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CYTOGENETIC AND KARYOTYPE ANALYSIS OF SAPODILLA (*ACHRAS ZAPOTA*)

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Abstract

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Sapodilla is a fruit plant developed potentially in Indonesia because in addition to being consumed as fruit, it can serve as medicinal plant as well. Genetic information particularly on the chromosome of sapodilla is still limited. This study aims to get morphological information on its chromosome such as number, size, form, and karyotype of sapodilla from Bojonegoro and Wonogiri. This research was conducted in the Laboratory of Biotechnology and Plant Physiology, Faculty of Agriculture, University of Sebelas Maret Surakarta. The chromosome number of sapodilla from Bojonegoro and Wonogiri had similarity: 2n = 26, metacentric in shape. The chromosome size of sapodilla from Bojonegoro ranged between 1.46 ± 0.38 μm and 3.06 ± 0.48 μm, while that of Sapodilla from Wonogiri ranged between 1.49 ± 0.19 μm and 3.58 ± 0.27 μm. The karyotype formula of Sapodilla from Bojonegoro and Wonogiri was 2n=26m consisting of 13 metacentric chromosome pairs.

Key words: chromosome; karyotype; mitotic; *Achras zapota*

Introducion

Sapodilla (*Achras zapota*) is one fruit crop in Southeast Asia. The plants are native to Mexico and America, but have spread in the tropics (Das and De, 2015). Sapodilla is a plant that can be developed in Indonesia because it tastes sweet and delicious compared to other tropical fruit (Woo et al., 2013). Sapodilla fruit is usually consumed as fresh fruit as a dessert (Hiremath and Rokhade, 2012). Sapodilla is an annual plant that contains polysaccharides, saponins, pilofenol and secondary metabolites, so it can be used as medicine (Chanda and Nagani, 2010). According to Srivastava et al. (2014), sapodilla can act as anti-inflammatory, antioxidant, antimicrobial and analgesic. According to Yuniastuti et al. (2016) young fruits and flowers sapodilla able to treat diarrhea, dysentery and lung disease. Barbalho et al. (2015) also mentions that sapodilla can be used as an alternative medicine cough and fever and can help prevent obesity, diabetes and dyslipidemia.

Sapodilla plants potentially serve as new varieties. In addition, the sapodilla is one commodity whose economic value of tropical fruit is quite high in Indonesia (Agustiningrum et al., 2014). However, the sapodilla plant has not been intensively cultivated. The number of sapodilla plants decreasing due to horizontal diversification. Thus, the need for efforts to develop plants sapodilla, one of them by doing research on genetic information, especially about chromosomes. Data of chromosome helpful as the cornerstone of cytogenetics and plant breeding, for example, the selection of appropriate species and varieties development with poliploidisasi or mutation (Al-Saghir et al., 2014; Venkatesh et al., 2015).Therefore, this study aimed to obtain information on the morphological characteristics such as chromosome number, size, shape and karyotype of sapodilla from Bojonegoro and Wonogiri so it can support plant breeding programs. Karyotype analysis on individual plant or population is very important because it can be used for multiple purposes such as studying cell function, taxonomic relationships and information about the evolution of a species (Zhang et al., 2010; Abdallaand El-Kawy, 2010).

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Materials and Method

Planting Material

Planting materials used are sapodilla plant seeds originating from Bojonegoro and Wonogiri. Meristematis root tip is used to make preparations in the observation of chromosomes.

Making Preparations

Making the chromosome preparation includes: making planting material, pre-treatment, fixation, hydrolysis, staining and squashing. Retrieval of materials is done at 5:30 to 6:30 pm with cutting edge roots of ± 5 mm. Pre-treatment is done by soaking the root tip in distilled water for 24 h at 5° C. Fixation farmer using a solution (ethanol:glacial acetic acid = 3:1) for 24 hours at room temperature. Hydrolysis carried out with HCl 1 N for 15 minutes at room temperature. Staining with acetoorcein 2% for 24 hours at room temperature. Squashing is performed by placing the roots in a glass preparations, spilled with 45% solution of acetic acid, covered with cover glass and then pressed with the thumb or wooden pencil.

