Starch and Karyotype Study of Taro (*Colocasia esculenta* L.) from West Sumatra, Indonesia

Ismed Wahidi and Nursyahra
Biology Education Department, STKIP PGRI Sumatera Barat, Padang, West Sumatra, Indonesia
Corresponing author: nursyahra13@gmail.com

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Abstract- The shape, amount, and distribution of starch granules are specific to each plant. Different plants will have different shapes, quantities, and distribution of starch granules. Based on the shape, amount and distribution of starch, the types of starch producing plants can be classified. Taro (*Colocasia esculenta* L .) is a starch-producing plant that has the potential to be developed in the future. *Colocasia esculenta* L is distributed in a wide area in West Sumatra, Indonesia so that genetic variations and variations in anatomical structure are interesting to know. The method in this study is the observation of the shape and distribution of starch, as well as the observation of chromosomal karyotypes. This study aims to determine the shape, amount, and distribution of starch granules and karyotypes from *Colocasia esculenta* L. *Colocasia esculenta* L has the form of round starch granules, rectangles, semicircles, polygonal and triangles. The amount of starch is 6-35 percent and the pattern of spreading irregular starch with cortical cells is not entirely filled with starch. The basic chromosome number of *Colocasia esculenta* L is n = 14.

Keywords- *Colocasia esculenta* L, identification, shape, starch, tuber, karyotype

I. INTRODUCTION

Starch is a carbohydrate in the form of granules in plant organs, such as cereal seeds (corn, wheat, sorghum, and rice), tubers (potatoes and cassava) and cassava roots (Samsuri, 2008). The form of specific starch granules in each plant, there are oval ones with long and elongated. Different plants will have different forms of starch granules. The number and distribution of starch granules found in plant tissues are also different.

A group of starch source tubers, such as *Colocasia esculenta* L, have not been widely used in West Sumatra, Indonesia. Taro (*Colocasia esculenta* L) is a local food ingredient that has the potential to be developed in the future. Taro (*Colocasia esculenta* L) is a tropical tuber crop largely produced for its underground corms contain 70–80 % starch and the corms of *Colocasia antiquorum* contain anthocyanins such as cyanidin-3-glucoside, pelargonidin-3-glucoside and cyanidin-3-chemnoside which were reported to have antioxidative and anti-inflammatory properties (Kaushal *et al*., 2015). *Colocasia esculenta* L is distributed in a wide area so that genetic variations and variations in anatomical structure are interesting to know. This study aims to determine the form of starch granules and karyotypes from *Colocasia esculenta* L.

II. METHODS

Observation Form and Distribution of Starch

Colocasia esculenta L was obtained from several regions in West Sumatra, Indonesia. *Colocasia esculenta* L was sliced thin and observed under a microscope then photographed with an Olympus microscope camera.

Chromosomal Observation

a. The acclimatization

The root was *Colocasia esculenta* Lsoaked in colchicine solution 0.01%; 0.02%; and 0.03% for 6 hours and then transferred to a single pot for acclimatization.

b. Make of preparations

1) Pre-treatment

The meristematic root of *Colocasia esculenta* L is cut along ± 5 mm from the root tip and washed with clean water. The tip of the root of *Colocasia esculenta* L is inserted into the bottle containing aquadest for 2-3 hours at 5-8 ° C. This activity is carried out to separate and decompose chromosomal density, cytoplasmic purification and soften the tissue.

2) Fixation

Fixation is done by soaking the material into carnoy 2 solution (6 ethanol: 3 chloroform: 1 pure 96% glacial acetic acid) and stored in a refrigerator at 5°C for ± 3 hours. The finished material was fixed, then washed with 70%, 50%, and 30% alcohol for 5 minutes, then washed with distilled water 3 times.

