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Morphological and cytological analysis of yellow skin dragon fruit (*Selenicereus megalanthus*)

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Abstract. *Setyowati A, Sukaya, Yuniastuti E. 2018. Morphological and cytological analysis of yellow skin dragon fruit (*Selenicereus megalanthus*). Cell Biol Dev 2: 8-14.* Dragon fruit is a tropical fruit that has the potential to be developed. Information on morphological and cytological characters of dragon fruit plants is still small and simple, so morphological and cytological analysis is necessary. The morphological and cytological analysis yielded useful information to support the dragon fruit plant breeding program. This study aims to study the morphological and cytological characteristics (karyotype) of the yellow skin dragon fruit (*Selenicereus megalanthus* (Schum. ex Vaupel) Moran). This study took samples from "Kusumo Wanadri Agrotourism" Glagah Indah Beach, Yogyakarta, Indonesia. Chromosomal identification was carried out at the Plant Breeding Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Indonesia. The research was carried out from December 2006 to June 2008. The methods used were field observations and treatment methods in water for 24 hours at a temperature of 5-8ºC, fixation in 45% acetic acid solution for 2 hours at a temperature of 5-8ºC, and hydrolysis in an aqueous solution. One (1) N HCl for 3-4 minutes at 60ºC, staining in 2% aceto-orcein solution for 24 hours at 5-8ºC and squashing. Morphological and cytological data were analyzed and presented descriptively. The morphology of the plant *S. megalanthus* is a white thread-shaped root with a fibrous plant root system. The stem is a smooth, faceted wet stem with concave edges. The fruit is oval, surrounded by short spines, the skin is yellow, and the flesh is white. The *S. megalanthus* has a tetraploid chromosome 2n = 4X = 44 with a chromosome length ranging from 2 + 0.098 m to 4.75 + 0.98 m. The karyotype formula for the *S. megalanthus* chromosome 2n = 4X = 44 = 40 m + 4 sm.

Keywords: Cytology, morphology, *Selenicereus megalanthus*

INTRODUCTION

Dragon fruit plants originating from Central and South America have not been widely cultivated. This plant has only been cultivated intensively in several countries such as Israel, Colombia, Nicaragua, Vietnam, Thailand, China, and Australia (Lichtenzveig et al. 2000).

Dragon fruit plants have tufts that resemble dragon scales. This plant is a vine-shaped tree with thorns that grow along the tendrils and look unique, especially if the fruit has appeared on the tendrils. At first, this plant was only used as an ornamental plant. However, after it was discovered that the fruit was delicious to eat, it started to be cultivated as a fruit crop.

Dragon fruit can be consumed in fresh or processed form. Dragon fruit has a fairly high nutritional and water content of about 90.20% of the fruit weight (Kristanto 2003; Aji et al. 2013). In addition, it tastes quite sweet because the sugar in the fruit is quite high. Besides being cultivated as a fruit plant, dragon fruit is also cultivated as a medicinal plant because it has properties for human health. These benefits include: balancing blood sugar, preventing colon cancer, protecting oral health, reducing cholesterol, preventing bleeding, and treating vaginal discharge complaints.

This plant was recognized and cultivated in Indonesia in 2000. However, the development of this plant cultivation is very slow, even though Indonesia's climatic conditions are very supportive of the development of this plant. In 2006, there were only a few areas that cultivated this plant, namely Malang, Kediri, Tawangmangu, Semarang, and Kulon Progo, with various planting areas. In recent years, after it was discovered that dragon fruit has medicinal properties, dragon fruit cultivation continues to be carried out because it is very profitable. However, the cultivation of yellow skin dragon fruit (*Selenicereus megalanthus* (Schum. ex Vaupel) Moran). is still rarely done. It is because *S. megalanthus* requires an environment to grow in the highlands, in contrast to other types of dragon fruit cultivated in the lowlands.

Introducing dragon fruit plants based on morphological and cytological characters will greatly support the success of the dragon fruit plant breeding program. However, until now, knowledge of dragon fruit plants' morphological and cytological characteristics is still small and simple. By knowing with certainty the morphological and cytological characteristics of a plant, genetic information of a plant can be known so that appropriate methods can be sought for its development and cultivation.