Observations Chromosomes

Observations were made with a microscope at this stage of prometafase or metaphase. Observed data includes the number, size and shape of chromosomes. Size include long chromosome long arm (q), the length of the short arm (p) and the total length (q+p). Classification of chromosome was performed by calculating the ratio of the long arm and short arm of chromosome follows the way Ciupercescu (1990) (Table 1).

Table 1
Forms Chromosome Based Chromosome Arm Ratio

Form Chromosome	Arm Ratio (r = q/p)
Metasentric	1.0-1.7
Submetasentric	1.7-3.0
Acrosentric	3.0-7.0
Telosentric	>7.0

The data were analyzed descriptively to determine the pattern of karyotype (chromosome composition) is done by arranging in sequence homologous chromosomes ranging in size from the longest to the shortest then compare the chromosome sapodilla Bojonegoro and Wonogiri based on the number and shape of chromosomes.

Asymmetry index calculations chromosome follow the way Romero (1986). Intrakromosomal asymmetry index $A_1 = 1 - \left[\sum_{n=1}^{i}\left(\frac{bi}{Bi}\right)/n\right]$, *bi* is the average short arm of each pair of homologous chromosomes, *Bi* which is the average length of each arm pairs of homologous chromosomes and *n* is the number of pairs of homologous chromosomes. Interkromosomal asymmetry index $A_2 = SD/\bar{x}$, with SD is a standard deviation the length of chromosomes in a karyotype and $\bar{x}$ which is the average length of chromosomes in a karyotype.

Results and Discussion

Chromosome number

The observation of the root tip cell chromosome indicates that the sapodilla from Bojonegoro and Wonogiri have the same chromosome number is 2n = 2x = 26 (Figure 1 and 2). Sapodilla plant belongs to the family Sapotaceae. In Tjitrosoepomo (2010), the species included in this family include *Manilkarakauki*, *Mimusopselengi*, *Chrysophyllumcainito* and *Palaquiumburckii* but have not found literature that reported the number of chromosomes and morphological characteristics of the other chromosomes in these plants. However, Syukur (2015) revealed that the basic chromosome number sapodilla ie n = x = 13.

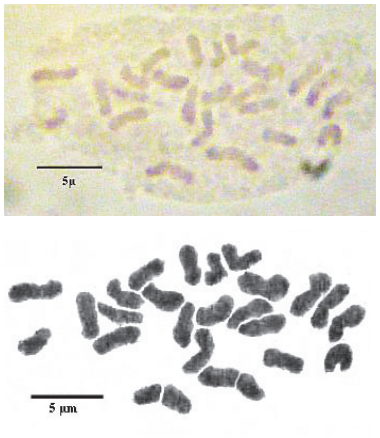
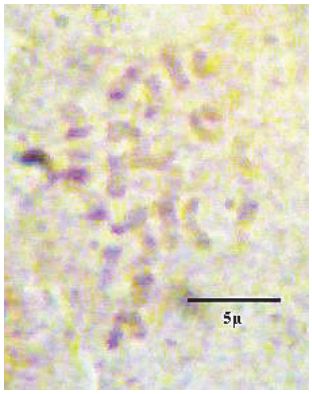


Fig. 1. Chromosome of sapodilla Bojonegoro

The number of chromosome ploidy on sapodilla plants is diploid. According Syukur (2015), most of the plants have diploid number of chromosomes in its somatic cell. Diploid iscontain two copies of the genome, so that the number of chromosomes is a multiple of the number of basic chromosomes.

Chromosome size

The results showed that the sapodilla from Bojonegoro (Table 2) has a length of chromosome 1.46 ± 0.38 µm until 3.06 ± 0.48 µm. The size of the long arm of chromosome ranges from 0.82 ± 0.22 µm until 1.74 ± 0.27 µm. The size of the short arm of chromosome ranges from 0.65 ± 0.17 µm until 1.33 ± 0.23 µm. Sapodilla from Wonogiri (Ta-



