

The rambutan (*Nephelium lappaceum* L.) chromosomes

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Abstract. Putri IIS, Yuniastuti E, Parjanto. 2022. The rambutan (*Nephelium lappaceum* L.) chromosomes. *Biodiversitas* 23: 2196-2202. Rambutan (*Nephelium lappaceum* L.) is an annual plant with a wide distribution, it generates this plant to have diversity. The diversity is more often found in morphological characters, in this case, its genetics has not been discovered. The genetics of rambutan could also be indicated by its chromosome karyotype. This research was conducted using the squashing method. The genetic material used was four rambutan varieties which are: Ace, Binjai, Lebak Bulus, and Rapih. For each variety, the study process will be repeated three times. The results of the study showed rambutan chromosome number in all varieties was 22 chromosomes ($2n=22$) with an average chromosome size of Ace was 1.777 ± 0.363 μm , followed by Binjai was 1.477 ± 0.392 μm , Lebak Bulus was 1.418 ± 0.320 μm , and Rapih was 1.443 ± 0.315 μm , and the chromosome shape is metacentric. The karyotype formula obtained was $2n=2x=22=11m$.

Keywords: Cytogenetics, karyotype, squash method

INTRODUCTION

Rambutan (*Nephelium lappaceum*) is a tropical fruit generally found in Southeast Asia, especially in Indonesia and Malaysia. Rambutan has a wide distribution throughout Indonesia. The areas where rambutan is grown are scattered in various regions, especially in Java, Kalimantan, and Sumatra. The spread of the rambutan cultivation can be found in almost all places on Java Island, from the West to the East. The centers of rambutan production in West Java are located in the Regencies of Bogor, Bekasi, Subang, and Purwakarta. Moreover, the production centers in Central Java are Semarang, Banyumas, Purbalingga, Purworejo, and Magelang. Therefore, the production centers in East Java are Jember, Blitar, and Lumajang Regencies. The centrum production in the Special Region of Yogyakarta is located in Sleman and Bantul Regencies, while the Special Capital Region of Jakarta is in Pasar Minggu (Rukmana and Oesman 2002). The wide distribution of rambutan in Indonesia makes rambutan have a high diversity. The diversity is built on genetic diversity and coupled with the cross-pollinating nature of the plants allows a reasonably high variation among the progeny. The process of pollination and fruit formation depends on insects (Muhamed and Kurien 2018).

The diversity of rambutans can be seen from the plant's structure and the characteristics of the fruit. Rambutan fruit has colors in each variety. There are dark-red, red, yellow, and also greenish-yellow. Each of these fruits contains excellent benefits for the health of the body. Rambutan fruit contains carbohydrates, protein, and several minerals and vitamins. From that nutrition richness, rambutan is beneficial for the human body. The rambutan seed can also be used for energy raw materials (bio-diesel), which can be the solution for the fuel in the future (Anggono et al. 2018).

Research on the study of rambutan chromosomes is still rare. It has only been found that the chromosome number is $2n=22$ (Sarip 1998). According to the previous studies on other plant species, different varieties usually do not change in the number of chromosomes, although the phenotypes are very different. The information about rambutan chromosomes is needed to see the rambutan karyotype pattern. The diversity of rambutan is relatively high, to increase the validity of the diversity, it is necessary to know its morphological and genetic characteristics. Plant species are often unique in terms of growth, habitat, and reproduction. Studying plant genomes can increase knowledge about some of the basic biological events that make individuals plant species special, and is able to develop agronomic plant species (Saxena et al. 2014). Knowledge of the karyotype pattern is also helpful to determine the chromosomal abnormalities in each variety of rambutan. This study aims to determine the chromosome karyotype of Ace, Binjai, Lebak Bulus, and Rapih rambutans.

MATERIALS AND METHODS

Plant materials

This chromosomal study was carried out using the squash method. The squash method is the production of chromosome preparations by squeezing the meristematic plant parts, so that the cells can spread out. This method uses a material in the form of a rambutan root tip. This root material originated from Karanganyar and Surakarta, Central Java, Indonesia.

Planting and cutting root tip

Prior to the squash method, plant the rambutan seeds until they are 20 days old. Therefore, cut off the root tips.

The tip of the rambutan root is cut along 1-2 cm. Root cuttings were carried out at 08:00-08:30 AM (Western Indonesian Time). Wash the cut roots with distilled water twice to remove the remaining soil.