There are 2 genera of dragon fruit cultivated in Indonesia, namely *Hylocereus* and *Selenicereus*. The dragon fruit cultivated is dragon fruit from the genus *Hylocereus*, namely *H. undatus* (Haw.) Britton & Rose (white flesh), *H. polyrhizus* (F.A.C.Weber) Britton & Rose (dark red flesh), and *H. costaricensis* (F.A.C.Weber) Britton & Rose (pink flesh), which are all diploid plants

with 2N = 2X = 22. Meanwhile, the *Selenicereus* genus cultivated is *S. megalanthus*, a tetraploid plant 2n = 4x = 44. However, although the ploidy is known, the karyotype is not known.

This study aims to study the morphological and cytological characteristics (karyotype) of the yellow skin dragon fruit (*S. megalanthus*).

MATERIALS AND METHODS

Materials

Plant material of yellow skin dragon fruit (*S. megalanthus*) obtained from "Agrowisata Kusumo Wanadri" Glagah Indah Beach, Kulon Progo District, Yogyakarta, Indonesia. Chromosomal identification was carried out at the Plant Breeding Laboratory, Faculty of Agriculture, Universitas Sebelas Maret, Surakarta, Central Java, Indonesia.

The main chemicals are 2% aceto-orcein, 45% acetic acid, and 1 N HCl solutions.

Research design

Morphology

Morphological research was carried out using survey observation methods in the field. The survey is aimed at knowing the conditions at the research site, including observing environmental factors and identifying dragon fruit plants. A sampling of 5 plants was done randomly (random sampling).

Cytology

Cytological research was carried out using the squashing method, a method for obtaining preparations by squeezing a piece of tissue or an organism. Thus, a dispersed preparation was obtained and thus can be observed under a microscope.

Morphological observation

Observations of morphological characteristics were carried out by randomly taking plant samples of 5 plants. The observed variables were based on plant morphology stated by Tjitrosoepomo (2003). These variables include the nature of roots, stems, fruits, and seeds.

Root morphology

The number of roots; (i) The observation was on the number of roots growing (very little, little, medium, a lot, or very much). (ii) Root form; The observation is on the shape of plant roots, whether they are spear-shaped (fusiform), top-shaped (napiform), or thread-shaped (filiform). (iii) Presence of root hairs; The observation was on the number of root hairs that grew (none, a little, moderate, a lot, or a lot). (iv) Root color; observations are on root color (white, yellowish white, yellow, or other colors). (v) Root system; The observation is on the root system, whether the taproot system or the fibrous root system.

Stem morphology

(i) Types of stems, observations were made by observing whether the stems were wet (herbaceus), woody stems (lignosus), grass stems (calmus), or *mendong* stems (calamus). (ii) The shape of the stem is observed by observing the shape of the stem in its cross-section, whether it is round (teres), square (angular), or flat. (iii) The surface of the stem, observations were made by observing the surface, whether it was smooth (laevis), ribbed (costatus), grooved (sulcatus), winged (alatus), hairy (pilosus), thorny (spinosus), showing leaf marks, showing traces of leaves. (iv) Leaf marks show many lenticels or conditions, such as crusting. In the branching of stems, observations were made by looking at the presence or absence of branching on the stem and then determining whether the branching method was monopodial, sympodial, or forking. (v) The number of branches was observed by counting the number of branches from the main trunk. (vi) Stem color (green, dark green, yellowish green, or other colors). (vii) The shape of the stem circumference observations was made by observing the shape of the stem circumference, whether it was curved inward (convex) or curved outward (concave).

Fruit morphology

(i) Fruit weight observations were made by weighing the observed fruit. (ii) Fruit shape is observed by observing its shape and determining whether it is round, elliptical, oval, or star. (iii) Observing fruit skin color is made by carefully observing the fruit skin color. (iv) The color of the fruit's flesh is done by carefully observing the color of the fruit's flesh. (v) The aroma of taste and observations are made by tasting the fruit's flesh, whether it tastes sour, sweet, or very sweet. The presence of thorns or tassels. (vi) The observations are made by observing whether the fruit's skin has thorns or tassels.

