BIODIVERSITAS
Volume 24, Number 1, January 2023
Pages: 40-46

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240106

Karyotyping of green, yellow and red matoa (*Pometia pinnata* J.R.Forst. & G.Forst.) from Central Java, Indonesia

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Manuscript received: 28 July 2022. Revision accepted: 26 December 2022.

Abstract. *Yuniastuti E, Masailla APD, Nandariyah, Rahmah N. 2023. Karyotyping of green, yellow and red matoa (*Pometia pinnata *J.R.Forst. & G.Forst.) from Central Java, Indonesia. Biodiversitas 24: 40-46.* Matoa (*Pometia pinnata* J.R.Forst. & G.Forst.) belongs to Sapindaceae family and is widely distributed naturally in Indonesia. However, research on matoa plants, especially for the genetic aspect is poorly reported. Genetic information, through chromosome analysis can be helpful in plant breeding programs. This study aimed to analyze karyotype of chromosomes green, yellow and red matoa collected from Central Java, Indonesia. Chromosome analysis in this study used the squash preparation method. This research was the first karyotyping of matoa using 3 types based on exocarp colors, namely green, yellow and red skin matoa. Each type of matoa was repeated 4 times. The results showed that three types of matoa skin colors have the same number of chromosomes, namely 2n=2x=22 with each chromosome size of green, yellow, and red matoa are 1.070±0.251 µm, 1.025±0.281 µm, 006±0.252 µm, respectively and all of matoa have a metacentric chromosome's form. The karyotype formula for each type of matoa is 2n=2x=22=11m.

Keywords: Central Java, chromosomes, karyotype, *Pometia pinnata*, squash method

INTRODUCTION

Matoa (*Pometia pinnata* J.R.Forst. & G.Forst.) belongs to the Sapindaceae family and is native to Sri Lanka to China (Yunnan) and South Pacific (POWO 2022). The species is widely distributed in both wild and cultivated status in Indonesia. Matoa grows naturally in warm to hot and humid subtropical and tropical zones at altitudes of 0-500 (-1700) m asl. with an annual rainfall of 1500-5000 mm. It grows on slightly acidic to neutral soils (pH 5-8) in lush from evergreen secondary to primary forests (Thomson and Thaman 2006). In cultivation, this species is adapting and breeding in warm-hot, humid, subtropical and tropical regions from 14°N to 20°S (Lin 2013).

Matoa fruit has even recently penetrated and been recognized throughout the country as an economically promising fruit. With its unique taste and texture, matoa fruit is very popular outside Papua and has good market potential. Matoa fruit also has the potential to be further processed into syrups, juices, and other useful processed products and will be a new economic opportunity for households and industries. This species also known as Kasai is very popular among the local Sarawakian population (Furay et al. 2018). The ripe matoa fruit contains many beneficial vitamins A, C, and E which are rarely found in other fruits. Some people in Indonesia use the matoa plant as medicine. The fleshy fruits contain compounds that are valued as traditional medicines for treatment of hypertension, gynecology, and stomach, such as stomach complaints, diarrhea, and dysentery (Suzuki et al. 2021). Almost all parts of this plant can be used as medicine, including leaves, fruit, bark, skin, and roots (Hanafi et al. 2020).

Matoa is a perennial plant and is up to 20-40 m tall. Based on the color of the skin, there are three types of commonly matoa fruit, namely red, green, and yellow matoa (Arumugam et al. 2021). Green matoa has a green to yellowish-green skin color when ripe, yellow matoa has a yellowish-green to completely yellow color, whereas red matoa has a green-reddish and up to blackish-red color when is riped.