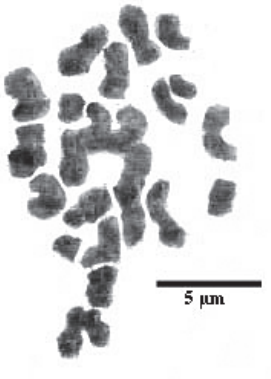


Fig. 2.Chromosome of sapodilla Wonogiri

ble 3) have a long-range chromosome between 1.49 ± 0.19 µm up to 3.58 ± 0.27 µm. The size of the long arm of chromosome ranges from 0.83 ± 0.13 µm until 2.07 ± 0.18 µm. The size of the short arm of chromosome ranges from 0.65 ± 0.07 µm until 1.51 ± 0.21 µm. Chromosomes are found in a cell's never the same size. Different cells will have a length of chromosomes different. This can occur because of differences in levels of chromosome condensation. Chromosome size difference shows the difference in the genetic information contained in it (Samadder et al., 2012). The size and morphology of chromosomes can help determine the evolutionary relationships of species of plants (Biswas et al., 2014).

Chromosomes shape

Chromosome can be distinguished based on the location of the centromere. Based on the location of the centromere, chromosome is divided into four, namely metacentric, submetacentric, acrocentric and telocentric. Determining the form of chromosomes is done by calculating the ratio of the long arm and short arm of chromosome follow the way Ciupercescu et al. (1990). Based on the calculation of the ratio of long arm and short arm of chromosome sapodilla Bojonegoro (Table 2) it can be seen that the sapodilla Bojonegoro has 13 pairs of chromosomes in the form metacentric. All pairs of chromosomes sapodilla Wonogiri also has a form metacentric (Table 3). According Suminah *et al.* (2002), the chromosomes are often found in plants are generally shaped metacentric.

Karyotype

Phenotype of chromosome karyotype is composed by pairs of homologous chromosomes and sorted by size of chromosomes from the longest to the shortest (Tjong et al., 2013). The composition of the karyotype can be presented in the form of chromosomes karyogram which is a photomicrograph of a single picture of the somatic metaphase cells are arranged based on similar size and shape. Knowledge of the karyotype is very important for plant breeding (Paknia and Karimzadeh, 2010). The results showed that the karyotype pattern Bojonegoro and Wonogiri sapodilla plants have in common is 2n = 26 m consisting of 13 pairs of chromosomes metacentric. Karyogram Bojonegoro and Wonogiri sapodilla plants can be seen in Figure 3 and Figure 4.

Chromosomal karyotype can also be presented in the form idiogram. Idiogram is a diagrammatic depiction of chromosomes or chromosome haploid gametes (n) of a species and is used to compare the karyotype of a species and another (Syukur, 2015). Making idiogram can help clarify the size and

Table 2
The size and shape of chromosomes sapodilla (*Achras zapota*) Bojonegoro

Chromosome Pairs	Chromosome Length (x ± SD, µm)			Arm Ratio (r=q/p)	Chromosome Shape
	Long Arm (q)	Short Arm (p)	Total Length (q+p)		
1	1.74 ± 0.27	1.33 ± 0.23	3.06 ± 0.48	1.31	Metasentric
2	1.67 ± 0.28	1.27 ± 0.24	2.94 ± 0.51	1.31	Metasentric
3	1.53 ± 0.25	1.28 ± 0.24	2.81 ± 0.44	1.20	Metasentric
4	1.57 ± 0.35	1.16 ± 0.16	2.73 ± 0.44	1.35	Metasentric
5	1.46 ± 0.25	1.22 ± 0.22	2.67 ± 0.46	1.20	Metasentric
6	1.49 ± 0.33	1.00 ± 0.15	2.50 ± 0.43	1.49	Metasentric
7	1.33 ± 0.29	1.05 ± 0.13	2.38 ± 0.41	1.26	Metasentric
8	1.22 ± 0.25	1.04 ± 0.18	2.26 ± 0.43	1.18	Metasentric
9	1.21 ± 0.26	0.96 ± 0.15	2.17 ± 0.40	1.26	Metasentric
10	1.21 ± 0.35	0.89 ± 0.13	2.10 ± 0.43	1.35	Metasentric
11	1.09 ± 0.17	0.83 ± 0.22	1.92 ± 0.37	1.32	Metasentric
12	0.95 ± 0.24	0.70 ± 0.13	1.65 ± 0.37	1.35	Metasentric
13	0.82 ± 0.22	0.65 ± 0.17	1.46 ± 0.38	1.26	Metasentric