Making chromosome preparation

After cutting the root tip, the root was soaked (pre-treated) in distilled water (5-10°C) for 24 hours. The next step was a fixation, which was using 45% acetic acid for 1 hour. After this process, rinse the roots with distilled water three times, then hydrolyzed with 1N HCl for 10 minutes at room temperature, and rinsed three times with distilled water. The next step is staining the roots with four drops of 2% Aceto-orcein, soaked for 24 hours. Therefore, cut the roots along 1-2 mm on a glass object, drop one drop of 45% acetic acid, then cover with a glass cover, and tap with a brush.

Observation

The cleavage phase observed was prometaphase. The genetic material used was Ace, Binjai, Lebak Bulus, and Rapih. Each was repeated three times so that there were 12 experimental units. Chromosome size is obtained by calculating the length of the long arm (q), the length of the short arm (p), and the total length (q + p) of the chromosomes. The shape of the chromosomes is obtained from the ratio of the chromosome arms ($r = q / p$) and categorized according to Ciupercescu et al. (1990) Table 1.

The karyotype is conducted by sorting the chromosomes from the longest to the shortest size and pairing the homologous chromosomes. Homologous chromosomes are determined according to the same size and shape. The ideogram arrangement is based on the average of chromosome pairs of the long arms (q) and short (p) arms and the shape of the chromosomes

The chromosome asymmetry index is calculated based on the formula of (Zarco 1986) intrachromosomal asymmetry index (A1), which was:

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

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

The interchromosomal asymmetry index is $A2 = SD/\bar{X}$. The standard deviation of the average length of chromosomes in a karyotype. X is the Average chromosome length in a karyotype.

Table 1. The type of chromosome shape based on chromosome arm length ratio ($r = q/p$)

Chromosome shape ($r = q/p$)	The ratio of arm length
Metacentric (m)	$1.0 < r \leq 1.7$
Submetacentric (sm)	$1.7 < r \leq 3.0$
Akrocentric (t)	$3.0 < r \leq 7.0$
Telocentric (T)	≥ 7.0

Data analysis

The data was obtained through the direct observation method and subsequently carried out descriptive chromosome analysis by presenting the data in pictures, tables, and descriptions to describe the karyotype of chromosomes (number, size, and shape) of rambutan. The data are processed in the Corel Draw X5 application to redraw the chromosome data obtained to see the number and shape more clearly. Chromosome size was obtained by calculating the long arm (q) and short arm (p), and the total length (q+p) in the Corel Draw X5 and Microsoft Excel applications by dividing the results of the micrometer calibration. The chromosome shape is obtained from the ratio of the length of the chromosome arm ($r = q/p$).

RESULTS AND DISCUSSION

Chromosome number

The results showed that the number of chromosomes from rambutan was 22 chromosomes or 11 pairs. The cytoplasm is clear, and the chromosomes are evenly distributed. The number of chromosomes could usually be seen and counted directly in the field of view (photo) thereunder. This makes the number of chromosomes a characteristic often used in cytology research. The rambutan chromosome shows in (Figures 1 and 2).

According to the research results, things that shall consider in cutting the roots are cutting time and plant conditions. Root cutting is a significant part of making chromosome preparations. Vigorous root growth was found at 5-10 mm from the root tip (Nair 2019). Each plant has a specific time of cell division, generally in the morning period. In Willie and Aikpokpodion's (2015) research, root cutting at 08:00 in legumes was founded on many mitotic phases, the most suitable phase for chromosomal observation. In addition, Sangur et al. (2021) research cut the *Cajanus cajan's* root at 08:00. The Rambutan plant has an active cell division time. There are many prometaphase cells from 08:00-08:30. At that time, the chromosomes appear shorter and more spread out, which makes observation easier. Ramkumar and Baum (2016) stipulate that the cell division phase requires a rapid and almost complete change in the structure, polarity, and shape of the cell to separate all the cellular components into two daughter cells. Another thing that can affect is the condition of the plant. Rambutan plants that experience poor growth tend to have less active cell division, which affects the quality of the chromosome preparations.

Chromosomes size and shape

The chromosome size variable can assist in the identification of the chromosome shape of the rambutan plant. Based on the size of the chromosomes, each variety has a chromosome size that is not much different. The shape of the chromosome from each variety is the same (Tables 2, 3, 4, and 5).

