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STERILE WILD BANANA (*MUSA ACUMINATA* VAR. *MICROCARPA* BECC.) OCCURRENCE IN INDONESIA

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SUMMARY

Indonesia and the Southeast Asian region have become the center of diversity for cultivated bananas and their wild relatives. Bananas with the highest diversity, both wild and cultivated, serve as a gene pool for banana breeding. The following study examined a sterile wild banana (*Musa acuminata* var. *microcarpa* Becc.) Nasution, accession LIPI-464, originating from Kalimantan, Indonesia. The said accession produces female flowers with no seeds. Observing the chromosome during metaphase I of meiosis of the pollen mother cells revealed abnormal chromosome configurations, such as univalent and trivalent, which suggested chromosomal translocation. The chromosomal translocation also reduces fertility. Despite showing high pollen viability using a staining test, pollination experiments showed seedless fruits, which confirmed the sterility of the accession. In this study, pollens with incomplete dissolved callose walls were evident, along with genetic abnormality in the degradation of callose walls during pollen development. This wild banana accession serves as a valuable resource for understanding the mechanism of sterility in bananas and the impact of chromosomal translocation on seed production, requiring considerations in future breeding programs on bananas.

Keywords: *Musa acuminata* var. *microcarpa* Becc., wild *Musa* diversity, sterility, meiosis, chromosome segregation, chromosomal abnormalities, seedless fruits

Key findings: This study is a new report on the wild sterile banana (*Musa acuminata*) with all its characteristics and will shed light on the evolution of the banana from fertile to sterile seedless banana.

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INTRODUCTION

In bananas, the sterility is mainly due to triploidy and the accumulation of chromosomal translocation, leading to unbalanced chromosome distribution during meiosis (Dodds, 1943; Shepherd, 1999). Cultivated bananas are also generally triploid, i.e., 'Pisang Ambon,' 'Pisang Kepok,' and 'Pisang Raja' (Simmonds, 1962; Ahmad *et al*., 2023). The translocation among chromosomes causes sterility even in diploid forms, for example, in the 'Pisang Mas' group (Poerba *et al.*, 2018). In cultivated bananas, the sterility trait is highly crucial because it provides the fruit with a seedless nature. However, the low fertility hampers the banana breeding program, resulting in a low seed set after pollination (Silva *et al*., 2001).

In contrast, the diploid banana wild relatives, such as *Musa acuminata* and *Musa balbisiana,* are fertile and seeded (Simmonds and Shepherd, 1955). Similarly, the translocation transpires in wild banana relatives but occurs at a low frequency, and fertility of these bananas remains high (Shepherd, 1999; Ahmad *et al*., 2020; Ahmad, 2021). Hence, it is necessary to understand translocation patterns in banana chromosomes to develop seedless banana breeding strategies. Cytogenetic studies to determine the chromosomal translocations in bananas, which have been conducted by observing the meiosis of pollen mother cells before the formation of pollen, revealed seven types of translocations (Shepherd, 1999).

In wild bananas and cultivars from the banana collection at Katholieke University of Leuven, the genomic studies revealed observations of nine types of translocations (Martin *et al*., 2020). In wild banana relatives, namely, *M. acuminata* and *M. balbisiana,* less is known about the types of translocations based on genome studies. Until now, detecting five types of translocations with seven chromosomes was successful based on studies carried out in seven subspecies of the wild banana (*M. acuminata*) (Šimoníková *et al*., 2020).

Indonesia and the Southeast Asian region are the areas called the center of diversity for cultivated bananas and their wild relatives (Simmonds, 1962). The highest diversity of bananas, both wild and locally cultivated, serves as a gene pool for the banana breeding program (Simmonds and Shepherd, 1955). The evolution of cultivated bananas has the belief of originating from the crosses between wild bananas, involving meiotic abnormalities, resulting in diploid gametes, pollen sterility, and complications arising from the appearance of parthenocarpy traits in these crosses (Simmonds and Shepherd, 1955; Shepherd, 1999; Heslop-Harrison and Schwarzacher, 2007).