Table 3
The size and shape of chromosomes sapodilla (*Achras zapota*) Wonogiri

Chromosome Pairs	Chromosome Length (x ± SD, µm)			Arm Ratio (r=q/p)	Chromosome Shape
	Long Arm (q)	Short Arm (p)	Total Length (q+p)		
1	2.07 ± 0.18	1.51 ± 0.21	3.58 ± 0.27	1.37	Metasentric
2	1.77 ± 0.16	1.28 ± 0.16	3.05 ± 0.23	1.38	Metasentric
3	1.67 ± 0.15	1.25 ± 0.11	2.91 ± 0.23	1.34	Metasentric
4	1.63 ± 0.14	1.15 ± 0.09	2.79 ± 0.21	1.42	Metasentric
5	1.47 ± 0.14	1.19 ± 0.12	2.66 ± 0.21	1.24	Metasentric
6	1.39 ± 0.13	1.05 ± 0.17	2.45 ± 0.17	1.32	Metasentric
7	1.32 ± 0.11	0.99 ± 0.15	2.32 ± 0.19	1.33	Metasentric
8	1.26 ± 0.08	0.96 ± 0.10	2.22 ± 0.16	1.31	Metasentric
9	1.26 ± 0.06	0.89 ± 0.16	2.14 ± 0.11	1.42	Metasentric
10	1.09 ± 0.13	0.89 ± 0.05	1.98 ± 0.12	1.22	Metasentric
11	1.03 ± 0.08	0.86 ± 0.12	1.89 ± 0.12	1.19	Metasentric
12	1.02 ± 0.14	0.72 ± 0.03	1.74 ± 0.15	1.41	Metasentric
13	0.83 ± 0.13	0.65 ± 0.07	1.49 ± 0.19	1.28	Metasentric