The research found that Ace rambutan has a chromosome size with an average chromosome length of $1.777 \pm 0.363 \mu\text{m}$. The total chromosome length ranges

from 1.217 ± 0.118 to 2.440 ± 0.185 μm . The length of the long arm was 0.690 ± 0.047 μm to 1.336 ± 0.138 μm , and the short arm length was 0.526 ± 0.071 μm to 1.105 ± 0.047 μm . Afterward, Binjai rambutan has an average chromosome length of 1.477 ± 0.392 μm . The total chromosome length ranged from 0.936 ± 0.043 μm to 2.167 ± 0.195 μm . The long arm length was 0.493 ± 0.016 μm to 1.143 ± 0.101 μm , and the short arm length was 0.443 ± 0.027 μm to 1.024 ± 0.094 μm . Lebak Bulus rambutan has an average chromosome length of 1.418 ± 0.320 μm . The total chromosome length ranged from 0.998 ± 0.017 μm to 2.104 ± 0.113 μm . The long arm length was 0.550 ± 0.064 μm to 1.139 ± 0.056 μm , and the short arm length was 0.448 ± 0.017 μm to 0.964 ± 0.057 μm . Rapih rambutan average chromosome length was 1.443 ± 0.315 μm . The total chromosome length ranged from 1.008 ± 0.016 μm to 2.045 ± 0.279 μm . The length of the arm was 0.532 ± 0.015 μm to 1.094 ± 0.153 μm , and the short arm length was 0.476 ± 0.001 to 0.952 ± 0.125 μm . Plants in one species usually have the same number of chromosomes, but the size and shape of the chromosomes can change. The variation usually involves numerical changes with insignificant chromosome shape and size differences (Gutiérrez-Flores 2018). These variations can be neutral, useful, or damaged to the plant (Saxena 2014).

Based on the results of observations (Tables 2, 3, 4, and 5). The calculation of the chromosome arm ratio shows that the chromosomes of Ace, Binjai, Lebak Bulus, and Rapih rambutans (*Nephelium lappaceum*) have a metacentric shape in all chromosome pairs. Metacentrics are formed

from helical coils of chromatin sequences (Beseda et al. 2020).

Karyotype chromosomes

A karyotype can be presented in two forms, karyogram and ideogram. The following is the result of compiling a karyogram (Figures 3, 4, 5, and 6). The karyogram that has been arranged shows the similarities in each pair. The similarities are in the size and the shape of the chromosomes from each pair. The shape of the chromosomes of each variety is the same, called a metacentric. The size of the chromosomes for each variety is not much different, but the largest size starts with the Ace, then followed by the Binjai, Rapih, and Lebak Bulus. However, in some chromosome pairs, the homologous chromosomes are almost the same between each pair. This can be seen on Binjai rambutan chromosomes number 11, 12, and 13, 14. Ideogram is a karyotype that is arranged in the form of a diagram. The ideogram arrangement can be seen in Figures 7, 8, 9, and 10.

According to the results, there are similarities between each pair from each variety. Ideograms can help to clearly show the shape and size of one set of chromosomes (Yuniastuti et al. 2018). The karyotype of each individual of one species generally has the same shape and size, but this does not rule out differences. These differences can occur due to changes in chromosome structure.

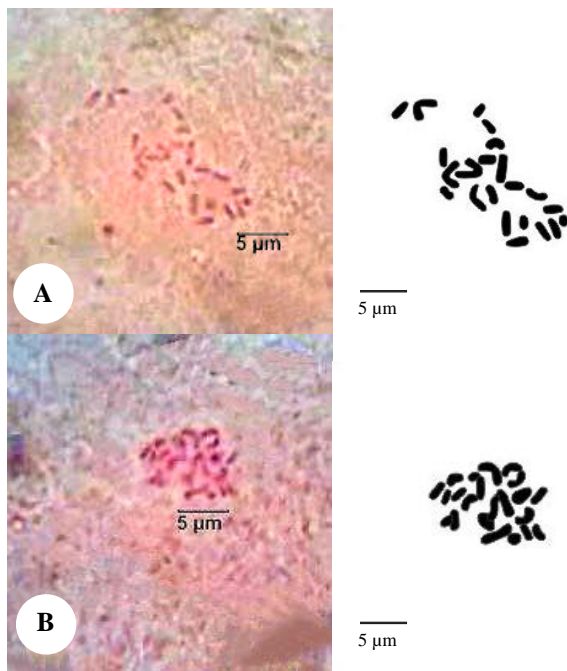


Figure 1. Chromosome results of rambutan (*Nephelium lappaceum* L.). A. Ace, B. Binjai