In the presented study, conducted observations and characterization succeeded on an Indonesian sterile accession of the wild banana (*M. acuminata* var. *microcarpa* Becc). Studying the chromosomes' structure and behavior during meiosis and pollen characteristics and conducting the crossing used this accession to confirm its sterility. This study will shed light on the evolution of bananas from fertile to sterile seedless bananas.

MATERIALS AND METHODS

Morphological characterization

A sterile accession, LIPI-464, of the wild banana (*M. acuminata* var. *microcarpa*) originated from a seed of the wild *M. acuminata* var. *microcarpa* accession PNK-16, collected from Desa Danau Sadar, Kecamatan Dusun Selatan, Kabupaten Barito Selatan, South Kalimantan province. Its planting continued at the Banana Experimental Plot, Soekarno Science and Technology Park, BRIN, Cibinong, Indonesia. The accession's observation for various morphological parameters ran from July 2022 to March 2023. The observed main morphological characters' description followed the Descriptors for Banana (*Musa* spp.) (IPGRI, 1996). For its sterility confirmation, a pollination test and its reciprocal took place using a fertile wild banana (*M. acuminata* var. *malaccensis*) as the male parent and the *M. acuminata* var. *microcarpa* as the female parent.

Ploidy evaluation

The ploidy evaluation engaged a flow cytometer (Partec CyFlow Space) with a standard CyStain PI absolute P (Sysmec) kit, following the protocol provided in the kit. The diploid *M. acuminata* var. *malaccensis* served as the diploid standard. The samples for flow cytometry comprised young banana leaves (cigar leaves). The collected leaves measured 0.5 cm × 0.5 cm before placing them in a Petri dish with 1.5 ml of UV-Ploidy Cystain buffer (Partec, Germany) and then chopping them with a razor blade. The chopped leaves entailed filtering with a 30-mesh filter, with the filtrate inserted into a cuvette tube for analysis. The reading of leaf samples had a wavelength of 440 nm and a flow rate of 1000 nuclei per second, with control samples of diploid plants calibrated on channel 200.

Sample fixation

For pollen and meiotic studies, the male flowers and young anthers collected from male buds in the morning (9:00 a.m.) were the samples used (Damaiyani and Hapsari, 2017). The male flowers and young anthers' fixative applied a solution of absolute ethanol to glacial acetic acid (3:1) for one hour at room temperature. The volume of the fixative used was approximately 20 times the sample volume. Subsequently, washing the materials three times with 70% ethanol ensued before storing them in 70% ethanol until observation.

Pollen measurement and viability test

From the fixed anthers of male flowers, squeezing the pollen out onto a slide used a needle. Adding two drops of lacto-phenol orcein (Sass, 1964) followed, leaving the slide for a minimum of 15 minutes. Subsequently, the pollens sustained scrutiny under a light microscope (Olympus CX22 LED) before being photographed. Using ImageJ, the study measured at least 250 pollens for diameter (Schneider *et al.*, 2012), and upon staining, partially colored, the number of stained pollens succeeded in recording. Viable pollens were indicative of the red color of the pollen.

Meiotic observations

The fixed young anthers with lengths between 7 and 10 mm, as selected, underwent water washing three times for one minute each time. Then, placing anthers in a 1.5 ml tube consisted of 200 µL of pectolytic enzyme solution containing 0.2% pectolyase Y23 (Sigma P-3026), 0.2% cellulase RS (Yakult 203.033, Yakult Pharmaceutical, Tokyo, Japan), and 0.2% cytohelicase (Bio Sepra 24.970–014) in 10 mM sodium citrate buffer (pH 4.5) before incubating for two hours at 37 °C. Subsequently, inactivating the enzyme was at 70 °C for one minute, with a chromosome spreading and staining method performed after (Ahmad, 2021). Microscopic observations proceeded under an Olympus BX-53 fluorescence microscope with DAPI filters and epifluorescence illumination. The brightness and contrast of the images taken from the microscope, when adjusted, employed the GIMP 2.10.32 to obtain the best images for observation without reducing the amount of the dynamic range of the image. Subsequently, this recorded each stage of the meiotic division.