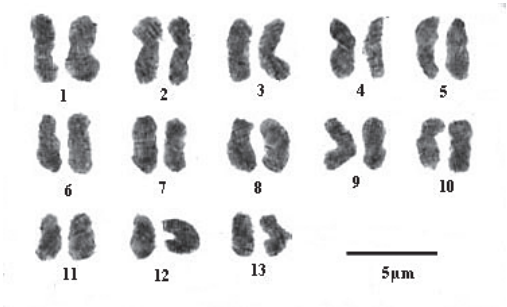


Fig. 3. Karyogram of sapodilla Bojonegoro

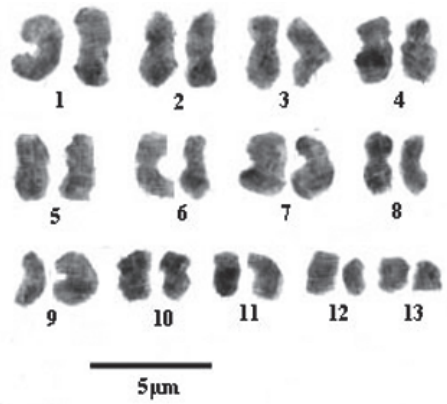


Fig. 4. Karyogram of sapodilla Wonogiri

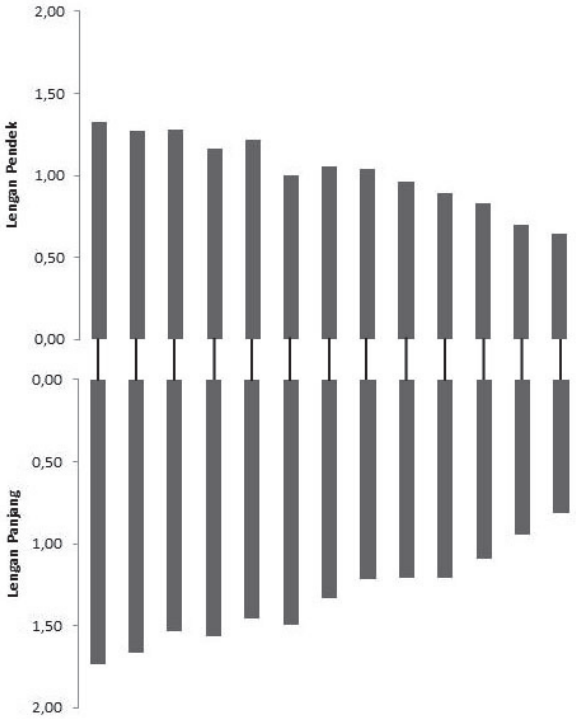


Fig. 5. Idiogram of sapodilla Bojonegoro

shape in a single set of chromosomes. Sawo Idiogram Bojonegoro and Wonogiri can be seen in Figure 5 and 6.

Asymmetry indexes Chromosome

Morphology of chromosomes can be analyzed further by calculating the asymmetry index of chromosomes consist-

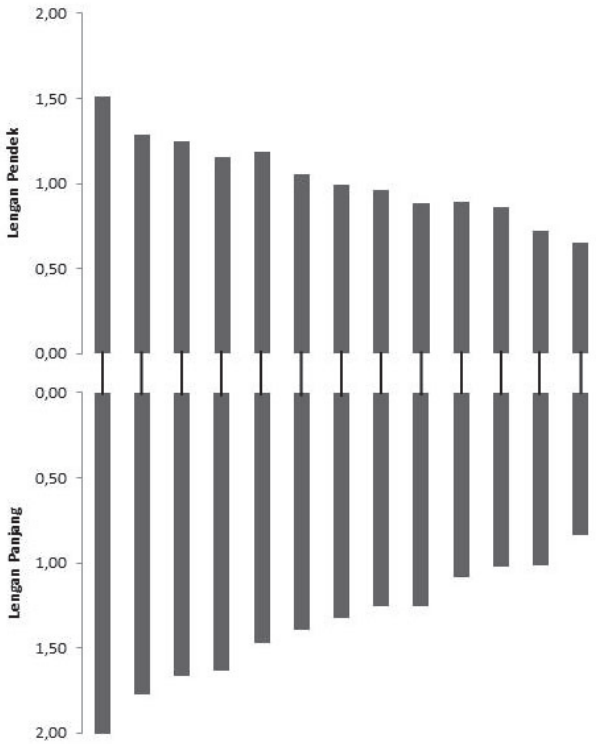


Fig. 6. Idiogram of sapodilla Wonogiri

ing of intrakromosomal asymmetry index (A_1) and interkromosomal asymmetry index (A_2). According Setiawati et al. (2013), the intrakromosomal asymmetry index value (A_1) is used to determine variations in the shape of a karyotype while interkromosomal asymmetry index value (A_2) is used to determine the deviation (dispersion) size of the chromosomes in the karyotype. Based on the calculation, sapodilla Bojonegoro has a value A_1 0.23 ± 0.05 and value A_2 0.19 ± 0.03. Sapodilla Wonogiri has A_1 0.24 ± 0.04 and value A_2 008 ± 0.02. Value A_1 of sapodilla Bojonegoro and Wonogiri small shows that the dominant form of sapodilla chromosomes are metacentric (Akhavan et al., 2015), while the value of A_2 small show chromosomal aberrations size sapodilla in the karyotype is not too large.

Conclusion

The number of chromosomes sapodilla from Bojonegoro and Wonogiri same that 2n = 2x = 26 with metacentric shape. Chromosome size of sapodilla Bojonegoro between 1.46 ± 0.38 µm until 3.06 ± 0.48 µm while the chromosome size of sapodilla Wonogiri between 1.49 ± 0.19 µm up to 3.58 ± 0.27 µm. Karyotype formula sapodilla Bojonegoro and Wonogiri have in common is 2n = 26m (consisting of 13 pairs of chromosomes metacentric). Sapodilla Bojonegoro has the value A_1 at 0.23 ± 0.05 and value A_2 0.19 ± 0.03 while sapodilla Wonogiri A_1 has a value of 0.24 ± 0.04 and value A_2 0.08 ± 0.02.

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