Seeds

(i) The number of seeds per fruit; observations were made by counting the number of seeds per fruit. (ii) Weight of 100 seeds, observations were made by weighing every 100 seeds. (iii) Seed shape, observations are made by observing the shape of the seed and determining whether it is round, elliptical, or oval. (iv) The color of the seeds is done by carefully observing the color of the seeds.

Nursery

Dragon fruit seeds are obtained from vine cuttings. Cuttings are grown in pots using a planting medium like Malang sand. After the roots appear, these cuttings are cut and used as material for making preparations.

Preparation making

Preparation of preparations using the squash method (squeezing) and sealing it semi-permanently, which is adapted from the method used by Anggarwulan et al. (1999) and Parjanto et al. (2003). Pre-treatment started with root cutting. First, the roots are washed with clean water. Then, the meristematic part of the root was cut about 5 mm

from the root tip and soaked in distilled water for 24 hours in the refrigerator at a temperature of 50º-80ºC.

Root pieces were fixed using a 45% acetic acid solution and stored in the refrigerator for 2 hours. Next, the root pieces were taken and washed with distilled water three times. Root pieces that have been fixed are then hydrolyzed with 1 N HCl solution for 3-4 minutes and stored in an oven at 60ºC. Then, 1 N HCl is removed and washed with distilled water three times (Anggarwulan et al. 1999).

Chromosomal staining was carried out by soaking the root pieces in 2% aceto-orcein solution (Anggarwulan et al. 1999; Parjanto et al. 2003) for 24 hours in the refrigerator. After this process, the root cap at the root tip is removed. Next, the meristematic part (approximately 0.5 mm from the root tip) was taken and placed on a glass slide. Next, the root pieces were covered with a cover glass placed on top of the root pieces and squeezed with the thumb or using the tip of a pencil slowly (Anggarwulan et al. 1999; Damayanti and Mariska 2003). Then the pressed preparations were sealed using clear nail polish (Anggarwulan et al. 1999) and observed using a light microscope at 1,000 times magnification.

Visible cells do not accumulate and exhibit prometaphase or metaphase are selected. At this stage, the chromosomes appear to be spread out well, making it easier to observe. Selected cells were photographed with a Nikon photomicroscope and micrographed. This method is a modification of the method used by Parjanto et al. (2003).

Chromosomal observation

Chromosomal morphology observations include:

Number of chromosomes

Observation of the number of chromosomes is done by taking pictures of them after they are visible on a light microscope and printing them with an enlarged print so that the number of chromosomes can be counted (Anggarwulan et al. 1999).

Chromosome size

On the chromosome image, the length of the two arms (Anggarwulan et al. 1999) and the length of the chromosome (the sum of the length of the long arm and the length of the short arm) were measured (Parjanto et al. 2003).

Chromosome shape

The location of the centromere determines the shape of the chromosome. The location of the centromere is determined by the ratio of the long arm to the short arm. Determination of the shape of this chromosome refers to the method of Ciupercescu et al. (1990) cit. Parjanto et al. (2003).

Karyotype

Chromosomes in prometaphase or metaphase showing the good distribution of chromosomes are photographed with a photomicroscope. The chromosomal images were then observed for morphology and arranged sequentially from the longest to the shortest size as a karyotype. The arrangement of the karyotype is made by pairing homologous chromosomes, which are determined based on the similarity of the size and shape of the chromosomes (Parjanto et al., 2003).

Data analysis method

Morphology

Morphological data were analyzed and presented descriptively based on the results of morphological observations in the field.

Cytology

Cytological data were analyzed and presented descriptively based on observations from the photographed chromosome images and observations of the length and shape of the chromosomes.

RESULTS AND DISCUSSION

Location

The land in "Kusumo Wanadri Agrotourism" Glagah Indah Beach, Kulon Progo District, Yogyakarta, Indonesia is a sandy land with an altitude of 5 m asl. The environmental conditions for growing dragon fruit plants in the study area have an average daily intensity of sunlight between 2,860-3,950 lux. The average daily temperature is between 27-3°C. The average daily humidity is between 88-93%. Rainfall is between 1,580-2,300 mm/yr. The growing environmental conditions are by the requirements for growing dragon fruit plants as stated by Kristanto (2003), that the ideal temperature for dragon fruit plants is between 26-36°C, and the humidity needed by plants is between 70-90%. However, the growth of *S. megalanthus* plants will be optimal if planted in cold areas with an altitude of more than 800 m asl.