Pollination test

For confirmation of the sterility in the assessed accession, the pollen of the *M. acuminata* var. *microcarpa* served to pollinate a fertile *M. acuminata* var. *malaccensis*. Pollination transpired in the morning between eight and 10 a.m. by rubbing the male flower onto the stigma until the pollen covers the stigma. In this experiment, researchers pollinated five hands of flowers of *M. acuminata* var. *microcarpa* and five hands of flowers of *M. acuminata* var. *malaccensis*. After 2.5 to 3 months of pollination, the harvesting of fruits continued to check for the presence of seeds, which indicates a fertile pollination.

Germination test

Pollens of two wild bananas (*M. acuminata* var. *microcarpa* and var. *malaccensis*) used in this study came from an experimental field. The pollen grains had a culture medium containing

0.01% H_3BO_3, 0.01% KNO_3, 0.03% $Ca(NO_3)_2.4H_2O$, 0.02% $MgSO_4.7H_2O$, and 15% sucrose and solidified with 2% agar (Brewbaker and Kwack, 1963). The culture remained at room temperature in the dark for one hour before counting the germinated pollen grains under a stereo microscope (magnification 4×). All the pollen grains on the dish underwent counting to evaluate the germination percentage in vitro, with the pollen grains considered germinated when the length of the pollen tube was at least as long as the diameter of the pollen grain.

RESULTS

Morphological characterization

The latest study was a follow-up to previous findings about the seedless and sterile accession of a plant that grew from an accession seed in Kalimantan and was planted in a banana experimental plot. Adaptation of the seedling from the seed was relatively low, and this was evident in the only accession that survived until the flowering stage. After flowering, scientists found the accession produced female flowers, but later, the fruits were undeveloped and seedless. In present observations, the habitus, the bud, and the male flower of the seedless accession were like the parent plant that was found in South Kalimantan and were typical of the *M. acuminata* var. *microcarpa* (Nasution, 1991).

Morphological traits of sterile var. *Microcarpa*

Plant: Leaf habit—erect, with no dwarfism; suckering—freely and close to its parent; pseudostem—height 2–3 m, slender to normal, colored reddish-purple on the outer layer and pink-purple in the underlying pseudostem, and shiny; sap color—milky; leaf sheaths—very few waxes; petiole—length is <50 cm, with a large dark brown blotch. The canal was straight with erect margins, and the leaf blade length was about 171–220 cm and the width was less than 70 cm. The upper surface was green, with a green midrib, and the lower surface was pink. The base shape was one side rounded and one side acute. The lower surface has weak waxiness, and the upper side was dull.

Inflorescence: Peduncle length is about 31–60 cm, and width was less than 6 cm; green, with pubescence present; bunch position—horizontal, and the bunch shape is cylindrical; rachis type—truncated and horizontal; male bud type—normal, intermediate in shape and size, about less than 20 cm; and the bract color—red-purple on the upper side and red on the inner side. The bract base shape was medium and pointed at the apex. The male flower falls with the bract.

Flower: The compound tepal base color was white with no visible sign of pigmentation; the lobe color of the compound tepal was orange. The free tepal was translucent white with an oval shape and developed on its apex; the filament was white, and the anther was cream; the style base color was white with no pigmentation. The style shape was straight with an orange stigma; the ovary's basic color was cream with no pigmentation. The dominant color of the male flower was white. The ovule arrangement comprised four rows. Fruit—the fruit numbered more than 17; fruit length is less than 15 cm. Shape—straight and slightly ridged in transverse section. Apex—blunt-tipped. Pedicel—length is between 11 and 20 mm and width is 5—10 mm, with a hairless surface. Pulp color—white, with no seed present. The morphology of the parent and the sterile *M. acuminata* var. *microcarpa* photographs appear in Figure 1.

