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KARYOTYPE ANALYSIS OF PACHIRA (*PACHIRA AQUATICA*)

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SUMMARY

Pachira aquatica belongs to the Bombacaceae group, the clades in the family Malvaceae. The subfamily contains about 304 species, most with high economic and medicinal values. Considering their importance, some of the plants attained special cultural status. Pachira comes from Central America and South America, introduced and spread to Europe, Africa, and Asia. The diversity of plant morphology can result from environmental factors interacting with genetic features, then expressed as phenotypes. Geographic isolation can prevent gene flow between populations. Geographic separation allows mutations to occur, and natural selection and genetic drift can alter allele frequencies differently in separate populations leading to allopatric speciation. The present study aims to determine the characteristics and chromosomal composition of *P. aquatica*. This study used the squashing method, often employed to obtain chromosomes by squeezing the preparations. The results showed that the number of chromosomes of pachira was 2n = 66. The chromosome length of pachira ranged from 0.812±0.009 to 1.955±0.009 µm. The chromosome shape of pachira is metacentric. The karyotype arrangement of pachira is 2n = 2× = 29m + 4sm, where 2n equals to 29 pairs of metacentric chromosomes and four pairs of submetacentric chromosomes. The intrachromosomal asymmetry index (A1) of the chromosome of pachira scored at 0.19±0.001, with the value of the interchromosomal asymmetry index (A2) at 0.224±0.021.

Keywords: Pachira (*Pachira aquatica*), cytogenetic, chromosome, squash method

Key findings: The value of the intrachromosomal asymmetry index (A1) indicates that the pachira chromosomes revealed mostly metacentric, and the value of the interchromosomal asymmetry index (A2) indicates that the deviation of chromosome size that occurs in the karyotype was small.

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INTRODUCTION

Pachira *(Pachira aquatica)*, a member of the Malvaceae, is a tropical wetland tree native to Central and South America (Robyns, 1964). According to another view based on palynomorphological characteristics exhibited by the members of this plant group, the subfamily could have a triphyletic origin with southern Central America, East Africa, Madagascar, and Southeast Asia as centers of origin (Fucha, 1967). In Asia, the stems of young seedlings pachira braid to make a braided bonsai money plant tree, which has become highly prized and in great demand in East Asia and Southeast Asia (Lim, 2012). The center of origin of the species of this plant group differs according to the genus. Two

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groups can categorize this plant group species, i.e., plants endemic to a certain area and plants widely distributed through an introduction.

Different cytotypes may coexist in a population, resulting in ecological and evolutionary variation, although the polyploids may diversify at lower rates (Mayrose *et al*., 2011). Hence, understanding these cytotypes and their distribution is necessary for explaining the evolutionary process of different taxa (Escudero *et al.*, 2012). Cytogenetic data already exist for many temperate plants, but neotropical taxa still lacked studies, causing a poor understanding of the evolution of ploidy levels in many groups (Husband *et al*., 2013). Rubio-De-Casas *et al*. (2007) stated that morphological variations of a population in different geographical conditions could result from differences in genetic structure and environmental conditions.

The species of *P. aquatica* varies in chromosome number from 2n = 88 (Baum and Oginuma, 1994), 2n = 92 (Costa *et al.*, 2017), and 2n = 34 (Lima *et al*., 2012). This difference may come from frequent fusion events, the same number of chromosomes can be a product of the convergence, and different numbers may appear among close relatives, resulting in a lack of phylogenetic signal for chromosome number. Chromosome number is one of the most required cytological data in comparative cytogenetics (Fishman *et al*., 2014). Traditional cytogenetic analyses in Bombacoideae suggest high intra- and interspecific karyotypic diversity (Baker and Baker, 1968). This study found the number of new chromosomes in *P. aquatica* plants by presenting the karyotype along with its shape and size.

