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Chromosome Characterization of *Bartek* (*Cucumis Melo* L. var. *Bartek*), Local Melon Variety from Pemalang

Karakterisasi Kromosom Timun Bartek (*Cucumis Melo* L. var. *Bartek*), Varietas Melon Lokal dari Pemalang

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ABSTRACT

Bartek is one of local melon varieties which mainly cultivated in Pemalang, Central Java. Bartek has three shapes of fruit variation; Long-Green, Ellipse-Green, and Yellow. Chromosome characterization of the Bartek was investigated to determine the genetic variation. The main purpose of this research was to determine the genetic characters of Bartek including chromosome number, mitosis, cell cycle, and karyotype. Squash method was used for chromosome preparation. The results showed that all of Bartek observed in this study have similar diploid (2n) chromosome number = 24. According to total number of chromosome, Bartek is more related to melon. The mitotic analysis exhibited that the Bartek has same Karyotype formula, 2n = 2x = 24m. According to the R value of the three kind of Bartek (R< 0.27), it has indicated that three kind of Bartek were considered to be originated from same species and one of melon varieties (*Cucumis melo* L. var. *Bartek*).

Key words: cucumber, bartek, chromosome, karyotype

INTISARI

*Timun Bartek adalah suatu varietas melon lokal yang umum dibudidayakan di Pemalang, Jawa Tengah. Timun Bartek memiliki bentuk dan warna buah yang bervariasi, yaitu Hijau panjang, Hijau lonjong, dan Kuning. Untuk mengetahui karakter dan variasi genetik pada timun Bartek, dilakukan pengamatan karakter kromosomnya. Penelitian ini bertujuan untuk mengetahui karakter kromosom timun Bartek meliputi waktu mitosis aktif dan prometafase, jumlah, bentuk, ukuran, susunan kromosom, serta karyotype. Metode yang digunakan untuk preparasi kromosom dalam penelitian ini adalah metode pencet (*squash*). Hasil yang diperoleh menunjukkan bahwa ketiga timun Bartek memiliki jumlah kromosom* diploid *(2n) yang sama yaitu 24. Berdasarkan jumlah kromosomnya, Timun Bartek lebih tepat dikelompokkan dalam spesies melon. Hasil analisis karyotype menunjukkan bahwa ketiga timun Bartek memiliki formula karyotype yang sama yaitu 2n = 2x = 24m. Berdasarkan perbedaan selisih nilai R dari ketiga timun Bartek (R<*

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0,27) menunjukkan bahwa ketiga timun Bartek tersebut digolongkan dalam spesies yang sama dan merupakan salah satu varietas melon (Cucumis melo L. var. Bartek.

Kata kunci *: mentimun, Bartek, kromosom, karyotype*

INTRODUCTION

Melon (*Cucumis melo* L.) is a climbing herbaceous annual fruit vegetable belonging to the family Cucurbitacea, which consists of 90 genera and 750 species. It is a cross-pollinated diploid ($2n = 2x = 14$) species. Melon was originally monoecious, as are many modern cucurbit plants, but gynoecious and andromonoecious cultivars were subsequently bred. Male and female flowers were typically borne at different nodes, with the female flowers at higher nodes than the male. Fruits are round to ellipse or narrowly cylindrical, with small tubercles and spines of trichome origin on the rind. *Cucumis* is a genus of about 25 species, mostly tropical African in distribution. Some are confined to tropical Asia (Robinson and Walters, 1999).