Description of root

S. megalanthus roots are similar to the roots of dragon fruit plants of the genus *Hylocereus*. These similarities include filiform roots, a moderate number of yellowish-white root hairs, and a fibrous root system. The root morphology of *S. megalanthus* also differs from the roots of dragon fruit plants from the *Hylocereus* genus in terms of the number of roots. The number of roots of *S. megalanthus* is not as much as the number of roots of dragon fruit plants of the genus *Hylocereus.*

The difference in the number of roots between *Selenicereus* and *Hylocereus* may affect plant growth. *Selenicereus* growth tends to be slower compared to *Hylocereus* growth. It is possible because of the influence of the number of minerals that plants can absorb that, it also affects the production of food substances that are distributed to all parts of the plant.

S. megalanthus roots are also the same as the roots of other types of dragon fruit, which are epiphytic, where the roots propagate and attach to other plants. In its cultivation, this plant requires a propagation medium. The required propagation medium can be concrete or bamboo. The roots

of dragon fruit plants are very drought tolerant and can not tolerate puddles.

Stem description

The stem is a very important part of the plant body. The stem can be equated with the body's axis and the plant's support. The stem of *S. megalanthus* is a type of herbaceous that contains a lot of water with a laevis surface because it is coated with wax. The shape of the stem is an angular rod, to be precise, a triangle. The stem of the *S. megalanthus* plant has a fork branching system in which 2 branches will appear on each stem. The stem of the *S. megalanthus* plant is green with a concave ridge shape. From the stem will come out short thorns from which flowers and fruit will grow.

The description of the stem of the *S. megalanthus* plant has similarities with the description of the dragon fruit plant of the genus *Hylocereus*, namely in terms of stem type, stem shape, stem surface, branching, number of branches on the stem, and the presence of thorns coming out of the stem. However, in addition to these similarities, there are differences in the stem's color and the rim's shape. The stem of the dragon fruit plant of the *Hylocereus* genus is dark green and has a convex edge shape.

The waxy coating that covers the stems of dragon fruit plants reduces plant evaporation. The stems have a high water content, so without the protection of the wax coating, the plant will lose a lot of water and become dry. Dragon fruit plants do not have leaves, so the stems do not grow leaves. Instead, the leaves have modified their shape into thorns that grow along the stems (vines). Similar to the wax coating, the spines function in reducing plant evaporation.

Fruit description

Dragon fruit plant fruit results from pollination, which causes the ovary to grow into fruit. Immature fruit skin is still light green. The color of the fruit gradually changes. Each type of dragon fruit has a different fruit color. For example, in *S. megalanthus* plants, the color will change to yellow. It differs from *H. undatus* whose skin color changes to pink, *H. polyrhizus*, whose skin color turns red, and *H. costarinensis*, whose fruit skin color turns dark red. The color of the fruit flesh of each type of dragon fruit plant is also different. *S. megalanthus* and *H. undatus* have the same flesh color, i.e., white. However, it differs from *H. polyrhizus*, which has pink flesh, and *H. costarinensis*, which has red flesh.

The shape of the fruit of each type of dragon fruit plant is not the same. For example, the fruit of *S. megalanthus* is oval. It is different from *H. undatus* and *H. polyrhizus*, which have elliptical fruit shapes, while the shape of *H. costarinensis* fruit is round.

Apart from being different in terms of fruit shape, skin color, and fruit flesh color, there is still one difference. There is no tuft on the fruit of *S. megalanthus* as in the three types of *Hylocereus*. The tufts on the fruit of *S. megalanthus* are replaced by the growth of short spines. The fruit of the dragon fruit plant appears along the stem, precisely near the thorns that grow on the stem. The fruit appears singly in groups of two or three or even more.