Based on its potential and benefits, information on matoa must be completed. However, research on genetic aspects of matoa, especially chromosome field is poorly reported. Marhold (2015) reported the number of matoa chromosomes was 2n=40. Based on previous research on the same species, there should be no change in genetic form, especially chromosome morphology. Genetic markers are important to determine the kinship of a species. Genetic markers can help plant breeding programs, especially in creating genetic diversity. Diversity is very important to provide information on the level of genetic diversity in the population for gene conservation targets, varietal improvement and plant breeding (Ifah et al. 2018). Genetic diversity can be analyzed by chromosome analysis. This study aims to analyze karyotype of chromosomes green, yellow and red matoa collected from Central Java, Indonesia.

MATERIALS AND METHODS

Plant materials

The research materials used are plants obtained from the germination of matoa seeds. The matoa fruits were obtained from cultivated status in Central Java, Indonesia (Figure 1). Green matoa was obtained from Mojosongo subdistrict, Surakarta; Red matoa was collected from the Jumantono field research in Karanganyar, Central Java; and Yellow matoa from Klaten, Central Java. This study was conducted from September 2021 to March 2022 at the Integrated Laboratory of Sebelas Maret University, Surakarta.

Procedures

Sample preparation

Meristematic root tips were taken from the plants in the morning at 8:00 am. The root tips were then pretreated with distilled water and stored at a low temperature of 4-5°C for 24 hours. Fixation with 45% glacial acetic acid solution for 1 hour at room temperature. After fixation, the roots were washed using distilled water with 3 repetitions. Then proceed with hydrolysis using 1N HCL for 10 minutes at room temperature. Before the last treatment, the root tips need to be washed again with distilled water 3 times. Staining the root tips using 2% aceto-orcein then stored for 24 hours at room temperature. Staining functions so that chromosomes can be seen compared to the cytoplasm (Harijati et al. 2017).

Observation

The preparation method used is the squash method. The squash method is known as a simple method and is a rapid way to visualize chromosomes and cell nuclei (Chirino et al. 2014). The squash method aims to get thin preparations and can be observed for a long period of time. Samples used for observation were 12 samples, obtained for each matoa type (green, yellow, red) which were repeated 4 times. The tip of root that has been colored is then dripped with 45% acetic acid (Yuniastuti et al. 2018). Followed by maceration (squash) using the tip of a brush or rubber eraser which has previously been covered with cover glass. Observation of root tip preparations was seen using a microscope and Optilab. The microscope magnification used is 1000x magnification. The required data were then noted and recorded. The chromosomes that appear on observation with a microscope are captured and from the images can be analyzed and the number of chromosomes counted.

Data analysis

Data analysis is based on descriptive data from selected chromosome images. The image data can then be interpreted based on observation variables with the help of several applications. Microscopic images of chromosomes were redrawn in the Corel Draw X5 application to produce a clearer number and shape of chromosomes. Chromosome size is measured by summing the long arm and short arm (q+p) using the grid in Corel Draw X5 and then calibrated with Microsoft Excel. Measurement of chromosome size is by summing the total of the long arm and short arm (q+p) with q is the long arm and p is short arm. Determination of chromosome shape is based on the ratio of chromosome arms (q/p). Identification of chromosome shape is calculated from the ratio of long arms and short arms using Levan et al. (1964) as presented in Table 1.

Measurement of chromosome length is based on the micrometer scale of the subject with the help of Corel Draw X7 software. The karyotype arrangement of matoa plants is expressed in the form of karyograms and idiograms. Karyotypes are arranged in chromosome order from shortest to longest, while ideograms are arranged by connecting pairs of chromosomes according to the length and overall shape of the average chromosome. Asymmetry chromosome is calculated using formulas A1 and A2 (Parjanto et al. 2003) as follows:

Intrachromosome asymmetry index:

$$A1 = 1 - [\sum_{n-1}^{i} \left(\frac{bi}{Bi}\right)/n]$$

Where: bi: average short arm of each homologous chromosome pair, Bi: average of the long arms of each homologous chromosome pair, and n: number of homologous chromosome pairs.