Ploidy evaluation

The wild banana was generally diploid. Given the sterile accession's morphological characteristic resembling its seed parents, an initial suspicion was that it was diploid as well. However, to confirm the ploidy level, the study performed a flow cytometry analysis by comparing it with a known diploid plant. Based on the flow cytometry results, the histogram for the sterile accession appeared in the same

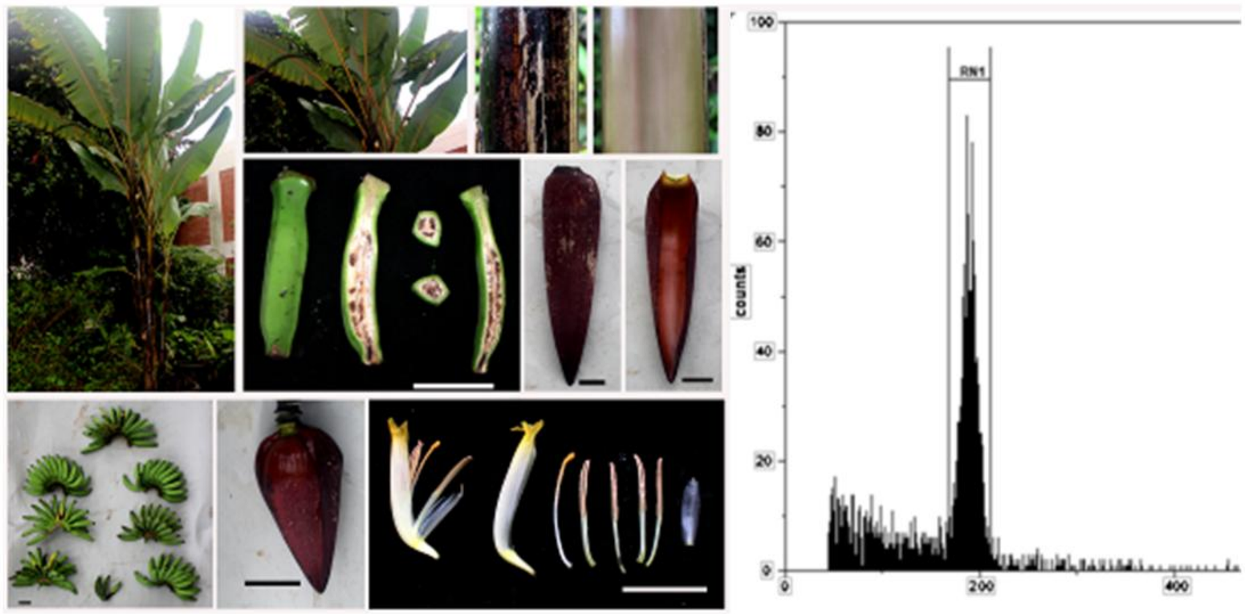

Figure 1. Habitus and parts of the *M. acuminata* var. *microcarpa* (Left) and ploidy confirmation showed that sterile *M. acuminata* var*. microcarpa* was diploid (Right). Bar = 3 cm.

channel, 200, as the diploid standard banana *M. acuminata* var. *malaccensis.* The result confirmed that the ploidy level of the studied accession was diploid (Figure 1).

Cytology characterization

Observing chromosomes during metaphase enabled scientists to examine the sterile *M. acuminata* var. *microcarpa* for abnormal chromosome repairing during pollen mother cell meiosis. However, only a few cells had visible chromosomes due to strong backgrounds blurring the images (Figure 2). Nonetheless, three cells showed an indication of abnormal chromosome configuration, with two univalents and two trivalents, while the remaining chromosomes formed seven bivalents (Figure 2).

Following the meiotic observation, the tetrad formation monitoring in pollen mother cells of the sterile *M. acuminata* var. *microcarpa* continued. The tetrad formation was evident in the anther, approximately 9–10 mm long. The classified tetrads totaled 124, classified into five types based on their wall separation configuration (Figure 2). Tetragonal (type I) was the most common at 46%, followed by linear (type II), T-shape (type III), pyramidal (type IV), and five-cell (type V) forms, with proportions of 46%, 29%, 17%, 6%, and 2%, respectively. Type I to Type IV likely represent the formation of four viable cells, while the five-celled Type V tetrad showed three small cells, suggesting abnormalities during tetrad development.

