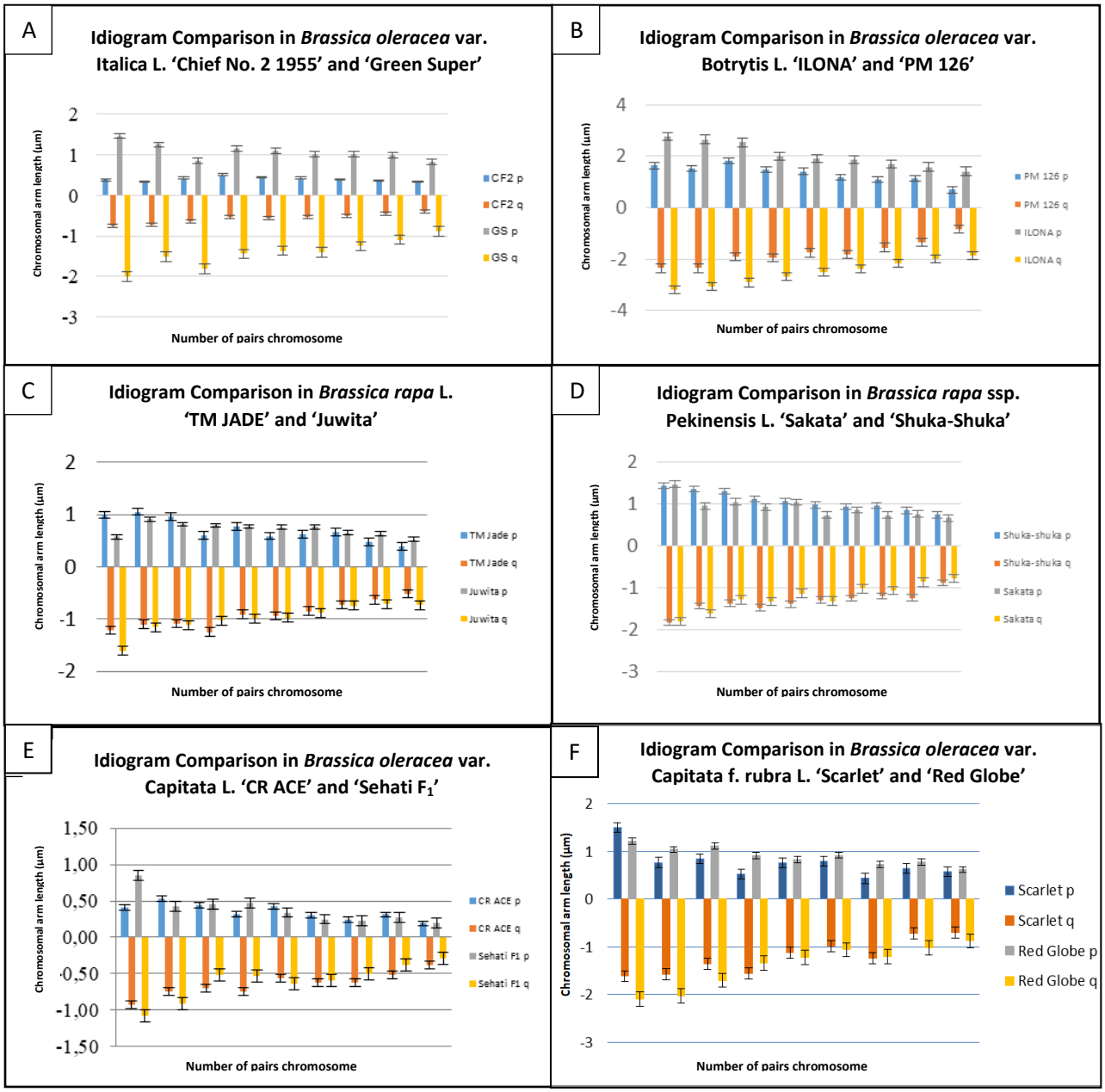

Figure-3. The results of ideogram comparison of six cultivar belonging to the Brassicaceae family. Broccoli (*Brassica oleracea* L. 'Chief No.2 1955' and 'Green Super' (A); Cauliflower (*Brassica oleracea* L. 'ILONA' and 'TM 126' (B); Green Mustard (*Brassica rapa* L. 'Juwita' (E) and 'TM Jade' (C); White Mustard (*Brassica rapa* ssp. Pekinensis 'Sakata' and 'Shuka-shuka' (D), White Cabbage (*Brassica oleracea* L. 'CR ACE' (I) and 'Sehati F_1' (E); and Red Cabbage (*Brassica oleracea* var. Capitata. f. *rubra* 'Scarlet' and 'Red Globe' (F)

Differences in the size and the arrangement of chromosomes will affect the location of genes at molecular level, and differences in chromosome level may have a large influence on the phenotype expressed. Although both cultivars are belonging to the same cultivar, they have different phenotypes due to differences in the chromosome level. The differences in phenotypic characters in plants are influenced by environmental factors. However, such differences are also

influenced by differences in the arrangement of genes on chromosomes which can express different phenotypic characters.

