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Stages of Microspore Development in Eggplant (*Solanum melongena L.*)

Devi Bunga Pagalla [1*]

[1] Program Studi Biologi, Jurusan Biologi, FMIPA, Universitas Negeri Gorontalo, Jl. Jendral Sudirman No. 6 Kota Gorontalo - Indonesia
[*] Correspondence: devibungapagalla@ung.ac.id

Abstract

Background: Microspores are small haploid spores that develop into the male gametophyte. Microsporocytes undergo meiotic division to form microspores. Microspores can be found in seedless and seed plants. The microspores in each flowering plant are different. This study aims to observe microspores on eggplant flowers. **Method:** Microspore observations were carried out on different flower bud sizes until the flower buds bloomed. **Result:** The results showed that microspores in Eggplant had various stages of development for each flower bud size. The stages of microspore development observed were Early uninucleate (Young microspore), Mid-uninucleate, Late uninucleate (Vacuolate microspore), early binucleate (Young bicellular pollen), mid-binucleate, and mature pollen. **Conclusion:** In eggplant microspore culture, anther length is a solid parameter to predict the stage of microspore development contained therein.

Keywords: Androgenesis; Anther; Developmental stage; Flower Bud; Microspore

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Introduction

In Flowering plants have anthers as a place for the development of pollen. Microspores are haploid cells with a single nucleus, which develop into pollen. Microspores are male gametes produced in the anther. According to Bhojwani & Bhatnagar (1999), microspores are immature pollen. Currently, eggplant cultivation can be done through anther and microspore culture to produce double haploid (DH) plants. Through anther culture, haploid pollen cells can be induced to become haploid plants. Microspore culture is an appropriate platform for rapidly producing large haploid plants in multiple plant species (Francios et al., 2014). Anther and microspore cultures use flower buds as a source of explants. Olmedilla (2010) said flower buds and microspore development stages strongly correlate with embryo formation through microspore embryogenesis induction. Parra-Vega et al., 2013 observed the microspore of Capsicum annuum L. measuring anther length, bud length, anther purple pigmentation, and the ratio between petal length and bud length as predictors of microspore development stages. In tomato anther culture (Adhikari & Kang, 2017) revealed that anther length is a decisive parameter for determining the stage of microspore development. Pagalla et al. (2020) showed that anther length is an essential parameter in determining the stage of development of microspores that can be induced to become embryogenic microspores.

The developmental stage of vacuolated microspores is a developmental stage that allows microspore reprogramming to occur in most species. Microspores have six stages: early uninucleate (a tetrad form), early uninucleate, mid uninucleate, late uninucleate,

binucleate, and trinucleate (Kott et al., 1998). Flower buds are usually harvested when the microspores are in the late uninucleate to early binucleate stages (Ferrie & Caswell, 2011). Eggplant flower buds containing about 70% late uninucleate and early binucleate microspores were the right phases for embryogenesis induction. Microspores at the young microspore (YM) and mid-microspore (MM) stages did not show a growth response after being transferred to the embryogenesis medium (Medium B). The optimal stage for inducing embryogenesis is not the transitional stage from vacuolate microspore (VM) to the young bicellular pollen/YBP stage (Salas et al., 2012). Selection of the right stage of microspore development for embryogenesis induction is one of the determining factors for the success of microspore culture. This study examines the microspore development stages observed in various sizes of eggplant flower buds.

Methods

The Flower bud of Eggplant is produced by cv. Planet Hijau is a source of explants. Fresh flower buds were picked and collected, then flower bud length, diameter, petala length, and anther length were measured using a digital caliper (NANKAI). The anthers from each flower bud were collected in a 35x15 mm petri dish, added with 1 ml of distilled water, and crushed using a stir bar until all the anthers were broken and the microspores came out. Anther morphology of each flower bud size was observed using a stereo microscope (Nikon, Japan) with 0.8 times magnification. Microspore observation was done using a Boeco binocular microscope (BM-180 SP, Germany) with ten times hyperbole. The data obtained is qualitative (stages of development at each flower bud size), and OptiLab 2.2 Miconos took images. The Quantitative data are percentages for each stage of microspore development observed on collected flower buds and were analyzed using Microsoft Excel 2019. Data were obtained from three replicates and presented as mean (± standard deviation) in the table.