The tufts on the *Hylocereus* dragon fruit are the same as the spines on the *S. megalanthus* fruit. Both are modifications of flower petals. The flower crown will wither and fall when the ovary begins to form. However, it is not the case with flower petals. The petals do not wither and fall in fruit growth but still grow and develop. It's just that in this growth, the flower petals have modified their shape. In the Hylcereus dragon fruit, the petals will be modified into tassels. At the same time, the fruit of *S. megalanthus* was modified into thorns. Both grow around the fruit.

In fruit morphology, fruit weight and aroma taste could not be observed. The observed fruit is still small and growing on the tree. In addition, the sample for observation of fruit is only one fruit. Therefore, it does not meet the sample requirements.

Description of seeds

Seeds are the primary means of reproduction for seed plants. Seed observation can only be done on *Hylocereus* dragon fruit. Meanwhile, observation of seeds on *S. megalanthus* could not be done because there were no samples. Dragon fruit seeds are small, so many seeds are in one fruit.

Based on observations, the average number of seeds of *H. undatus* was 4,399, *H. polyrhizus* was 4,790, and *H. costaricensis* was 5,232. Meanwhile, the average weight of 100 seeds of *H. undatus* was 0.2005 g, *H. polyrhizus* was 0.1783 g, and *H. costaricensis* was 0.1973 g. The shape of the seeds of the three *Hylocereus* species is the same: oval. Similarly, the color of the seeds. All three types of *Hylocereus* have black seeds.

Number of chromosomes

The number of chromosomes is a characteristic of chromosomes that is easy to observe and stable. The results showed that the *S. megalanthus* had a chromosome number of 2n= 4X = 44. The chromosome number of *S. megalanthus* in Figure 1 shows that the number of chromosomes is quite large. Therefore, there are still chromosomes attached.

The number of chromosomes *S. megalanthus* is tetraploid, where 1 set of chromosomes consists of 4 homologous chromosomes. This number differs from the number of chromosomes of the dragon fruit genus *Hylocereus*, which is 2n= 2X = 22 (diploid). The difference in the number of chromosomes will be expressed in several plant morphological characters.

In general, tetraploid plants produce larger fruit than diploid plants. However, this is not the case with *S. megalanthus.* The size of the fruit of this plant is even smaller than the size of the fruit of the *Hylocereus* type. Furthermore, *S. megalanthus* fruit is not surrounded by tufts as in the *Hylocereus* type fruit but is surrounded by thorns. In addition, the appearance of the stem of *S. megalanthus* is also different from the dragon fruit of the genus *Hylocereus*. The stem of *S. megalanthus* is relatively thinner and has a concave rim, while the stem of the dragon fruit genus *Hylocereus* is thicker with a convex rim.

Another difference is that *S. megalanthus* has slower growth than the dragon fruit of the *Hylocereus* genus.

Chromosome size

One of the criteria for identifying chromosome morphology is chromosome size. Observations of chromosome size include the total length of the chromosome (q+p), the length of the long arm of the chromosome (q), and the length of the short arm of the chromosome (p).

The length of the *S. megalanthus* chromosome ranged from 2 + 0.098 µm to 4.75 + 0.98 µm. The length of the short arm of the chromosome (p) ranged from 0.76 + 0.053 µm to 2.09 + 0.053 µm, while the length of the long arm of the chromosome (q) ranged from 1.05 + 0.06 µm to 2.85 + 0.06 µm.

Based on the average length of chromosomes, *S. megalanthus* has a fairly large chromosome size. In addition, it has many chromosomes compared to the dragon fruit of the *Hylocereus* genus. The length of the DNA sequence will determine the size of the chromosome, and it varies from species to species. Thus, the longer the chromosome size, the longer the DNA sequence. According to Damayanti et al. (2005), the amount of DNA content in the cell nucleus positively correlates with the length and total volume of chromosomes.

Chromosomal shape

Determination of the shape of the chromosome can be based on the location of the centromere. The location of the centromere is one of the important morphological characteristics of chromosomes in identifying chromosomes. The classification of the shape of the chromosomes is based on the ratio of the chromosome arms (r = q / p).