Figure 3 in line with the comparison results of the ideogram of six cultivar belonging to the Brassicaceae family shows that *Brassica oleraceae* of Green Super cultivar has a longer chromosome size both in the short and long arms of the chromosome in general when compared with Chief No.2 cultivar. Ilona and PM 126 cultivars have a longer chromosome size. Vegetables of *Brassica rapa* L. of TM Jade and Juwita cultivars have the short arms of chromosome which are not much different, but for long arms of chromosome, the Juwita cultivar has a longer long arm when viewed in general. *Brassica rapa* ssp. Pekinensis L of Shuka-Shuka varieties has a longer chromosome size compared with chromosome size of the Sakata variety in both long arms and short arms. *Brassica oleracea* of both cultivars namely CR ACE and Sehati F_1 has short arm lengths of chromosomes that are not much different from each other, but for the long arm of the chromosome, CR ACE cultivars generally have a longer size than Sehati F_1 cultivar. Red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*) and Red Globe cultivars tend to have a longer chromosome size both in short arms and long arms compared with Scarlet cultivars.

Cultivated plants of the Brassica genus may be diploid or amphiploid plants in nature. Basic diploid cultivars are including *B. nigra* (BB, 2n=16), *B. oleracea* (CC, 2n=18) and *Brassica campestris* (AA, 2n=20). The *B. campestris* cultivar has another name, namely *B. rapa*, consisting of green mustard, chicory/white mustard, and etc. *B. oleracea* consists of some members such as broccoli, cauliflower, cabbage, and etc. Amphidiploid cultivar may include *B. carinata* (AABB, 2n=34), *B. juncea* (BBCC, 2n=36), and *B. napus* (AACC, 2n=38). Cros-breeding between basic cultivar is supported by dihaploid genome behavior (Ramanujam and Srinivasachar, 1943). In addition, from the data we can also show that *B. Juncea* is an amphidiploid of *B. campestris* X B. nigra. *B. napus* is an amphidiploid of *B. campestris* X B. oleracea, and *B. carinata* is an amphidiploid of *B. campestris* X B. Nigra. *Arabidopsis thaliana* is a wild cultivar of *Brassicaceae* which is an ideal plant for genetic and molecular studies (Lan et al., 2000).

Regarding the results of the research, the plants belonging to the *Brassica* genus that have been used as seeds for agricultural cultivation as a result of breeding used in the study have the same number of chromosomes sets as their ancestor. Both ancestors are in the *Brassica* genus, *B. rapa* and *B. oleracea*. Thus, it can be said that the cultivated plants belonging to the Brassica genus have primitive properties based on their chromosome analysis, or they tend to maintain their ancestor properties. This is because there are no many numbers of chromosome sets (amphiploid) as in *B.*

carinata, *B. juncea*, and *B. napus* which are classified as modern cultivar of the Brassica genus in cultivated plants.

In Brassicaceae, even the scant data on karyotype evolution based on chromosomal data, it has provided some cytogenetic signatures supporting and re-defining the proposed phylogenetic topologies. Chromosome morphology and structure of most of the crucifer cultivar are closely linked to the discrete distribution of repetitive DNA elements along a longitudinal chromosome axis. Additional, chromosome karyotype of most cultivar influences how long cultivation and breeding. Base chromosome numbers vary from x = 4–17 with more than one-third of the taxa having karyotypes based on x = 8 (Warwick and Al-Shehbaz 2006), implying that x = 8 is most likely an ancestral chromosome number of the whole family. Base chromosome numbers are practical in recognizing diploids (2x) from higher ploidy levels (3x, 4x, etc.) within a given taxon.

Moreover, generic base numbers based on a lowest chromosome count available do not reflect the true nature of diploid-like genomes which were often influenced by paleo- and mesopolyploid events followed by subsequent diploidization base chromosome numbers vary from x = 4–17 with more than one-third of the taxa having karyotypes based on x = 8 (Warwick and Al-Shehbaz 2006), implying that x = 8 is most likely an ancestral chromosome number of the whole family. Base chromosome numbers are practical in recognizing diploids (2x) from higher ploidy levels (3x, 4x, etc.) within a given taxon. However, frequent auto- and allopolyploid events increasing the number of chromosome sets have been followed by cultivar and lineage-specific chromosome reshuffling. Chromosome fusions and fissions are causing an intra-generic numeric variation known as descending and ascending dysploidy (Lysak and Koch, 2010). Therefore, several crucifer genera (e.g., *Brassica*, *Cochlearia*, *Diplotaxis*, *Erysimum*, and *Physaria*) are polybasic, i.e., characterized by multiple base chromosome numbers (Warwick and Al-Shehbaz 2006). This makes the base number concept impractical in some genera. Moreover, generic base numbers based on a lowest chromosome count available do not reflect the true nature of diploid-like 1 Phylogeny, Genome, and Karyotype Evolution of Crucifers (Brassicaceae) 15 genomes which were often influenced by paleo- and mesopolyploid events followed by subsequent diploidization.

CONCLUSION

The mitotic time range, chromosome number, Karyotype and Ideogram in six cultivars of the Brassicaceae family are different and have their own uniqueness. Broccoli cultivar (*Brassica oleracea* L. var Italica 'Chief No.2 1955' and 'Green Super') and cauliflower (*Brassica oleracea* L. var. Botrytis 'ILONA' and 'TM 126') have a

mitotic time range from 04.00 to 09.00 a.m. with 2n chromosome number=18. Green mustard cultivar (*Brassica rapa* L. 'Juwita', 'TM Jade') and white mustard (*Brassica rapa* ssp. var. pekinensis 'Sakata' and 'Shuka-shuka') have a mitotic time range from 3.00 to 8.00 a.m. with 2n chromosome number=20. White cabbage cultivar (*Brassica oleracea* L. var. Capitata 'CR ACE' and 'Sehati F_1') has a mitotic time range from 04.00 to 09.00 a.m and red cabbage (*Brassica oleracea* var. *capitata* f. *rubra* 'Scarlet' and 'Red Globe') has a mitotic time range from 09.00 to 10.00 a.m. with 2n chromosome number=18.

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