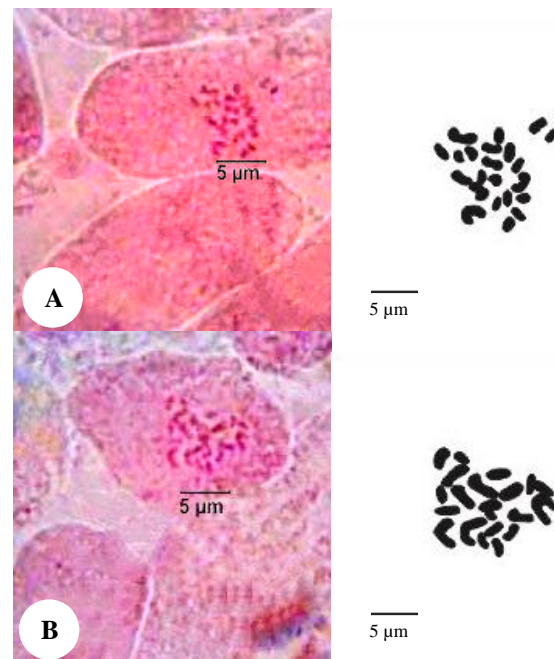


Figure 2. Chromosome results of rambutan (*Nephelium lappaceum* L.). A. Lebak Bulus, B. Rapih

Table 2. Chromosome shape and size of Ace rambutan (*Nephelium lappaceum* L.)

Chromosome pair	Chromosome length ($\bar{x}\pm SD, \mu\text{m}$)			Ratio ($r=q/p$)	Chromosome shape
	Long arm (q)	Short arm (p)	Total (q+p)	($\bar{X}\pm SD, \mu\text{m}$)	
1	1.336±0.138	1.105±0.047	2.440±0.185	1.207±0.073	Metacentric
2	1.157±0.094	0.976±0.054	2.133±0.148	1.185±0.031	Metacentric
3	1.083±0.084	0.990±0.047	2.074±0.131	1.093±0.033	Metacentric
4	1.061±0.002	0.883±0.057	1.944±0.059	1.203±0.076	Metacentric
5	1.031±0.003	0.893±0.057	1.924±0.061	1.157±0.070	Metacentric
6	0.902±0.024	0.817±0.009	1.720±0.014	1.105±0.042	Metacentric
7	0.867±0.006	0.786±0.033	1.653±0.028	1.105±0.055	Metacentric
8	0.825±0.045	0.736±0.037	1.561±0.082	1.121±0.005	Metacentric
9	0.807±0.057	0.721±0.037	1.529±0.094	1.118±0.022	Metacentric
10	0.781±0.040	0.574±0.024	1.355±0.064	1.361±0.015	Metacentric
11	0.690±0.047	0.526±0.071	1.217±0.118	1.318±0.088	Metacentric

Table 3. Chromosome shape and size of Binjai rambutan (*Nephelium lappaceum* L.)

Chromosome pair	Chromosome length ($\bar{x}\pm SD, \mu\text{m}$)			Ratio ($r=q/p$)	Chromosome shape
	Long arm (q)	Short arm (p)	Total (q+p)	($\bar{X}\pm SD, \mu\text{m}$)	
1	1.143±0.101	1.024±0.094	2.167±0.195	1.116±0.004	Metacentric
2	1.170±0.039	0.919±0.027	2.089±0.012	1.274±0.079	Metacentric
3	0.935±0.086	0.756±0.045	1.690±0.131	1.235±0.039	Metacentric
4	0.896±0.032	0.704±0.049	1.600±0.081	1.276±0.043	Metacentric
5	0.880±0.076	0.676±0.006	1.555±0.082	1.302±0.100	Metacentric
6	0.779±0.030	0.685±0.035	1.463±0.066	1.138±0.014	Metacentric
7	0.686±0.047	0.595±0.034	1.281±0.081	1.152±0.014	Metacentric
8	0.704±0.025	0.533±0.047	1.237±0.072	1.322±0.070	Metacentric
9	0.650±0.030	0.506±0.022	1.156±0.008	1.287±0.116	Metacentric
10	0.610±0.013	0.468±0.042	1.077±0.056	1.307±0.089	Metacentric
11	0.493±0.016	0.443±0.027	0.936±0.043	1.115±0.031	Metacentric

Table 4. Chromosome shape and size of Lebak Bulus rambutan (*Nephelium lappaceum* L.)