Table 1. Chromosome shape based on chromosome arm ratio

Chromosome shape	Arm ratio (*r=q/p*)
Metasentric (m)	$1.0 < r \leq 1.7$
Submetasentric (sm)	$1.7 < r \leq 3.0$
Akrosentric (t)	$3.0 < r \leq 7.0$
Telosentric (T)	≥ 7.0

A **B** **C**

Figure 1. Fruit of matoa (*Pometia pinnata*). A. Green skin matoa. B. Yellow skin matoa. C. Red skin matoa

Asymmetric index between chromosomes:

$A2 = SD/\overline{X}$

Where: SD: Standard deviation of chromosome lengths within a karyotype and $\overline{x}$: Average length of chromosomes in a karyotype.

RESULTS AND DISCUSSION

Chromosome number

Chromosome number is one of important genetic information needed for breeding programs. Observation of chromosome numbers in plant can be seen clearly in the prometaphase phase of cell division. The chromosomes of green matoa are shown in Figure 2, yellow matoa in Figure 3, and red matoa in Figure 4.

Observations of the number of chromosomes obtained from image data which was then redrawn with Corel draw to get a clearer number of chromosomes. Three types of matoa have the same number of chromosomes, 2n=22 (Figures 2-4). The number of matoa chromosomes observed in this study is different from the study conducted by Marhold (2015) which states number of chromosomes of *P. pinnata* is 2n=40. Several factors can affect the difference in the number of chromosomes in one species, one of which is that the diversity of matoa is still not widely studied so there are variations that have not yet been found. It is revealed by Xin et al. (2020) that species with close relatives generally have the same number of chromosomes, although it does not rule out the possibility of microevolution. The results instead show that matoa has close relationships to rambutan (*Nephelium lappaceum*) so it has the same number of chromosomes 2n=22 as reported by Putri et al. (2022).

Chromosome size

The calculation will produce long arm, short arm, total length, long arm and short arm ratio (q/p). The ratio of long and short arms is used to determine the chromosome shape (Table 1). The size and shape of green, yellow, and red matoa are presented in Tables 2, 3, and 4.

The green matoa chromosomes have an average size of 1.070±0.251µm. The shortest chromosome size is at 0.776±0.061µm and the longest is at 1.630±0.166 µm. From the calculation of the ratio of the long arm and short arm of the chromosome in the range of the lowest value of 1.089±0.069 to the largest 1.307±0.253 (Table 2). The yellow matoa chromosomes with an average chromosome size of 1.025±0.281 µm. The shortest chromosome size is 0.700±0.081 µm and the longest is 1.705±0.3656 µm. The calculation of chromosome ratio ranged from 1.083±0.118 to 1.250±0.167, indicating that the chromosome shape of yellow matoa is metacentric because the value is not more than 1.70 (Table 3). The red matoa chromosomes show an average size of 1.006±0.252 µm, and have a range of chromosome sizes between 0.666±0.087 µm to 1.528±0.056 µm. The ratio value of the long arm and short arm of the red matoa chromosome is between 1.050±0.058 to the largest 1.338±0.236, indicating that the size of the red matoa chromosome is metacentric (Table 4).

Karyotype

The karyotype of matoa chromosomes is presented in the form of karyograms and ideograms. The arrangement of karyotypes is based on the longest to shortest size. In karyotyping, all measurements can be saved and used to calculate karyotype features and create feature maps. Karyograms and Ideograms of matoa chromosomes for each type (green, yellow, and red) are shown in Figure 5 and Figure 6, respectively.

The ideogram of matoa chromosomes was prepared using Microsoft Visio 2010 application. The ideogram was prepared based on the order of the chromosome pairs arranged in the previous karyogram. Each 1 bar on the ideogram indicates the average length of 1 homologous chromosome pair. There are differences in each average chromosome pair. It concluded that the karyotype formula of the 3 types of matoa (green, yellow, and red) is the same, namely 2n=2x=22=11m (Figures 5 and 6).