Pollen viability

Viable pollen was obvious with the pollen stained red or dark red. In contrast, non-viable pollen emerged transparent and less stained by the Lacto Phenol Orcein (Figure 4). A total of 1284 pollens of the sterile *M. acuminata* var. *microcarpa* were notable for viability observations. We observed 843 stained pollens (66%), indicating viable pollen with a diameter between 90 and 250 µm, and 441 (34%) unstained pollens indicated as not viable pollens with a range of 40–150 µm in diameter. The average diameter of the viable pollen was larger at 170 µm than the non-viable pollen at 70 µm. (Figure 3). Visualizing the level of viability of the sterile *M. acuminata*

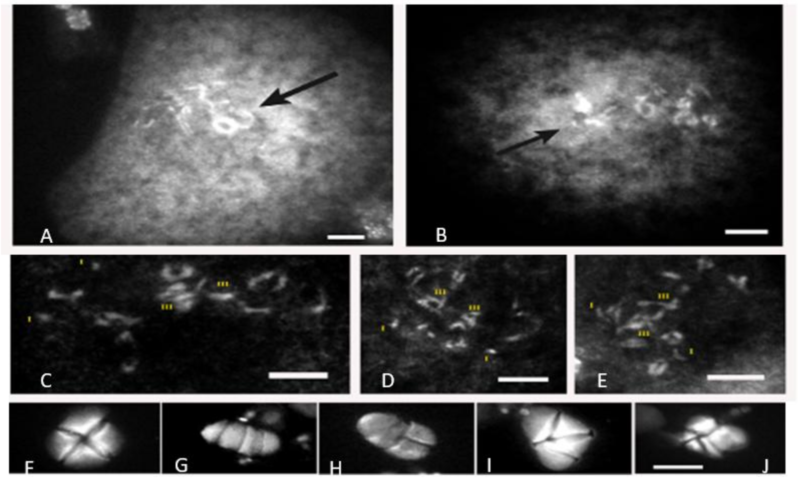

Figure 2. The cytoplasm of pollen mother cells in *M. acuminata* var. *microcarpa* obscured the chromosome during meiosis. Univalent (arrow) was present but hardly visible (A and B). In the first metaphase, a single pollen mother cell of sterile *M. acuminata* var. *microcarpa* showed abnormal chromosome configurations (arrow indicates univalent and trivalent) (C,D,E). Five pollen mother cell tetrad shapes appear in sterile *M. acuminata* var. *microcarpa*: Type I - tetragonal (F), Type 2 - linear (G), Type III - T-shape (H), Type IV - pyramidal (I), and Type V – Five cells (J). Bar = 10 μm.