Result

Microspore culture is an alternative in vitro plant breeding. Healthy flower buds are the best source of explants to start microspore culture. Microspores is a male gametophyte cell or immature pollen. In normal development (gametophytic, in vivo), microspores will be differentiated as mature pollen and produce two sperm cells, which can be converted by in vitro culture technique into embryogenic microspores (sporophytic). The developmental stage of microspores and stress became a prominent role in inducing embryogenic microspores. Embryogenic microspores may allow us to regenerate haploid plants (Touraev et al., 1997).

The most apparent difference in flower bud morphology (Figure 1) of the three eggplant cultivars is the shape of the flower buds—Eggplant Flower Bud produced by cv. Fabian F1 tends to be oval, while the Eggplant Flower Bud is produced by cv. Green Planet and Eggplant Flower Buds produced by cv. Egg-shaped wren. The emergence of flower buds on one plant is not simultaneous, so flower buds of various sizes will be found (Figure 2).

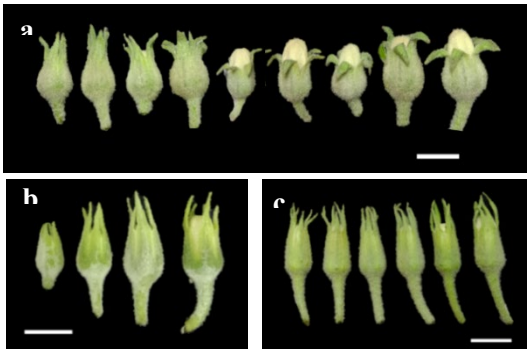

Figure 1. The Differences of Morphology in eggplant flower bud. a) cv. Gelatik; b) Planet Hijau; c) cv. Fabian F1. Bar scale 10 mm.

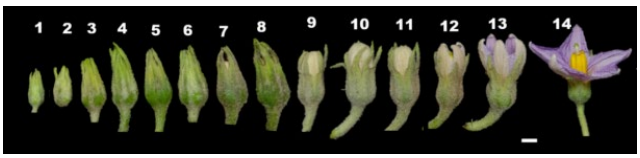

Figure 2. Developmental of Eggplant flower bud cv. Planet Hijau. Flower buds 1-8: the petals are not visible; 9-14 petals are visible until the flowers bloom, Bar scale 10 mm.

Eggplant flower bud cv. Planet Hijau that has been collected is then measured. The measurement results of flower bud length show that different flower bud lengths have different anther lengths (Figure 3). One flower bud of Eggplant generally has five anthers, but during the observation, it was also found flower buds with four, six, seven, eight, and even ten anthers. This depends on the type and variety of Eggplant and the size of the flower bud diameter.

Figure 3. Morphology of flower buds and anthers of eggplant cv. Planet Hijau, (A) The different sizes of Flower buds; (B) The petals of flower buds; (C) Anther inside the flower bud; (D) The morphology of Eggplant anther without filament. (A, B) Bar scale 5 mm; C: Bar scale 500 µm; D: Bar scale 100 µm.

Table 1. The Percentage of Microspore Development Stages in Flower Buds

Length of flower bud (mm)	Petal length (mm)	Anther length (mm)	Tetrad/ Meiocyte	Uninucleate			Binucleate	Mature Pollen
				Early	Mid	Late		
8	4	3.00	100 ± 0					
10	5	3.99		81.25 ± 2.35	18.75 ± 0.42			
14	6	4.30		47.57 ± 2.42	28.16 ± 1.85	24.27 ± 1.08		
18	7	6.00		35.16 ± 0.53	14.84 ± 0.32	50 ± 1.07		
15	7.5	6.00		23.76 ± 1.35	21.78 ± 1,03	54.46 ± 2.07		
16	7	5.97					50.49±3.51	49.51 ± 4.22
17	8.5	6.50		23.71 ± 1.16	21.65 ± 1,29	54.64 ± 1.57		
18	10	7.20		2.89 ±0.42	10.36 ± 1.42	55.42 ± 3.92	25.79±3.56	5.54 ± 1.42
19	15	8.00						100 ± 0
20	13.5	8.50						100 ± 0
20	17	8.00						100 ± 0
21	Bloom	9.00						100 ± 0
24	Bloom	9.00						100 ± 0

Discussion

Measurement of the morphology of flower buds (Figure 3A) was picked randomly (Table 1) and was carried out before observing the microspores found on the anthers. The measurement results show that the anther as the primary source of explants to start microspore culture is an important parameter to determine the stage of microspore development in the anther (Figure 4).