Melon is commonly planted in several regions in Indonesia. In Java and Sumatera, melon planted in low land area. It is usually used for table fruit, fresh consumed fruit, and cocktail. However, improvement of melon is slow, especially for local variety of melon, because it has small and un-similar fruit shape. Most of farmers choose hybrid melon for this economic reason (Threhane *et al.*, 1995). Bartek is one of local melon varieties which is mainly cultivated in Pemalang, Central Java. Bartek is usually planted in low land farm on the dry season with 21ºC-27ºC temperature and it needs 2 months to be harvested. Bartek is a unique local melon, since it has three shapes of fruit in one cultivar; Long-green, Ellipse-green, and Yellow. However, the genetic identity of Bartek has not been studied yet. Hence, chromosome studies of Bartek are absolutely needed due to their implications for melon breeding program in this country. In addition, the melon industry is today critically dependant on genetically superior cultivars to improve fruit quality, disease resistance, and ability to tolerate in environmental stresses. The objective of this research was to examine mitosis period, chromosome number, chromosome size, and karyotypes of Bartek. The findings of the research are expected to enrich valuable information concerning the genetic identity of the Bartek to develop local melon quality through breeding program.

Chromosome characterization can be used to determine the genetic differences among plant cultivars. Stace (1979) stated that closer relation among the cultivars will make higher similarity in their chromosome number. The chromosome number inside the nucleus was generally stabile in each species (Kimball, 1983). Chromosome characterization usually done by study the mitotic cell division, because the morphological characters of chromosome in this cell division is stabil and can be seen clearly (Min *et al.*, 1984). The whole components of chromosome inside the cell is called karyotype. Karyotype is important to determine the identity of organisms, such as identify the taxonomical unit, biological diversity, evolution process analisys, and to determine the genetic abnormality of organisms (Jones and Luchsinger, 1979).

MATERIALS AND METHODS

1. Chromosome Preparation

A chromosome preparation procedure of the Bartek followed those described in Jahier and Tanguy (1996). Seeds of Bartek collected from Pemalang, Central Java were sown and germinated in Petri-dishes at Genetics Laboratory, Faculty of Biology, Gadjah Mada University, Yogyakarta. Chromosome preparation was conducted from 08.00 a.m. to 11.00 a.m. (WIB) with 15 minutes interval. Fresh root tips from germinated seeds of the Bartek were fixed in 45% acetic acid at 4°C for 15 minutes. Fixed root tips were then macerated in 1 N hydrochloric acid for about 11 minutes at 55°C. The root tips were stained in 1% aceto-orcein for about 24 hours before they squashed. The slides were then photographed using Olympus C-35-AD-4 and Fuji ASA 200 film.

2. Chromosome Analysis

The measurement of chromosome size was made on the chromosomes observed at prometaphase using Adobe Photoshop CS2 for Windows program. Centromere position of chromosome was classified by centromeric index calculated by short arm/ total length accorded to Levan *et al*. (1964) : metacentric chromosome with centromeric index of 37.50-50.00; submetacentric chromosome with centromeric index of 25.00-37.49; subtelocentric chromosome with centromeric index of 12.5-14.99; and telocentric chromosome with centromeric index of 0-12.49. Data of chromosome size and centromere position of chromosomes were then arranged to construct karyogram using Adobe Photoshop CS2 for Windows program, and ideogram using CorelDRAW Graphic Suite X3.

RESULTS AND DISCUSSION

Bartek has three shapes of fruit; Long-Green, Ellipse-Green, and Yellow. However, there are no specific morphological differences of the plants that produce those kinds of fruit. Bartek is generally used as a mix of a cocktail. The stems of Bartek are usually herbaceous, climbing, and branched. It can reach 138-215 cm in length with 0,7-1 cm diameters. The leaf of Bartek is about 13-18 cm long with 14-18 cm wide. The fruits of Bartek have many variations in size, but commonly, it is about 10-12 cm in length. The characters that differ the three kinds of Bartek's fruit are the color and shape of the fruits. The color of the flesh fruits of Bartek usually change from white or pale-yellow to orange when it starts to mature.

The results exhibited that mitosis period of Bartek mainly occurred from 08.00-11.00 a.m. (WIB). The finding of mitosis period of Bartek investigated in this study is useful to obtain prometaphase stages used to examine chromosome characters. Prometaphase stage of Yellow Bartek was found at 08.50 a.m. (WIB), whereas prometaphase stage of Long-green and

Elips-green Bartek were found at 09.00 a.m. (WIB).