Chromosome pair	Chromosome Length ($\bar{x}\pm SD, \mu\text{m}$)			Ratio ($r=q/p$)	Chromosome shape
	Long arm (q)	Short arm (p)	Total (q+p)	($\bar{X} \pm SD, \mu\text{m}$)	
1	1.139±0.056	0.964±0.057	2.104±0.113	1.182±0.013	Metacentric
2	0.902±0.051	0.829±0.094	1.731±0.094	1.093±0.063	Metacentric
3	0.898±0.057	0.731±0.024	1.629±0.024	1.230±0.118	Metacentric
4	0.788±0.044	0.745±0.051	1.533±0.051	1.058±0.013	Metacentric
5	0.804±0.008	0.631±0.063	1.435±0.063	1.280±0.142	Metacentric
6	0.752±0.067	0.615±0.066	1.368±0.066	1.224±0.021	Metacentric
7	0.711±0.005	0.632±0.035	1.343±0.035	1.126±0.055	Metacentric
8	0.651±0.019	0.519±0.020	1.170±0.020	1.256±0.085	Metacentric
9	0.731±0.040	0.473±0.032	1.204±0.032	1.547±0.019	Metacentric
10	0.586±0.062	0.499±0.042	1.085±0.042	1.175±0.024	Metacentric
11	0.550±0.064	0.448±0.017	0.998±0.017	1.227±0.097	Metacentric

Table 5. Chromosome shape and size of Rapih rambutan (*Nephelium lappaceum* L.)

Chromosome pair	Chromosome length ($\bar{x}\pm SD, \mu\text{m}$)			Ratio ($r=q/p$)	Chromosome shape
	Long arm (q)	Short arm (p)	Total (q+p)	($\bar{X}\pm SD, \mu\text{m}$)	
1	1.094±0.15	0.952±0.125	2.045±0.279	1.148±0.011	Metacentric
2	0.993±0.129	0.808±0.028	1.801±0.158	1.226±0.117	Metacentric
3	0.838±0.010	0.779±0.028	1.617±0.018	1.077±0.052	Metacentric
4	0.845±0.016	0.755±0.029	1.600±0.047	1.121±0.022	Metacentric
5	0.745±0.090	0.707±0.070	1.452±0.162	1.053±0.023	Metacentric
6	0.743±0.043	0.708±0.063	1.451±0.107	1.051±0.034	Metacentric
7	0.692±0.032	0.633±0.053	1.325±0.087	1.095±0.042	Metacentric
8	0.666±0.032	0.556±0.112	1.223±0.145	1.216±0.186	Metacentric
9	0.643±0.035	0.545±0.057	1.188±0.092	1.182±0.060	Metacentric
10	0.613±0.048	0.546±0.085	1.160±0.135	1.129±0.088	Metacentric
11	0.532±0.015	0.476±0.001	1.008±0.016	1.117±0.029	Metacentric

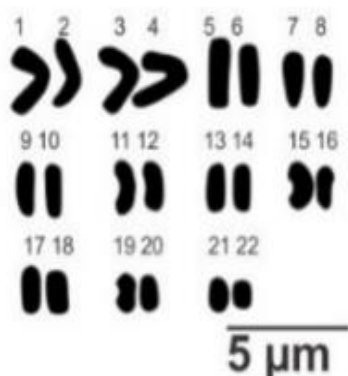


Figure 3. Chromosome karyogram of Ace rambutan (*Nephelium lappaceum* L.)

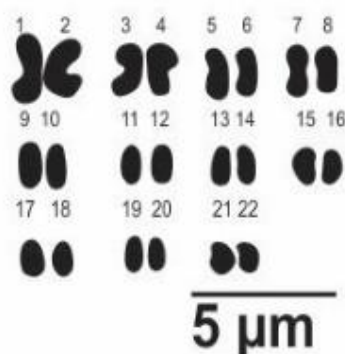


Figure 5. Chromosome karyogram of Lebak Bulus rambutan (*Nephelium lappaceum* L.)