MATERIALS AND METHODS

Plant material

Materials used were *P. aquatica*'s seeds collected from Garut, Jawa Barat, Indonesia. The obtained pachira seeds came from pachira fruits that were ready for harvest and separated from the pulp. Keeping seeds moist avoided damage from fungi and other organisms. The study used roots to determine the number, shape, size, and type of chromosomes from the pachira plant. Root cutting is an essential step in the manufacture of chromosomal preparations (Putri *et al.,* 2022).

Study species

Pachira plants grow in partial shade or full sun, depending on climatic conditions. The pachira plant has a large crown and dense leaves, round in shape, with a stem diameter of 25–90 cm. The bark of pachira is thick, smooth, and gray to brownish. Pachira plants flower at different times of the year depending on the growing region, but can be observed from September to November or from December to August (Hernández-Montero and Sosa, 2016).

Pachira plants have compound leaves arranged circularly in shiny green, with five to seven lanceolate leaflets (Azizah *et al.,* 2021). Pachira flower buds are yellowish, narrowly cylindrical, and slightly curved at the tip. The flowers are showy and long, with narrow petals and hair-like yellowish-orange stamens. Pachira seeds shaped a medium-sized ellipse. *Pachira aquatica* includes Euphorbiaceae, such as, rubber plants and castor oil, with seed structure containing elaiosome oil. Pachira seeds, mostly consumed raw or as roasted nuts, taste with a chestnut flavor. Pachira leaves and young flowers served widely as vegetables (Lorenzi, 1992).

Chromosome observation

Pachira aquatica seeds germination used a plastic box after soaking in PGPR for 6 h, with watering as needed. Sprout shoots from previously treated seeds served as the material for this study. The shoots cut at 5 mm long underwent soaking in distilled water at a temperature of 5°C - 7°C for 24 h. Fixation was done by soaking the shoots in 45% acetic acid solution for 1 h at room temperature, then washed with distilled water three times. The next step includes hydrolysis with 1N HCl solution for 1 min at room temperature, then washing the shoots with distilled water three times. Squashing the shoots followed using a drop of 45% acetic acid with a covered glass, squeezing with the thumb, then pressing with a blunt brush tip to make it more spreadable. Sealing the preparations used a clear nail polish to protect the preparations. This study used a binocular microscope with 1000× magnification and documented using the Optilab app.

Karyotype analysis

The preparations received immersion oil drops for easier observations under a microscope with a magnification of 100×. The photographed image used the Optilab app.

Data processing used the Image Raster application for calibrating the observations and the CorelDraw X7 application to redraw the chromosome data to make it look clearer. The obtained karyotype came from sorting the chromosomes from the longest to the shortest total length using CorelDraw X7 and Microsoft Visio.

The process of making a karyotype begins with the identification of homologous chromosomes. The chromosome sorting started from the longest to the shortest mean of the absolute chromosome arm (Billa *et al.,* 2022). Mismatches in determining homologous chromosome pairs can result in an error in the karyotype of chromosomes in a plant. Sorting the pairs of chromosomes from the longest to the shortest size based on the homogeneity of shape and size helped arrange karyotype. The intrachromosomal asymmetry index (A1) determined a karyotype's variation of chromosome shape. Intrachromosomal asymmetry index (A1) calculation used a formula based on Romero (1986) and Parjanto *et al.* (2003).

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

Where:
bi = average short arms of each pair of homologous chromosomes
Bi = average long arms of each pair of homologous chromosomes
n = number of pairs of homologous chromosomes

The interchromosomal asymmetry index (A2) determined the size deviation of chromosomes in a karyotype. The interchromosomal asymmetry index (A2) calculation also used a formula based on Romero (1986) and Parjanto *et al.* (2003).

$$A2 = \frac{SD}{\bar{A}}$$

Where:
SD = Standard deviation of chromosome length in a karyotype
$\bar{\mathbf{A}}$ = Average length of chromosomes in a karyotype

The intrachromosomal asymmetry index (A1) helped determine the variation of the chromosome shape of a karyotype. The interchromosomal asymmetry index (A2) determined the dispersion or deviation of chromosome size from a karyotype (Hartati *et al.,* 2014). The values for the intrachromosomal asymmetry index (A1) ranged between zero and one, and the values for the interchromosomal asymmetry index (A2) were the same, ranging between zero and one. The results of the A1 calculation show a number that is close to zero. According to Ningsih *et al.* (2015), the value of the intrachromosomal asymmetry index (A1), between 0–1, indicates that the plant's chromosomes are metacentric. This statement gained support from the study results showing that the chromosome shape of the *P. aquatica* plant was mostly metacentric. The shape of the chromosome is determined by the ratio of the long arm to the short arm. The preparation of the idiogram used the length and shape of the chromosomes of the 38 somatic cells.