Figure 1. The shape and color of Bartek's fruits

Three kinds of Bartek investigated in this study had similar chromosome numbers, 2n = 24. This count was not similar from the previous studies reported by Robinson and Walters (1999), who recorded that the diploid chromosome number of cucumber species was 14. However, Robinson and Walters (1999) also explain that the most common haploid numbers of Cucurbitaceae's chromosome were 11 and 12. Therefore, it could be concluded that Bartek was Cucurbitaceae family member and one of melon varieties. According to the research of PI 371795 melon and American muskmelon reported by Winarsih (2007) and Dewei *et al*. (2005) the chromosome number of Bartek is more related to Melon (2n=2x=24). In previous studies, the *karyotype* formula of several species in *Cucurbitaceae* family had been investigated. In this case, melon used as the comparison of Bartek because their similarity in chromosome number (2n=2x=24). The *karyotipe* formula of several cultivars of *Cucurbitaceae* showed variation. *Karyotype* formula that same to Bartek belongs to PI 371795 melon (2n=2x=24). This result was reported by Winarsih (2007). While, the different karyotype formula belongs to *American muskmelon* (*Cucumis melo* var. *reticulatus*) which has 2n=2x=20m+2sm+2sm(SAT) chromosome formula, and Hetian melon (*Cucumis melo* spp. *melo* Pang) which has 2n=2x=24=18m+4M+2sm chromosome formula (Dewei *et al.*, 2005). Those *karyotype* formula showed that *American muskmelon* and *Hetian melon* have two types of chromosome, metacentric and submetacentric chromosome, which then determined to be symmetry and asymmetry. The close relation between Bartek and Melon can also bee seen from the shape of the seed. The seed of Bartek is more similar to melon than cucumber.

Figure 2. The comparison of the seed's shape of cucumber, Bartek, and melon

Singh (1999) stated that symmetry *karyotype* was more primitive than the asymmetry *karyotype*. Therefore, it could be known that plant varieties with asymetric chromosome in its *karyotype* have better step of evolution than plant varieties with symetric chromosom in all of its part. It also could be revealed that Bartek has ancient evolution step because it doesn't have asymetric chromosome in its *karyotype*.

Moreover, the chromosome number of Bartek is also similar to white Bartek (Ariwarsi, 2008) and Timun Suri (Wicaksono, 2008). In addition all chromosome of Bartek also appeared to have the centromere in the median region and were thus classified as metacentric according to Levan *et al*. (1964) displaying similar karyotype formula 2n = 2x = 24 = 24m. In addition, autotetraploid chromosome numbers were appeared in Ellipse-Green Bartek (2.22%). It was small number compare to diploid chromosome (97.78%). Autotetraploid chromosome indicates natural mutation because Bartek has not been developed using polyploidization mutation agent. The centromeric index of Yellow, Ellipse-Green, and Long-Green Bartek was about 43.283-48.065; 42.404-47.531, and 41.649-47.460 respectively. The finding of metacentric chromosomes in Bartek revealed that Bartek investigated in this study have symmetry *karyotypes* indicating that Bartek have not been widely developed through breeding program. The phylogenetic research reported by Renner, *et al.* (2007) showed that the relation between cucumber and melon is not too close. Based on congruent nuclear and chloroplast phylogenies, they concluded that *Cucumis* comprises an old Australian/Asian component that was heretofore unsuspected. *Cucumis sativus* evolved within Australian/Asian clade is phylogenetically far more distant from *Cucumis melo* than implied by the current morphological classification. Hence, since Bartek has similar chromosome numbers with melon, it could be revealed that Bartek and melon are same species.