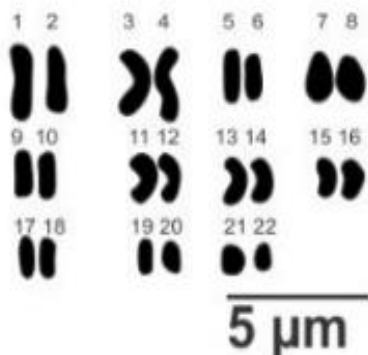


Figure 4. Chromosome karyogram of Binjai rambutan (*Nephelium lappaceum* L.)

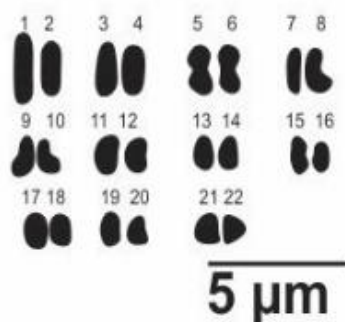


Figure 6. Chromosome karyogram of Rapih rambutan (*Nephelium lappaceum* L.)

Chromosome asymmetry index

Chromosome Asymmetry Index (A1) functions to explore variations in the shape of chromosomes in a karyotype. Tabur et al. (2012) also mentioned that the A1 value is helpful to determine the evolution relation between plant groups. The A1 value should be between 0 and 1. The observations found that the A1 Ace rambutan value was 0.147 ± 0.005 , Binjai rambutan was 0.183 ± 0.005 , Lebak Bulus rambutan was 0.170 ± 0.007 , and Rapih was 0.110 ± 0.004 .

The Interchromosome Asymmetry Index (A2) functions to detect any dispersion or deviation of chromosome size in a karyotype. The observations obtained were the A2 value of Ace rambutan was 0.204 ± 0.052 , Binjai rambutan was 0.265 ± 0.053 , Lebak Bulus rambutan was 0.225 ± 0.031 , and Rapih rambutan was 0.218 ± 0.076 .

Discussion

Based on the results of chromosome photos, it was found that the chromosomes had good distribution and clear appearance. This is because the time of cutting the roots is during the time that the dominant cell is prometaphase. In the prometaphase, the spindle threads of

the chromosomes are shorter and denser, resulting in clear and spread-out chromosomes. This certainly facilitates the process of observing the morphology of the chromosomes. This is suitable with the Aristya et al. (2019) stipulated that in the prometaphase, jerky movement causes the condensed chromosomes to spread out in the cytoplasm so that the counting of the number of chromosomes becomes more accessible because there are no overlapping chromosomes. Ou et al. (2017) mentioned the phase observed in the making of chromosome preparations is the prometaphase in the mitotic phase. In mitotic chromosomes, the chromatin threads are more flexible. Ferreira and Maiato (2021) also mentioned prometaphase means the assembly of the mitotic spindle that mediates the interception of chromosomes in their kinetochores and peaks during all chromosomes head toward the equatorial plane, so that the chromosome looks clear and shorter.

Determination of the number of chromosomes plays an essential role in studying evolution and the genome (Rice and Mayrose 2021). The results show that the rambutan has a chromosome number of 22 ($2n=22$) diploid in each variety (Shown in Figure 1). This is suitable with the established facts from previous studies by Sarip (1998) that rambutan (*Nephelium lappaceum* L.) has 11 pairs of

chromosomes. Although the phenotypes look different, different varieties usually do not make any changes to the chromosome number. The number of chromosomes tends to be more stable than the morphological characteristics, so it can help to determine kinship relationships (Yuniastuti et al. 2021). Another plant in the same genus as rambutan, lychee (*Nephelium litchi* Camb.) has $n=15$ chromosome number (Mehra, 1972). Differences in the number of chromosomes are often found in different species, and these differences are able to arise in speciation events (Lukhtanov et al. 2011).