The shape of the *S. megalanthus* chromosome is metacentric and submetacentric. The chromosome form of *S. megalanthus* consists of 40 metacentric chromosomes and 4 submetacentric chromosomes. The *S. megalanthus* chromosomes which have a metacentric shape are on chromosomes number 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, while the *S. megalanthus* chromosome which has the shape submetacentric, namely on chromosome numbers 21, 22, 23, and 24. In general, plants have metacentric chromosomes.

Karyotype

A karyotype is an arrangement of chromosomes in order from longest to shortest. Karyotyping is done by pairing one chromosome with a homologous chromosome. The result of this pairing of chromosomes is called a karyogram. The determination of homologous chromosomes is based on the similarity of the shape and size of the chromosomes. Karyogram is useful for knowing chromosomal aberrations in the number and structure of chromosomes that occur during cell division. In addition, it can be searched for its relationship with abnormalities found in a living creature's anatomy, morphology, and physiology.

Based on the number of chromosomes of *S. megalanthus* in the research results, 2n = 4X = 44, and the chromosomes of *S. megalanthus* are tetraploid so that each set of chromosomes consists of 4 homologous chromosomes. The karyogram of *S. megalanthus* can be seen in Figure 2.

The karyotype formula can be made from the karyotype arrangement of *S. megalanthus*. The formula for the karyotype of *S. megalanthus* is 2n = 4X = 40 m + 4 sm with m = metacentric chromosome and sm = submetacentric chromosome. The arrangement of chromosomes in the form of an idiogram can be seen in Figure 3.

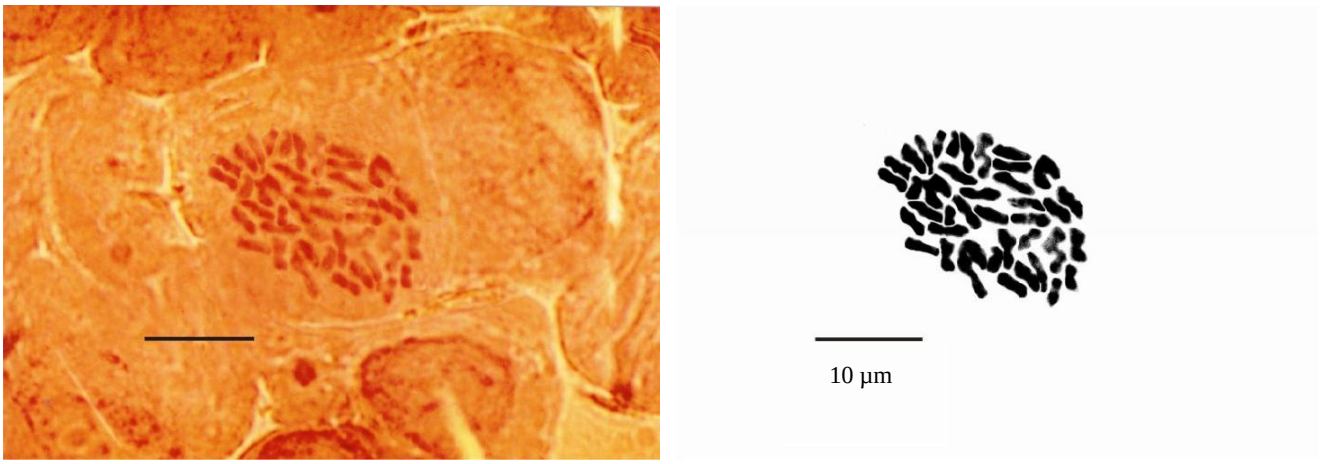

Figure 1. Chromosomes of yellow skin dragon fruit (*S. megalanthus*) are tetraploid 2n = 4X = 44. Bar 10 um