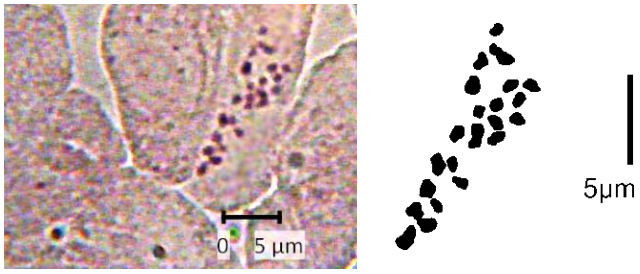

Figure 2. Chromosome of green matoa

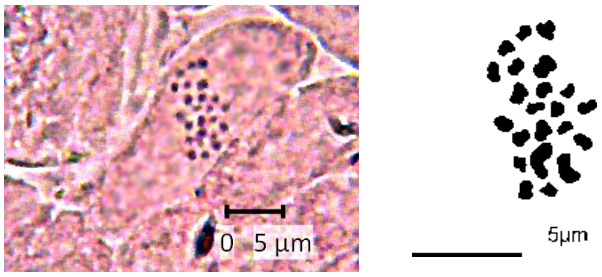

Figure 3. Chromosome of yellow matoa

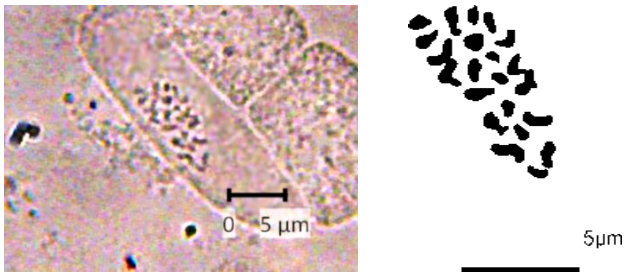

Figure 4. Chromosome of red matoa

Chromosome asymmetry index

The Intrachromosomal Asymmetry Index or A1 serves to determine the form of chromosomal variation in one karyotype. The value and index of asymmetry between chromosomes (A2) are used to determine the bias (dispersion) of chromosome size in one karyotype. The results showed that the A1 value of green matoa was 0.924±0.006, yellow matoa was 0.920±0.004, and red matoa was 0.921±0.002. The A2 value in green matoa is 0.216±0.017, yellow matoa is 0.275±0.037, and red matoa is 0.255±0.032.

Discussion

The results showed that the three types of matoa (green, yellow, and red) had the same number of chromosomes, namely 22 chromosomes with 11 pairs. According to Yuniastuti et al. (2018), the number of chromosomes in one species generally has the same number, shape, and size. The chromosome pairs are known as homologous or diploid (2n) chromosomes. Parjanto et al. (2003) also explained that the shape, size and number of chromosomes in one species are always constant, so knowing the genetic properties of a plant species is very important. So it can be formulated as 2n=2x=22. Compared to Putri et al. (2022), chromosomes in *Nephelium lappaceum*, which is in the same family as matoa have the same number of chromosomes. Juan (2007) explained that the Sapindaceae family has a basic chromosome number of x = 7, 9, 10, 11, 12, 14, 15, and 16.

Table 2. Size and shape of green matoa chromosomes

Chromosomes pair	Chromosomes length (x ± SD µm)			Ratio (r = q/p) (µm) ± SD µm	Chromosome shape
	Long arm (q)	Short arm (p)	Total (q+p)		
1	0.419±0.051	0.357±0.044	0.776±0.061	1.201±0.226	Metasentric
2	0.439±0.048	0.393±0.050	0.832±0.057	1.136±0.243	Metasentric
3	0.460±0.035	0.424±0.030	0.884±0.059	1.089±0.069	Metasentric
4	0.493±0.013	0.427±0.072	0.921±0.069	1.208±0.250	Metasentric
5	0.552±0.046	0.444±0.052	0.996±0.087	1.250±0.118	Metasentric
6	0.567±0.062	0.449±0.058	1.016±0.107	1.270±0.125	Metasentric
7	0.583±0.050	0.465±0.075	1.047±0.105	1.277±0.193	Metasentric
8	0.593±0.043	0.506±0.106	1.100±0.149	1.208±0.160	Metasentric
9	0.665±0.045	0.532±0.099	1.197±0.088	1.304±0.303	Metasentric
10	0.763±0.073	0.605±0.135	1.368±0.195	1.307±0.253	Metasentric
11	0.879±0.092	0.752±0.100	1.630±0.166	1.183±0.147	Metasentric