The shape of the chromosomes can determine the kinship between individual plants. According to the results, it was found that there were similarities in the shape of the chromosomes in each variety which is metacentric. Although the varieties and morphology of the fruit are different, plants with one species generally have the same chromosome shape. Yuniastuti et al. (2018) stipulated that even though they are in different species, the shape of the

chromosomes could be exact in one family. The A1 value of each variety getting closer to zero, according to Parjanto et al. (2003) whereas the proportion of the metacentric chromosome forms is getting bigger. These observations conclude that the four varieties have metacentric chromosome forms. The result showed that the A2 value has a small value (closer to zero). The smaller value of A2 indicates that the size deviation in the karyotype is getting smaller. Based on the results obtained, the A2 value in each variety is not much different. Parjanto (2003) stipulated that indicate the size deviation was not too large.

The conclusion of this research was Ace, Binjai, Lebak Bulus, and Rapih rambutans (*Nephelium lappaceum* L.) have a karyotype formula of $2n=2x=22=11m$. Rambutan has the same chromosome morphology even though the variety is different, there is a necessity for research on other different varieties in order to add variations to the data.

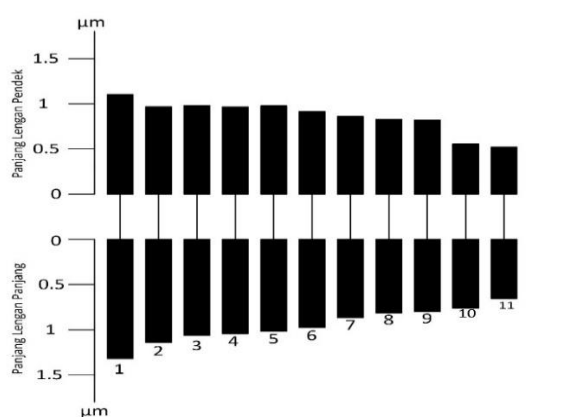


Figure 7. Chromosome ideogram of Ace rambutan (*Nephelium lappaceum* L.)

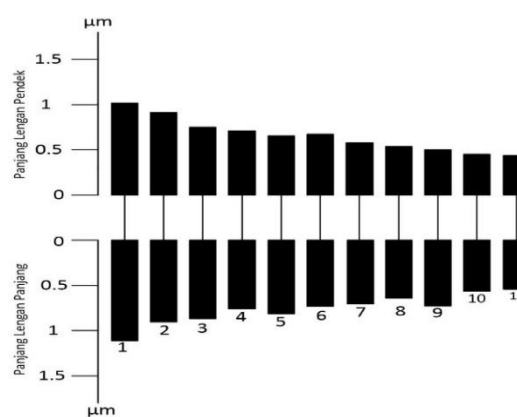


Figure 9. Chromosome ideogram of Lebak Bulus rambutan (*Nephelium lappaceum* L.)

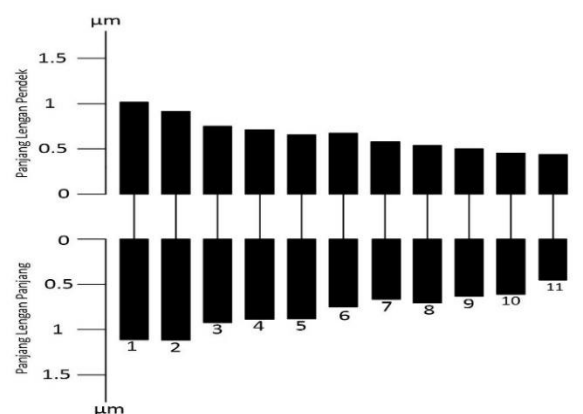


Figure 8. Chromosome ideogram of Binjai rambutan (*Nephelium lappaceum* L.)

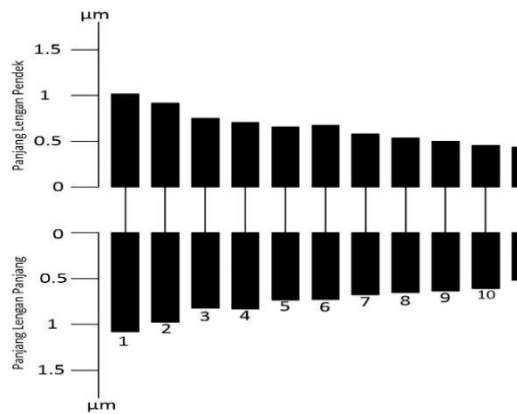


Figure 10. Chromosome ideogram of Rapih rambutan (*Nephelium lappaceum* L.)

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