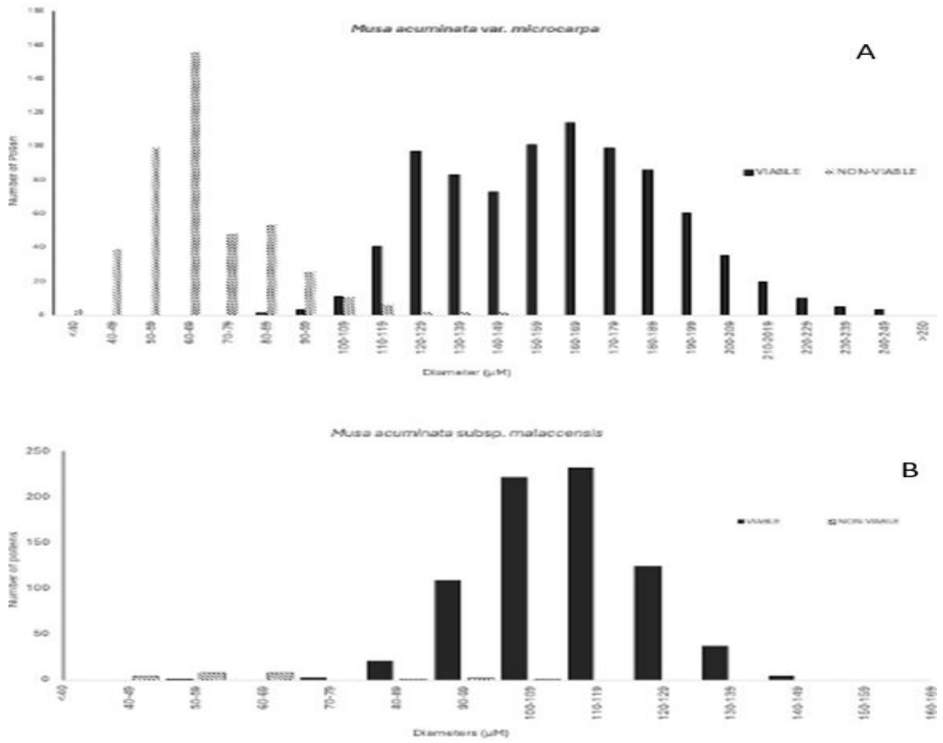

Figure 3. Pollen diameter range of *M. acuminata* var. *microcarpa* shows viable pollen (black bar) is larger than the non-viable pollen (gray bar), on average (A); For *M. acuminata* var. *malaccensis*, non-viable pollen is scarce, and viable pollen (black bar) is larger than the non-viable pollen (gray bar), on average (B).

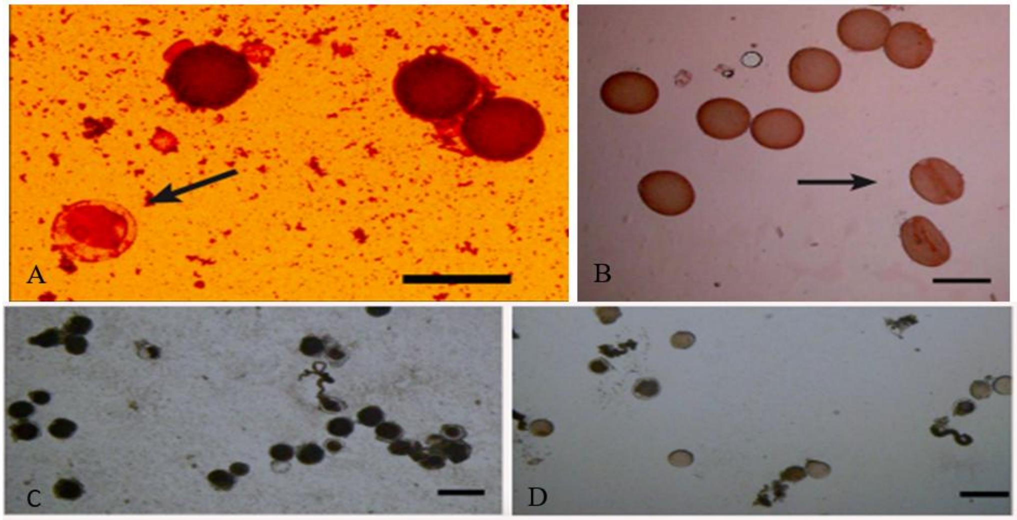

Figure 4. Viable pollen is stained, while non-viable pollen (arrow) remains unstained. Bar = 200 μm (A); Pollen grains with an equatorial line-like structure (arrow) suggest imperfect callose wall dissolution. Bar =100 μm (B); Germinated sterile (*M. acuminata* var. *microcarpa*) (C), and fertile (*M. acuminata* var. *malaccensis*) (D) pollen in Brewbacker and Kwack medium, with arrows indicating germinated pollen with tubes. Bar = 200 μm.

var. *microcarpa*, the pollens of a fertile *M. acuminata* var. *malaccensis* accession was examined. Out of 778 pollens of *M. acuminata* var. *malaccensis*, 754 pollens (96.9%) were viable, and 24 pollens (3.1%) were not viable (Figure 4).

Among the normal, rounded, viable, and non-viable pollens, a few pollens with an equatorial line-like formation were also prominent (Figure 4). These pollen grains received a viable classification. However, their shape was oval with a wall-like structure along the longest diameter. The frequency of this type of pollen was low, with only 20 (1.1%) out of the total observed pollen grains.

Pollen germination

In the progressive study, a germination test of the pollens ensued to estimate the germination rate of the sterile accession compared with the fertile wild banana *M. acuminata* var. *malaccensis*. Consider the pollen grains as germinated if the pollen tube length was at least as long as the pollen diameter (Kakani *et al*., 2005). Pollen germination tube shape was mostly curly, and the results showed the pollen germination percentage of the sterile *M. acuminata* var. *microcarpa* was 16.37%, which was less than half of the *M. acuminata* var. *malaccensis* germination rate (37.98%) (Figure 4).