Table 3. Size and shape of yellow matoa chromosomes

Chromosomes pair	Chromosomes length (x ± SD µm)			Ratio (r = q/p) (µm) ± SD µm	Chromosome shape
	Long arm (q)	Short arm (p)	Total (q+p)		
1	0.378±0.058	0.322±0.048	0.700±0.081	1.188±0.239	Metacentric
2	0.435±0.049	0.390±0.084	0.826±0.119	1.150±0.238	Metacentric
3	0.436±0.049	0.399±0.085	0.834±0.118	1.125±0.250	Metacentric
4	0.446±0.068	0.417±0.059	0.863±0.122	1.083±0.118	Metacentric
5	0.477±0.128	0.436±0.049	0.913±0.175	1.083±0.167	Metacentric
6	0.535±0.095	0.435±0.049	0.971±0.133	1.229±0.158	Metacentric
7	0.544±0.094	0.436±0.049	0.980±0.131	1.250±0.167	Metacentric
8	0.544±0.094	0.488±0.075	1.033±0.157	1.125±0.160	Metacentric
9	0.609±0.165	0.509±0.113	1.119±0.272	1.193±0.142	Metacentric
10	0.694±0.156	0.638±0.136	1.331±0.291	1.088±0.031	Metacentric
11	0.910±0.185	0.796±0.194	1.705±0.365	1.155±0.160	Metacentric

Table 4. Size and shape of red matoa chromosomes

Chromosomes pair	Chromosomes length (x ± SD µm)			Ratio (r = q/p) (µm) ± SD µm	Chromosome shape
	Long arm (q)	Short arm (p)	Total (q+p)		
1	0.381±0.078	0.285±0.010	0.666±0.087	1.338±0.236	Metacentric
2	0.381±0.078	0.363±0.062	0.744±0.139	1.050±0.058	Metacentric
3	0.439±0.032	0.406±0.034	0.845±0.047	1.088±0.118	Metacentric
4	0.447±0.046	0.415±0.025	0.862±0.038	1.083±0.167	Metacentric
5	0.465±0.050	0.431±0.019	0.896±0.065	1.077±0.090	Metacentric
6	0.517±0.039	0.439±0.032	0.956±0.047	1.185±0.128	Metacentric
7	0.568±0.030	0.456±0.045	1.024±0.075	1.252±0.060	Metacentric
8	0.585±0.042	0.490±0.042	1.076±0.066	1.205±0.112	Metacentric
9	0.621±0.074	0.567±0.055	1.188±0.111	1.104±0.125	Metacentric
10	0.673±0.031	0.613±0.053	1.286±0.050	1.108±0.127	Metacentric
11	0.837±0.048	0.690±0.056	1.528±0.056	1.225±0.155	Metacentric