3) Hydrolysis

Hydrolysis is done by soaking the material into 1 N HCL solution and stored in an oven with a temperature of 60^{o} C for ± 5 minutes. When finished, the ingredients are washed with aquadest 3 times. Hydrolysis is done to get cells that spread in the observation of chromosomes. Hydrolysis can use acid or hydrolase enzymes, one of which is hydrochloric acid (HCl). Hydrochloric acid has the ability to dissolve the middle lamella very high. A concentration of 1 N is the optimum concentration. At lower concentrations, the power of the work decreases, so it must be soaked longer. At higher concentrations it can decipher the nucleus and chromosomes in it, so that the shape of the nucleus extends and the chromosome cannot be observed perfectly.

5) Staining

Staining is done by soaking the material into 2% aceto-orcein solution and storing it in a refrigerator at 5o C for ± 24 hours. Aseto-orcein is commonly used in chromosomal staining. Aceto-orcein colors the core so that the chromosomes can be seen clearly.

6) Squashing

Squashing done by taking a cross-section along the root tip meristematic ± 0.5 mm from the root tip and placed on glass preparations. Furthermore, spilled with 45% acetic acid solution and covered with a glass lid and then push it*(squash)*with your thumb or using a pencil knock gently tap, then preparations squashing results sealed using clear nail polish. According to Setyawan and Sutikno (2000), the purpose of *squashing* is to make the cells separate from each other, but not lose their original shape and spread evenly over glass objects. The quality of *squash* determines the quality of the preparation. *Squash* that is good produces preparations that only consist of a layer of cells, separated, not overlapping, and not fragmented. After the process *squash*, the edge of the cover glass is sealed with clear nail polish. This sealing aims to make preparations last longer and prevent dryness of preparations.

c. Observation Variable Chromosome

Observation of the number of chromosomes is done directly and indirectly. Direct observation was carried out by calculating the number of chromosomes seen during observation with a magnification of 40X, 100X, 1000X. Indirect observations are carried out by calculating the number of chromosomes contained in the results of the shooting (printed image).

III. RESULTS AND DISCUSSION

A. The shape and distribution of starch

The result of the incision of *Colocasia esculenta* L seen to contain starch which is spread in the cortex. The form of starch *Colocasia esculenta* L is round, rectangle (rectangle), half circle, polygonal and triangle. Research that has been carried out on the form of starch *Colocasia esculenta* L has different results from the results obtained by Susiana, et al., (2013) in the form of round and oval starch granules in five types of taro, namely white talas (*Xanthosoma sagittifolium*), black talas (*Xanthosoma violaceum*), beetle talas (*Colocasia esculenta* cv. 1), taram talas (*Colocasia esculenta* cv. 2) and yellow talas (*Colocasia esculenta* cv. 3). According to Elida (1994) the size and morphology of starch granules depending on the type of plant and its shape can be in the form of circles, ellipses, oval, polyhedral or polygonal, and irregular shapes irregular

Patterns of starch dispersion with cortical cells are not entirely filled with starch. The amount of starch in one cell ranged from 6-35 starch granules which in one cell contained single starch and compound starch. Starch in *Colocasia esculenta* L is starch sentries, the hilum is located in the middle of the granule, with the hilum surrounded by lamellae (Figure 1). Mulyani (2006) and Hidayat (1995) say that plants have 2 types of starch, namely starch centric and eccentric starch. Essau (1962) states that there are two starch grains in plants, namely single starch, and compound starch.

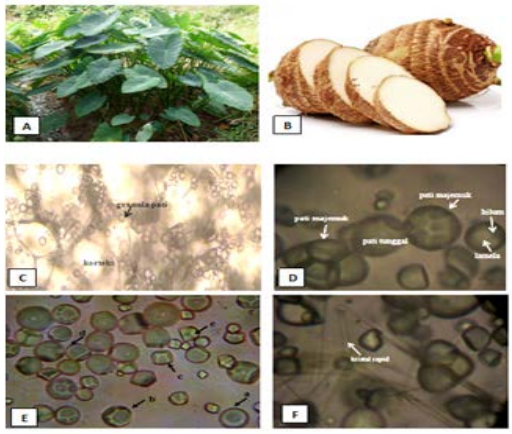

Figure 1. Starch form of *Colocasia esculenta* L.
a. Round, b. Polygonal, c. Rectangle, d. Half circle, e. Triangles