RESULTS

Chromosome number

Chromosome number is the most easily observed chromosome characteristic compared with other chromosomal characteristics, such as, chromosome size and karyotype. An imperative vital data in cytological research requires data on the chromosome number. The active mitotic time of the pachira plant ranges between 9.30–10.30 WIB, when the cells are in the prometaphase. According to Yuniastuti *et al.* (2021), the number of chromosomes occurs at the prometaphase. Research results on the chromosomes of the root tip cells of the *P. aquatica* amounted to 2n = 66 (Figure 1). It indicates pachira's chromosome number as diploid, with n = × = 33 (Figure 2).

Chromosome size and shape

The total chromosome length of the pachira plant ranges from 0.812±0.009 to 1.955±0.009 µm. The short arm of the chromosome ranged from 0.370±0.009 to 0.844±0.037 µm, while the long arm ranged from 0.442±0.018 to 1.110±0.028 µm. This result differs from the study of Lima *et al.* (2012), which reported that the total chromosome length of the pachira plant ranges from 0.70 to 6.25 µm. The short arm of the chromosome ranges from 0.20 to 3.08 µm, and the long arm ranges from 0.50 to 3.25 µm. The average length of homologous chromosome pairs' description appears in Table 1.

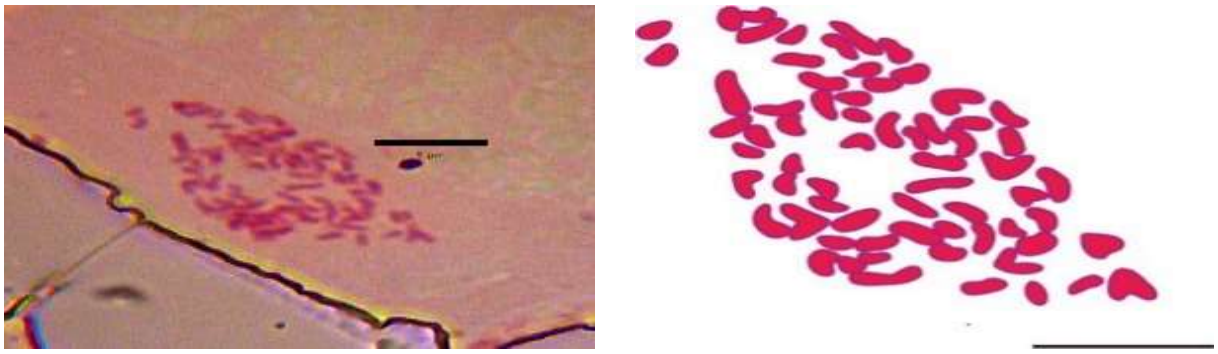

Figure 1. Chromosome of pachira (*Pachira aquatica*).

Table 1. Chromosome size of pachira (*Pachira aquatica*).