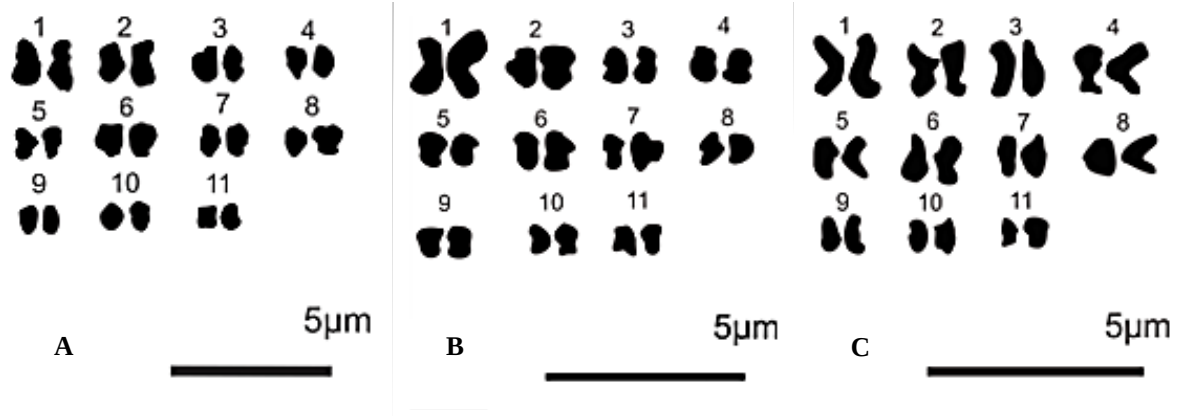

Figure 5. Karyogram matoa chromosomes. A. Green matoa. B. Yellow matoa. C. Red matoa

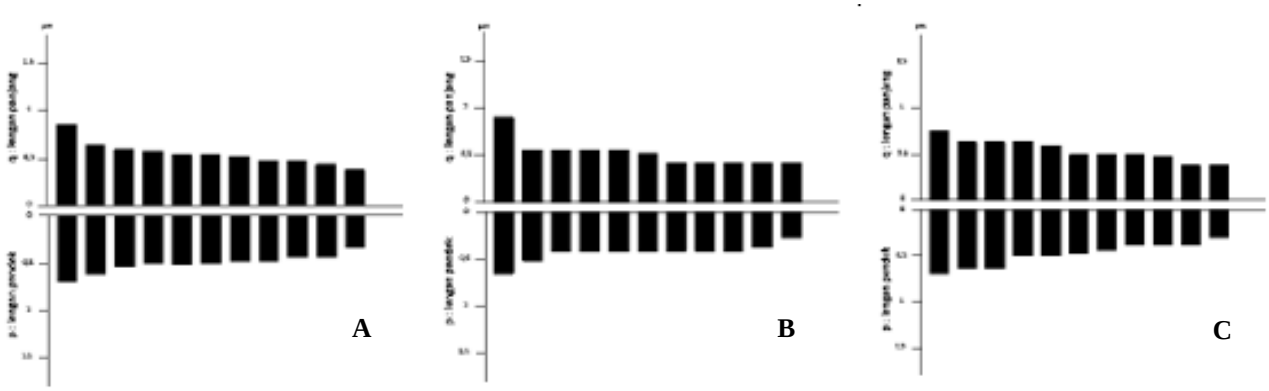

Figure 6. Ideogram of matoa chromosomes. A. Green matoa. B. Yellow matoa. C. Red matoa

When cells divide, their chromosomes change dramatically condensing before forming a pair of sister chromatids showing the well-known X shape of mitosis. Zhang and Wolynes (2016) stated that good chromosome distribution can be observed during mitotic division especially prometaphase. Ferreira and Maiato (2021) explained that prometaphase refers to the assembly of the mitotic spindle, which mediates the capture of chromosomes at their kinetochores and ends, while all chromosomes are orientated towards the equatorial plane, so that chromosomes appear sharp and shorter. The time of plant mitosis varies for each species. Sangur et al. (2021) suggested the best time for cutting root tips is 08.00 am. However, Syakhril et al. (2020) reported the best time to produce mitotic cell division is around 08.00-12.00 am.