Pollination test

The conducted experiment also assessed the fertility of var. *microcarpa* by crossing it with the pollen from *M. acuminata* var. *malaccensis* and by self-pollination. The fertility of *M. acuminata* var. *malaccensis* reached confirmation by allowing its female flower to be naturally pollinated and setting the seeds (Figure 5A). In contrast, self-pollination and crossing of the sterile *M. acuminata* var. *microcarpa* produced no seed, confirming its sterility (Figures 5B and 5C). Furthermore, the fruits from the selfing and crossing were undeveloped, indicating the banana was not parthenocarpic. As an additional observation, the morphology of the female flower of the sterile *M. acuminata* var. *microcarpa* incurred comparison with the fertile *M. acuminata* var. *malaccensis*. The female flower of both accessions had similar morphology, with a developed ovary containing rudimentary anthers.

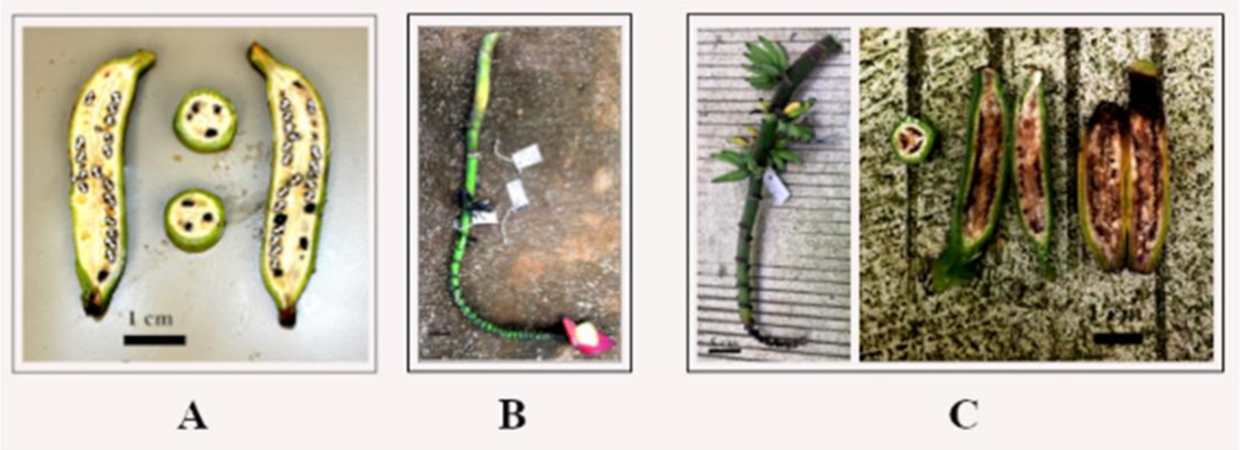

Figure 5. Fertility confirmation: Seed-filled fruit of *M. acuminata* var. *malaccensis* (pollen source) (A); Sterility of *M. acuminata* var. *microcarpa*: No seeds from self-pollination (B); No seeds from crossing (C).

DISCUSSION

In general, all the wild bananas are fertile and produce the seeds. However, this was not the case for the *M. acuminata* var. *microcarpa*, accession LIPI-464, as evidenced by its inability to produce seeds and its pollen's non-functionality in seed production when used to pollinate fertile wild banana accessions. Based on Nasution's (1991) description of var. *microcarpa* found in Kalimantan (Borneo), the latest observations confirm that the sterile *M. acuminata* var. *microcarpa* has a similar morphological habit to other wild *M. acuminata*. These bananas have a slender stature and upright leaves. However, *M. acuminata* var. *microcarpa* differed by producing seedless and fleshless fruits. In this accession, the failure of fruit development indicates no parthenocarpy.