Chromosome Pair	Chromosome Length ($\bar{X}$ ± SD, µm)			Ratio (r=q/p) ($\bar{X}$ ± SD, µm)	Chromosome Shape
	Long Arm (q)	Short Arm (p)	Total (q+p)		
1	1,110±0,028	0,844±0,037	1,955±0,009	1,317±0,090	m
2	1,019±0,028	0,786±0,101	1,805±0,073	1,311±0,204	m
3	0,948±0,018	0,734±0,046	1,682±0,028	1,295±0,106	m
4	0,916±0,046	0,714±0,055	1,630±0,009	1,288±0,164	m
5	0,877±0,009	0,701±0,055	1,578±0,046	1,254±0,112	M
6	0,890±0,009	0,669±0,028	1,558±0,018	1,332±0,069	M
7	0,825±0,064	0,708±0,009	1,532±0,073	1,165±0,076	M
8	0,838±0,009	0,669±0,009	1,506±0,018	1,252±0,003	M
9	0,799±0,028	0,662±0,018	1,461±0,009	1,207±0,075	M
10	0,786±0,009	0,656±0,009	1,442±0,018	1,198±0,003	M
11	0,786±0,028	0,623±0,018	1,409±0,009	1,262±0,081	M
12	0,760±0,028	0,604±0,009	1,364±0,018	1,259±0,065	M
13	0,727±0,018	0,617±0,064	1,344±0,046	1,187±0,153	M
14	0,766±0,037	0,552±0,009	1,318±0,028	1,389±0,090	M
15	0,695±0,028	0,584±0,055	1,279±0,083	1,192±0,065	M
16	0,675±0,018	0,578±0,009	1,253±0,009	1,169±0,050	M
17	0,662±0,037	0,578±0,009	1,240±0,046	1,146±0,045	M
18	0,675±0,018	0,539±0,009	1,214±0,009	1,253±0,055	M
19	0,669±0,009	0,539±0,009	1,208±0,018	1,241±0,004	M
20	0,649±0,018	0,526±0,009	1,175±0,009	1,235±0,056	M
21	0,617±0,028	0,539±0,009	1,156±0,018	1,145±0,071	M
22	0,617±0,028	0,500±0,009	1,117±0,018	1,234±0,078	M
23	0,597±0,037	0,487±0,028	1,084±0,009	1,231±0,145	M
24	0,597±0,018	0,461±0,009	1,058±0,009	1,296±0,066	M
25	0,591±0,009	0,468±0,018	1,058±0,028	1,264±0,030	M
26	0,552±0,009	0,468±0,018	1,019±0,009	1,182±0,066	M
27	0,565±0,009	0,442±0,018	1,006±0,009	1,281±0,074	M
28	0,526±0,028	0,442±0,018	0,968±0,009	1,194±0,112	M
29	0,526±0,009	0,429±0,018	0,955±0,009	1,229±0,074	M
30	0,506±0,018	0,396±0,009	0,903±0,009	1,280±0,076	M
31	0,474±0,028	0,416±0,018	0,890±0,009	1,143±0,117	M
32	0,487±0,009	0,383±0,009	0,870±0,018	1,271±0,007	M
33	0,442±0,018	0,370±0,009	0,812±0,009	1,194±0,079	M

Figure 2. Karyogram chromosome of pachira (*Pachira aquatica*).

Karyotype

The karyotype is the arrangement of chromosomes based on the number, length, and shape of an organism's chromosomes from predetermined standards. Karyotyping compiles chromosome pairs from an organism to reveal its structural characteristics (O'Connor, 2008). Yuniastuti *et al.* (2018) reported that karyotype arrangement presentation forms a chromosomal karyogram, a photomicrograph of a single picture of somatic metaphase cells arranged based on similarity in size and shape. The *P. aquatica* consists of 33 pairs of chromosomes with two sets of homologous chromosomes. The chromosome form of the pachira plant is mostly metacentric.

Chromosome asymmetry index

Chromosome morphological characters can be described by the degree of symmetry of the karyotype, namely, the intrachromosomal asymmetry index (A1) and the interchromosomal asymmetry index (A2). The results showed that the A1 is 0.19±0.001, and the value of the A2 is 0.224±0.021.