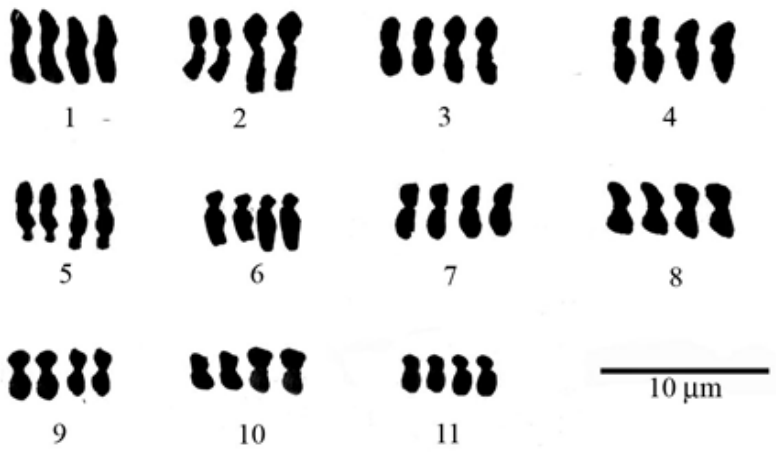

Figure 2. Karyogram of yellow skin dragon fruit (*S. megalanthus*)

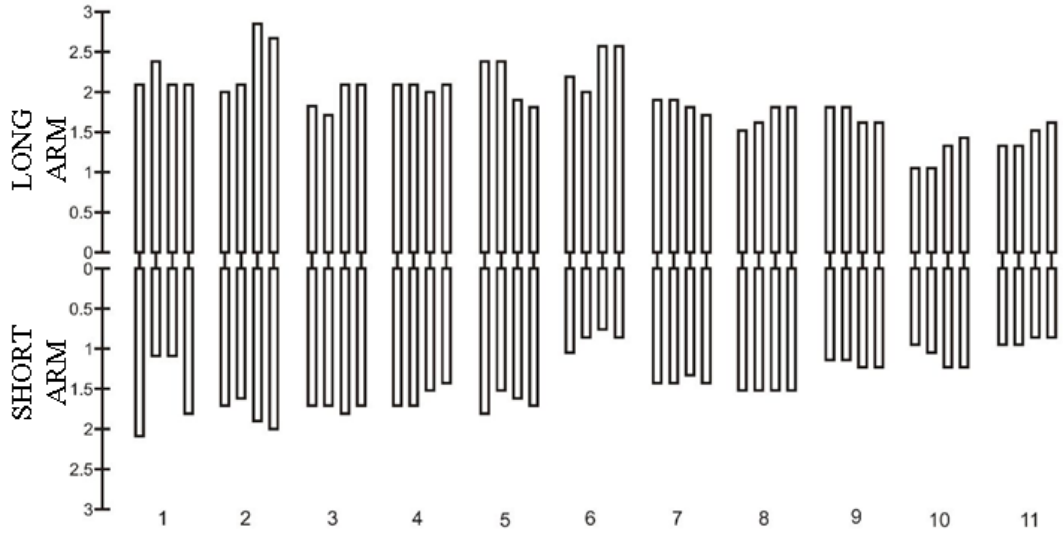

Figure 3. Idiogram of yellow skin dragon fruit (*S. megalanthus*)

Chromosomes paired with homologs often have similar shapes and sizes, making it difficult to determine homologous pairs. Therefore, it is necessary to identify chromosomes using a chromosome banding technique to overcome this. Thus, the identification of individual homologous chromosomes can be carried out so that the determination of homologous chromosome pairs can be carried out accurately (Parjanto et al., 2003).

Based on the research that has been done, it can be seen: (i) The roots of *S. megalanthus* are filiform in shape, have a moderate amount of root hairs, the root color is yellowish white, has a fibrous root system. The number of roots is not as much as the number of roots of dragon fruit plants genus *Hylocereus*. (ii) The stem of *S. megalanthus* is a type of herbaceus with a laevis surface because it is coated with wax. The trunk shape is an angular fork branching system where each stem will appear in 2 branches. The stem of the *S. megalanthus* plant is green with a concave ridge shape. (iii) The color of the fruit skin of *S. megalanthus* is yellow, and the color of the flesh is white. The *S. megalanthus* has oval fruit. On the fruit of *S. megalanthus*, there are no tufts but short spines. (iv) *S. megalanthus* has a tetraploid chromosome number of 2n = 4X = 44 with a chromosome length ranging from 2 ± 0.098 m to 4.75 ± 0.098 m. The *S. megalanthus* has 40 metacentric chromosomes and 4 submetacentric chromosomes with the karyotype formula 2n = 4X = 44 = 40 m + 4 sm.

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