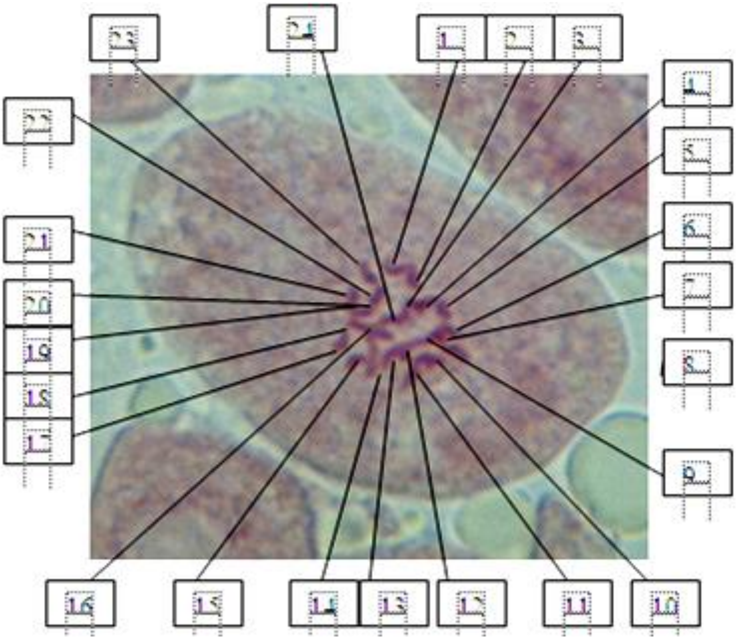

Figure 3. Total number of diploid chromosomes (2n) = 24 in Bartek

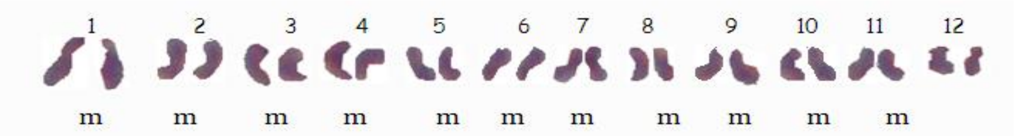

Figure 4. Karyotype of Yellow Bartek. Scale bar correspond to 5µm. (m = metacentric chromosome; number under the karyotype correspond to chromosome pairs)

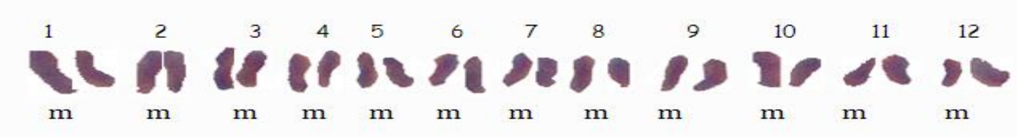

Figure 5. Karyotype of Ellipse-Green Bartek. Scale bar correspond to 5µm. (m = metacentric chromosome; number under the karyotype correspond to chromosome pairs)

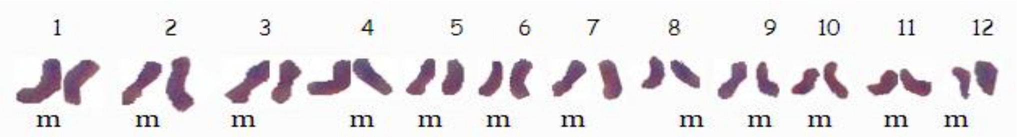

Figure 6. Karyotype of Long-Green Bartek. Scale bar correspond to 5µm. (m = metacentric chromosome; number under the karyotype correspond to chromosome pairs)