DISCUSSION

The basic chromosome of about 120 species representing 25 genera of the family Malvaceae, according to Skovsted (1935), has five series: (a) a five series (5, 10, 15, 25); (b) a six series (12, 18, 36); (c) a seven series (7, 14-, 21, 28, 35, 42, 56); (d) an 11 series (11, 22, 33); and (e) a 13 series (13, 26, 39, 65). Species belonging to the Malvaceae family have high chromosomal diversity. The number of chromosomes of *P. aquatica,* according to Lima *et al.* (2012), is 2n = 34, 2n = 88 for Baum and Oginuma (1994), and Costa *et al.* (2017) amounted to 2n = 92. Hazra and Sharma (1971) argue that the cytotaxonomy of Malvaceae has a basic chromosome seven, which is more prevalent and deep-seated in the majority of genera, and should be regarded as the basic number for the family from which other numbers (7, 8, 9, 15, 17) have evolved.

The Bombacoideae are predominantly neotropical, whereas putatively early-branching Malvoideae have a predominantly Asian or Australasian distribution (Pfeil *et al*., 2002). Plants of Bombacoideae are usually perennial tall trees with swollen tree trunks. The Bombacoideae species has high chromosome numbers. For example, the lowermost chromosome numbers in Bombacoideae occurred in Bombax insigne (2n = 18) from India and Pachira macrocarpa (2× = 26) from China, with uppermost numbers documented in Eriotheca species (6× = 276) came from Brazil (Marinho *et al.*, 2014). According to Marinho *et al*. (2014), most of the Malvoideae have chromosome numbers lower than the Bombacoideae, indicating that the origin of these latter taxa represents an important transition in the group as a whole. Biogeographic hypotheses need evaluation on a more inclusive phylogenetic data set than the previously considered.

Another example in the genus Adansonia, the baobab trees of Africa and Madagascar, up to 2n = 160 chromosomes have been reported (Baum and Oginuma, 1994). Meanwhile, report of Faridi *et al*. (2020) have the same number of chromosomes, 2n = 4× = 168. In Adansonia, the recently found diploid *Adansonia kilima* has a restricted distribution, whereas *Adansonia digitata*, which seems to comprise recent neopolyploids, is much more widespread (Pettigrew *et al*.,

2012). Recent polyploidization events involve cytotype formation and demographic establishment (Ramsey and Schemske, 2002), and populations of neopolyploids usually have mutations or special gene combinations that are fixed even when phenotypic changes are small, due to reproductive isolation (Soltis *et al.*, 2009).

The results of chromosome observations in pachira plant cells provided easy detection because of the good staining results of the preparations. Research results on the chromosomes of the root tip cells of the pachira plant scored 2n = 66. Pachira's chromosome number is diploid, with n = × = 33. Pachira basic chromosomes in this study amounted to 33, according to the study of Skovsted (1935), belonging to the 11 series (11, 22, 33).

The chromosome size of the *P. aquatica* varies. Differences in chromosome size can occur from differences in the division during the mitotic phase (Siagian, 2006). The total chromosome length of pachira plants ranged from 0.812±0.009 to 1.955±0.009 µm. The short arm of the chromosome ranged from 0.370±0.009 to 0.844±0.037 µm, with the long arm from 0.442±0.018 to 1.110±0.028 µm. This result differs from research by Lima *et al.* (2012) on the total chromosome length of pachira plants, with ranges from 0.70 to 6.25 µm. The short arm of the chromosome ranges from 0.20 to 3.08 µm, while the long arm ranges from 0.50 to 3.25 µm. This opinion also gained support from Moynihan and Mahon (1983) stating, a change between the length of the chromosome and the volume of the chromosome during the mitotic phase exists.

The chromosome arm ratio of the pachira plant was 1.143±0.117 to 1.317±0.090 µm. The chromosome shape of the pachira plant, based on the ratio of the chromosome arms, are metacentric and submetacentric because the arm ratio ranged between 1.0–1.7 µm (Figure 3). Based on the karyotype and chromosome ideogram of the pachira plant, the karyotype formula of the *P. aquatica* plant is 2n = 2× = 29m + 4sm, where × is the basic chromosome, m is the metacentric chromosome, and sm a submetacentric chromosome. However, *P. aquatica,* according to Lima (2012), has 2n = 34 chromosomes with four metacentric and 13 submetacentric chromosomes. These differences in chromosomal structure may occur through inversion, exchange, centric fission, Robertsonian fusion, deletion, duplication, and doubling of the genome encountered as a process of evolutionary change (Weiss-Schneeweiss and Schneeweiss, 2012).