Each type of matoa chromosome has a size that is not much different (Tables 2-4). Within one type of matoa, there are variations in size from the shortest to the longest. Differences in chromosome size can be caused by various factors, such as the stage of cell division. Parjanto et al. (2003) stated that in different cells, there can be differences in chromosome length due to the level of chromosome condensation. This opinion is also reported by Budi et al. (2019) that variations in chromosome size are caused by mitotic division factors. The shape of chromosomes in all types of matoa, whether green, yellow, or red is the same, which is metacentric. This similarity in chromosome shape can occur because they are still in the same species. Putri et al. (2022) reported the similarity of chromosomes in several varieties of *Nephelium lappaceum*. This result is supported by Yuniastuti et al. (2018) that in general the shape of chromosomes has similarities even within one family. The shape of the chromosomes can also be influenced by the translucency of the chromosomes when they are killed. A clear picture of the chromosomes is presented in Figures 2-4, caused by the chromosome coloring agent of 2% aceto-orcein. According to Ram et al. (2021), aceto-orcein has the advantage of chromatin homogeneity and rapid staining in seconds which further helps in reducing the time required.

The karyotype is displayed in two forms, namely karyogram (Figure 5) and ideogram (Figure 6). The karyotype of matoa chromosomes is composed of 11 chromosome pairs, each containing 2 chromosomes. Each type of matoa has a karyotype arrangement that is quite different in terms of shape. The different chromosome shapes are due to the different condensation of each chromosome. It is very difficult to find centromeres for

some plant species due to the thick and short (condensed) size of the chromosomes. Nagaki et al. (2012) reported that the location of centromeres is the basis for determining the shape of chromosomes. Most of the Matoa chromosomes found are short and thick so centromeres are difficult to determine (Figure 4). The three types of matoa have the same karyotype formula of 2n=2x=22=11m, where m is metacentric. Karyotype differences within a species are very likely to occur, because the level of condensation of each chromosome is different, so the accumulation of chromatin or heterochromatin is different. Feitoza et al. (2017) explained that along chromosomes there can be variations in the degree of chromatin condensation in prophase-prometaphase cells resulting in certain condensation patterns. Murray (2013) explains the difference between orthotopic karyotype selection (maintenance of karyotype consistency through the occurrence of characteristic structural mutations) and karyotype maintenance (maintenance of similar karyotype morphology in different taxa through the absence of structural mutations). The difference itself can occur due to overlapping chromosomes, causing difficulties in determining the number, size, and shape of chromosomes and making ideograms. This condition requires a proper squash technique to produce a good chromosome spread.

The Intrachromosomal Asymmetry Index or A1 serves to determine the form of chromosomal variation in one karyotype. Tabur et al. (2012) argue that the A1 value is used to determine the kinship and development of a plant. The results showed that the A1 value of each type of matoa has less than 1. It means that all type of matoa has metacentric shapes. Ningsih et al. (2015) stated that the plant chromosome intrachromosomal asymmetry index value (A1) is between 0 to 1 indicating that all chromosomes of the plant species have a metacentric shape. The research results obtained for the A2 value on each matoa are, on green matoa 0.216±0.017, yellow matoa 0.275±0.037, red matoa 0.255±0.032. Based on these results, it can be concluded that the A2 value in each variety is not much different, indicating that the size deviation is not too wide.

In conclusion, green, yellow, and red skin types of matoa have the same number of shapes, sizes, and karyotypes. The number of matoa chromosomes has 2n=2x=22 with the overall shape being metacentric. Green matoa has an average chromosome length of 1.070±0.251 μm, yellow matoa has an average chromosome length of 1.025±0.281 μm, and red matoa has an average chromosome length of 1.006±0.252 μm. The three types of matoa also have the same karyotype formula, which is 2n= 2x=22=11m. The A1 value in green matoa is 0.924±0.006, yellow matoa 0.920±0.004, red matoa 0.921±0.002 which indicates that the proportion of metacentric chromosomes is large. The A2 value in green matoa is 0.216±0.017, yellow matoa is 0.275±0.037, red matoa is 0.255±0.032 indicating that the deviation of the chromosomes in each karyotype is small.

ACKNOWLEDGEMENTS

We thank Integrated Research Excellence of Sebelas Maret University, Surakarta, Indonesia with contract number 254/UN27.22/PT.01.03/2022 for funding this research.

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