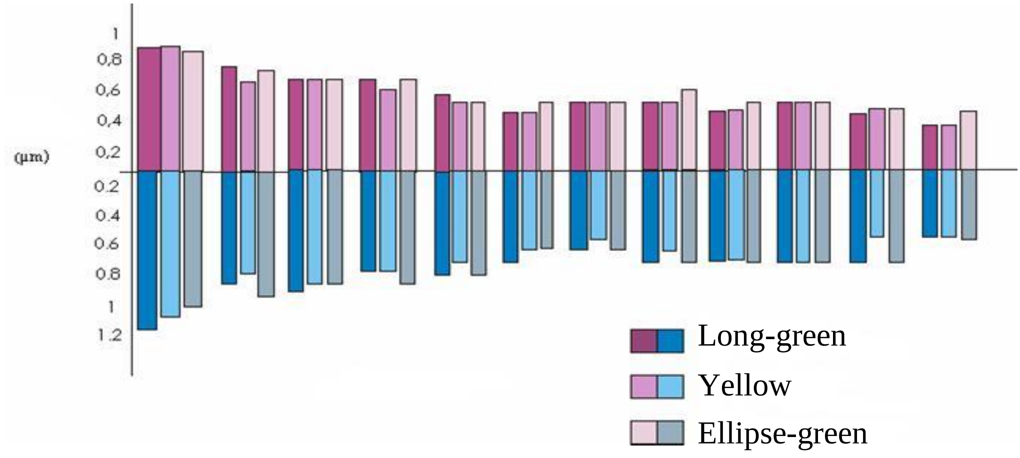

Figure 7. Ideogram showing comparison chromosome size among Yellow, Elips-green, and Long-green Bartek

As shown in Figure 1, 2, and 3, it could be seen that no satellites found in all karyotypes. Even though three types of Bartek showed similar chromosome numbers and karyotypes, they revealed different chromosome size from each other (Figure 4 and Table 1, 2 and 3). The largest chromosome total length of Yellow, Ellipse-Green, and Long-Green Bartek was 1.845 µm, 1.844 µm, and 1.887 µm respectively, whereas the smallest chromosome total length of Yellow, Ellipse-Green, and Long-Green Bartek was 1.167 µm, 1.118 µm, and 1.114 µm respectively. Short arm chromosome of Long-Green Bartek was smaller than the others. While the long arm chromosome of Ellipse-Green Bartek was longer than the others. The smallest short arm chromosome of Yellow, Ellipse-Green, and Long-Green Bartek was 0.529 µm, 0.512 µm, and 0.511 µm respectively, while the largest short arm chromosome of Yellow, Ellipse-Green, and Long-Green Bartek was 0.859 µm, 0.887 µm, and 0.884 µm respectively. The smallest of long arm chromosome of Yellow, Ellipse-Green, and Long-Green Bartek was 0.617 µm, 0.605 µm, and 0.603 µm respectively whereas the largest long arm of Yellow, Ellipse-Green, and Long-Green Bartek was 0.923 µm, 1.062 µm, and 1.004 µm respectively. The result showed variation with the previous studies reported by Chen, *et al* (1998), who recorded that the chromosome number of *Cucumis* is 2n=2x=14.

The result also exhibited that the proportion of the largest chromosome total length with the smallest chromosome total length (R) of of Yellow, Ellipse-Green, and Long-Green Bartek was 1.581, 1.649, and 1.694 respectively. This data revealed that chromosome size of three types of Bartek had similar variation compare to the others. The close genetic relationship among three kinds of Bartek gave valuable information concerning to breeding program. According to several researches, R value that same as 0.27 or lower can be used to strengthen the species placement. For example between *Ranunculus ternatus* (R=1.70) and *R. polii* (R=1.96) (Liang et al., 1991), *Arthiolaxis cupressoides* (R=2.05) and *A. selangoides* (R=2.32) (Lin-Chu,1992), *Larix sibirica* (R=1.85) and *L. olgensis* (R=2.06) (Lin-Chu, 1993), *Cupressus duclauxiana* (R=1.54) and *C. lusitanica* (R=1.79)

(Lin-Chu dan Yu-Xi, 1996), *Amentoraxis argotaenia* (R=2.71) and *A. yunnanensis* (R=2.59) (Qi-Xing et al.,2000). Furthermore, on the basis of the difference of R value in the three kinds of Bartek (R< 0.27) indicated that three kinds of Bartek had close genetic relationship.

Further chromosome studies on other *Cucumis* species from Indonesia will be needed to identify more precisely genetic identity of cucumber species of this country. This is due to plant breeders have always been appreciating enhancement of the existing gene pool, and chromosome studies would be helpful in the enrichment of the existing *Cucumis* germplasm resources of the country to develop *Cucumis* industry.