Flower buds with an anther length of 3.00 mm contain 100% microspores in the tetrad stage. The early uninucleate stage is thought to be found in anthers >3.00-6.00 mm long. Based on the data in Table 1, 81.25% of the early uninucleate microspores were found at

anther length of 3.99 mm, and mid-uninucleate microspores were found with a percentage of 18.75%. Late uninucleate microspores were found highest at anther length of 7.20 mm. Still, at anther length of 5.97 mm, no late uninucleate microspores were found, which were binucleate microspores and mature pollen. This is thought to influence the age of the flower bud donor plants. In this study, the effect of donor plant age on eggplant microspore quality had not been observed.

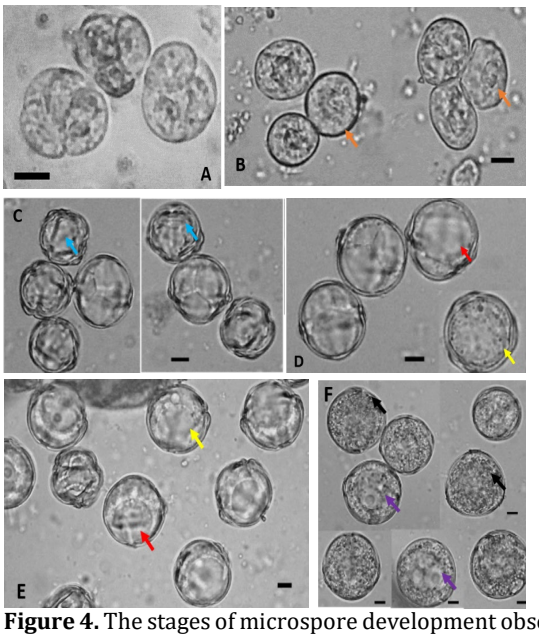

Figure 4. The stages of microspore development observed in flower buds of eggplant cv. Planet Hijau: A. Tetrad stage; B. Early uninucleate (Orange arrow); C. Mid-uninucleate (Blue arrow); D. Late uninucleate (Red arrow); E. Early binucleate (Yellow arrow); and F.), Mid-binucleate (Purple arrow), and Mature pollen (Black arrow).

The mature pollen is 100% found in buds that have bloomed and anther length ≥ 8.00 mm. In this study, there was a correlation between the length of flower buds and the stage of microspore development. Still, the size of flower buds was considered inappropriate as a parameter to determine the stage of microspore development. Anther length shows a stronger correlation in determining the set of microspore development. The same thing happened in Immonen & Antila (1998) for wheat microspore culture, anther length was considered an appropriate morphological marker to determine the specific development of microspores. According to Adhikari & Kang (2017), anther length and microspore development stages in Campari tomato plants indicate that these parameters are more suitable for predicting microspore development stages than flower bud length.

Embryos in large quantities can be obtained from responsive stages, namely anthers containing microspores at the late uninucleate stage and before mitosis (Dunwell, 1996). Each plant has specific criteria in determining the stage of microspore development which can be induced to form embryos such as rice plants (Suaib & Arma, 2012), the panicle morphology of the three varieties used contained microspores >50% mid- to late uninucleate stages when the flag leaves were as tall as 5-10 cm above the tongue (auricle) of the last leaf. Adhikari & Kang (2017) used flower buds 3.55 mm-18.38 mm to observe the proper stages of microspore development for embryogenesis induction.

Conclusions

A microspore or anther culture's success depends on using suitable microspores to induce embryogenesis. In this case, flower buds containing the right microspores will be cultured. In eggplant microspore culture, anther length is a solid parameter to predict the microspore development stage.

Declaration statement

The authors reported no potential conflict of interest.

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