The type of starch in *Colocasia esculenta* L is a single starch or monoadelf and polyadelf. Monoadelf is a starch which has a hilum surrounded by lamella and compound starch. Polyadelf is a starch which has more than one hilum, each surrounded by a lamella and outside is not surrounded by lamella. Compound starch in *Colocasia esculenta* has 2 forms. According to Mulyani (2006) the starch which has a hilum surrounded by lamella is called single starch *(monoadelf*), starch which has more than one hilum, each surrounded by a lamella and outside surrounded by a lamella joint called half-compound starch (*diadelf*) and starch which has more than one hilum, each surrounded by a lamella, and outside is not surrounded by a joint lamella called compound starch (*polyadelf*). In *Colocasia esculenta* L there are also rapid crystals scattered in the cortex tissue. Mulyani (2006) and Hidayat (1995) say that crystals are one of ergastic substances. Various types of crystals in plants are sand crystals, rapid crystals, drush crystals, prism crystals, and stiloide crystals. Rapid is a long and slender crystal with both pointed ends. Cells containing rapid often are typically distributed in plants.

B. Karyotype

The result of analyzing the number of chromosomes of *Colocasia esculenta* L is n = 14. The number of chromosomes is used in taxonomy because of the number chromosomes are one of the most fixed markers because all individuals in one type generally have the same number of chromosomes even though there are some exceptions (Figure 2). The number of chromosomes is the data that is most often used in taxonomic research because the observations are easy to do. Cytology data can be used at various levels in the taxonomic hierarchy, especially at the level of type because it has a close relationship with reproductive factors (Stuessy, 1990 cit. Sujadmiko and Sutikno, 1990).

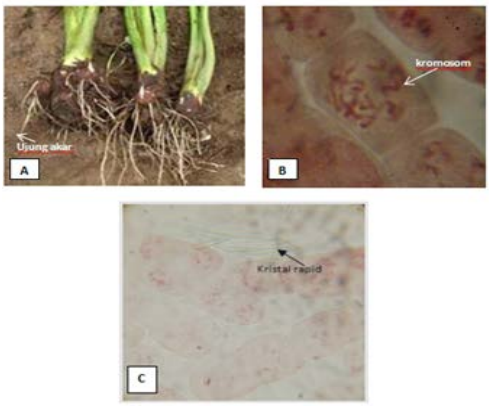

Figure 2. Chromosome of *Colocasia esculenta* L

Chromosome of *Colocasia esculenta* L diploids (2n=2x=28) and triploids (2n=3x=42) chromosomes in taro while diversity study using simple cytological techniques (Mace and Godwin, 2002). However, Dastidar (2009) reported existence of taro chromosome number 2n=14, 28, and 42 and 2n=36 and 48 in India and suggested as the genetic instability might be due to cultivation for long period of time in the region of center of diversity. Furthermore, Quero-Garcia et al., 2006 stated as taro is highly polymorphic, allogamous and protogynous species. This is in accordance with the results of research that has been conducted. Suryo (2003) states that the number of chromosomes of all individuals of a species is fixed from generation to generation. This consistency strengthens that chromosomes as one of the important taxonomic characters.

IV. CONCLUSION

Based on the research that has been done it can be concluded that *Colocasia esculenta* L has the form of round starch granules, rectangles, semicircles, polygonal and triangles. The amount of starch is 6-35/cell and the pattern of spreading irregular starch with cortical cells is not entirely filled with starch. The number of basic chromosomes of *Colocasia esculenta* L is n = 14

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AUTHORS

First Author – Ismed Wahidi, Biology Education Department, STKIP PGRI Sumatera Barat, Padang, West Sumatra, Indonesia.
Second Author – Nursyahra, Biology Education Department, STKIP PGRI Sumatera Barat, Padang, West Sumatra, Indonesia.

Correspondence Author – Nursyahra, email address: nursyahra13@gmail.com, contact number: +6285271652859