Table 1. Chromosome length, centromeric index, and chromosome types of Long-Green Bartek

Chromosome Pairs	Chromoaome Length (µm)			Centromeric Index	Type
	Short Arm (p)	Long Arm (q)	Total length (p+q)		
1	0.884±0.022	1.004±0.051	1.887±0.074	46.887	M
2	0.832±0.014	0.928±0.001	1.761±0.004	47.333	M
3	0.791±0.017	0.919±0.028	1.710±0.011	47.460	M
4	0.780±0.002	0.877±0.001	1.657±0.014	47.088	M
5	0.754±0.031	0.853±0.015	1.608±0.015	46.767	M
6	0.679±0,001	0.871±0,001	1.551±0.009	43.621	M
7	0.651±0.019	0.841±0.001	1.493±0.007	43.473	M
8	0.631±0.027	0.811±0.014	1.441±0.012	43.458	M
9	0.587±0.024	0.809±0.002	1.397±0.022	41.868	M
10	0.559±0.034	0.775±0.016	1.339±0.046	41.724	M
11	0.516±0.010	0.726±0.031	1.242±0.042	41.649	M
12	0.511±0.026	0.603±0.019	1.114±0.045	45.951	M

Table 2. Chromosome length, centromeric index, and chromosome types of Ellipse-Green Bartek

Chromosome Pairs	Chromoaome Length (µm)			Centromeric Index	Type
	Short Arm (p)	Long Arm (q)	Total length (p+q)		
1	0.887±0.036	1.062±0.115	1.844±0.040	47.274	m
2	0,809±0.007	0.893±0.009	1.702±0.017	47.531	m
3	0.771±0.011	0.874±0.012	1.643±0.022	46.956	m
4	0.728±0.006	0.875±0.007	1.604±0.013	45.385	m
5	0.721±0.013	0.835±0.001	1.554±0.017	46.327	m
6	0.669±0.043	0.822±0.012	1.500±0.019	44.803	m
7	0.651±0.009	0.808±0.007	1.459±0.017	44.047	m
8	0.623±0.002	0.779±0.021	1.405±0.021	44.258	m
9	0.570±0.018	0.774±0.009	1.344±0.009	42.404	m
10	0.549±0.009	0.736±0.026	1.286±0.035	42.815	m
11	0.523±0.011	0.694±0.013	1.221±0.027	42.979	m
12	0.512±0.015	0.605±0.034	1.118±0.051	45.947	m

Table 3. Chromosome length, centromeric index, and chromosome types of Yellow Bartek

Chromosome Pairs	Chromoaome Length (µm)			Centromeric Index	Type
	Short Arm (p)	Long Arm (q)	Total length (p+q)		
1	0.859±0.019	0.987±0.019	1.845±0.038	47.274	m
2	0.841±0.001	0.923±0.033	1.763±0.034	47.531	m
3	0.807±0.014	0.887±0.003	1.695±0.017	46.956	m
4	0.781±0.003	0.884±0.005	1.664±0.008	45.385	m
5	0.751±0.026	0.856±0.021	1.627±0.017	46.327	m
6	0.761±0.016	0.826±0.007	1.588±0.011	44.803	m
7	0.695±0.003	0.829±0.016	1.525±0.018	44.047	m
8	0.641±0.024	0.835±0.005	1.476±0.019	44.258	m
9	0.633±0.004	0.783±0.029	1.416±0.025	42.404	m
10	0.584±0.009	0.763±0.027	1.347±0.017	42.815	m
11	0.547±0.014	0.713±0.047	1.259±0.062	42.979	m
12	0.529±0.009	0.617±0.027	1.167±0.010	45.947	m

CONCLUSION

All types of Bartek showed similar diploid chromosome number and karyotypes composed of 24 metacentric chromosomes. According to the total number of chromosome Bartek is much more closely related to melon than to cucumber. On the basis of the difference of R value, all types of Bartek have close genetic relationship and considered to be cultivated from same species or variety.

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