The value of the interchromosomal asymmetry index (A2) of the *P. aquatica* plant is 0.224±0.021. The smaller A2 value indicates a relatively small chromosomal aberration in a karyotype. The calculation results of the A2 value showed a number close to zero, which means that the pachira plant chromosomal aberrations are small.

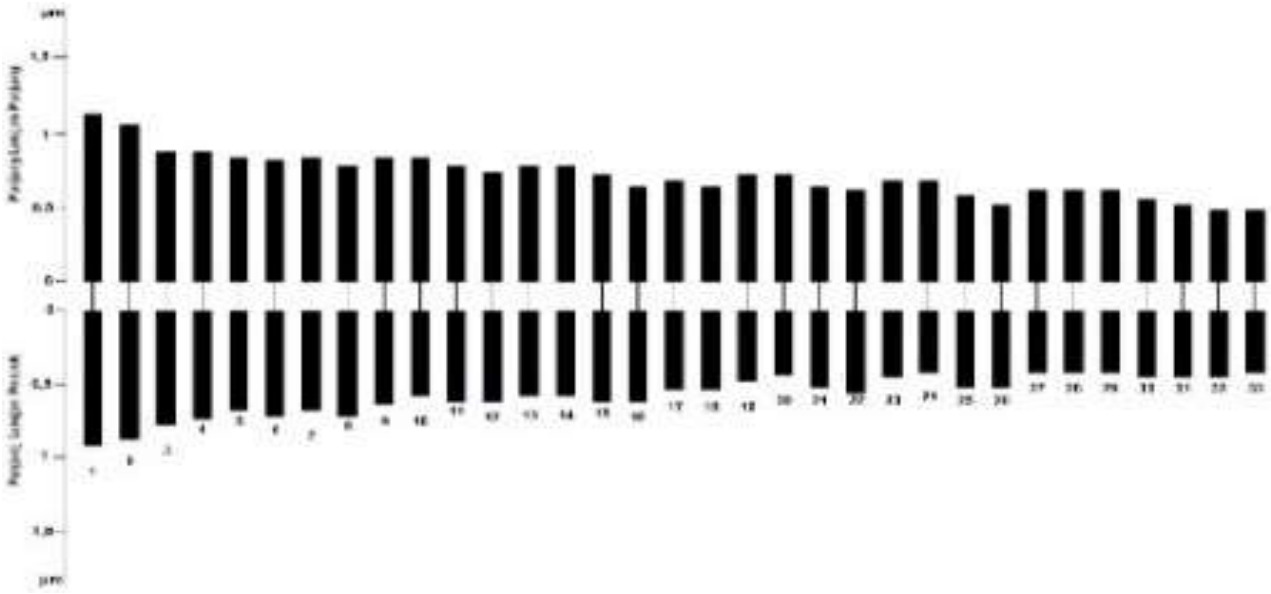

Figure 3. Ideogram chromosome of pachira (*Pachira aquatica*).

CONCLUSIONS

Based on this research, the conclusion shows that the number of chromosomes of the pachira plant is 2n = 66. The karyotype arrangement of pachira plant chromosomes is 2n = 2× = 29m + 4sm, where m is the metacentric chromosome and sm as the submetacentric chromosome. The intrachromosomal asymmetry index (A1) value between 0 and 1 indicates the pachira chromosomes are mostly metacentric. The value of the interchromosomal asymmetry index (A2) at 0.224±0.021 indicates that the deviation of chromosome size in the karyotype is small. Chromosome numbers remain uncertain and, thus, can tell little about biogeographic patterns or taxonomic affinities in the family. Additional research needs continuance to clarify the cytology of the Bombacaceae. Further counts on genera, as well as, recounts of previously studied groups require more research. In addition, studies of both meiotic and mitotic mechanisms would serve valuable to determine whether accessory chromosomes are present and whether aneusomatic divisions occurred.

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