Unlike cultivated banana cultivars like 'Pisang Rejang' and 'Pisang Mas,' they are sterile but possess parthenocarpy traits, allowing fruits to develop without seeds. These results suggested parthenocarpy genes might exist as traits found in some wild bananas (Simmonds, 1953; Ruan *et al*., 2012), although parthenocarpy was common in cultivated bananas but was not typical in wild ones. However, Ahmad *et al.* (2022) recently reported one wild accession of *M. acuminata* var. *malaccensis* that exhibited the parthenocarpy trait, as evidenced by fruit flesh formation from unpollinated flowers.

The encouraging results suggested total sterility in the accessions, where both ovules and pollen were non-functional for seed production. Its confirmation also prevailed by the failure of seed formation in crossing the sterile accession with fertile plants and pollinating fertile plants with pollen from the sterile accession. The sterility observed in these diploid wild banana accessions indicates it was not because of triploidy and polyploidy. In contrast, diploid cultivated banana plants proved typically sterile.

In this accession, the observations on meiosis from pollen mother cells revealed the presence of univalent and trivalent, which signify chromosomal translocation. Formations of univalent and trivalent result in unbalanced chromosome polarization during anaphase, leading to the formation of non-functional gametes (Shepherd, 1999). Sterile bananas exhibit this abnormal chromosome formation, and the higher the number of chromosome translocations, the higher their sterility level (Dodds, 1943; Shepherd, 1999). Dodds (1943) described a sterile diploid cultivated banana variety from Trinidad, accession I.R.11, with a chromosome formation like that in the presented study (seven bivalents, two trivalents, and two univalents). In this study, we were unable to observe the anaphase stage of pollen mother cells (PMC) with observable chromosomes during the anaphase stage. Therefore, further analysis of the estimated chromosome distribution after chromosome polarization at both cell poles was not possible.

Seedless cultivated bananas lose their ability to produce seeds due to accumulating chromosomes with translocations, resulting in most gametes being non-functional (Shepherd, 1999). Translocations in bananas can be homozygous, where one pair of homologous chromosomes has the same translocation, and heterozygous, where one homologous chromosome does not undergo translocation while its homologous chromosome undergoes translocation. Shepherd (1999) reported meiosis from pollen mother cells of various bananas and described at least seven types of translocations. Subsequently, based on genome sequences, Martin *et al*.'s (2017) findings showed translocations on chromosomes 1 and 4 in *M. acuminata* var. *malaccensis* 'DH Pahang.'

Ahmad (2021) stated another accession of *M. acuminata* var. *malaccensis,* LIPI-10, having heterozygous translocations, and the chromosomes with translocation were 1T4 and chromosomes 1 and 4 were without translocation. Interestingly, although this accession has chromosomes with translocations, it still produces seeds and progeny. This suggests that the fertility of this *M. acuminata* var. *malaccensis* was still high, with the possibility of a viable gamete formation above 50% (Martin *et al*., 2017), and based on its pollen viability, it was still high (Ahmad *et al*., 2020). Using chromosome painting, Šimoníková *et al*. (2020) observed various types of translocations involving chromosomes 1, 2, 3, 4, 7, 8, and 9 in nine wild bananas and six cultivated bananas. These results indicated the chromosome rearrangement was common in bananas.

The determination of the level of sterility in the accessions used in the presented study led to observing the pollen viability, pollen germination rate, and tetrad formation and conducting pollination tests using fertile wild bananas. In this study, most of the observed pollen was viable. Viable pollens had an average size twice as large as non-viable pollens. Smaller, shriveled, and non-viable pollens were also evident in studies on wild bananas, *M. acuminata* var. *malaccensis*, var. *breviformis*, var. *sumatrana*, var. *zebrina*, and cultivated bananas 'Rejang' (Ahmad, 2021).

Pollens from *M. acuminata* var. *microcarpa* exhibited a significantly lower percentage of pollen germination than the fertile *M. acuminata* var. *malaccensis* 'LIPI-10,' indicating that low fertility also affects the pollen germination. These results suggested when using the sterile *M. acuminata* var. *microcarpa* pollen for pollination, it also has a low fertilization ability. In *M. acuminata,* the normal size of pollen was around 100 µm (Ortiz, 1997). Based on the study results, viable pollen sizes were mostly in the range of 170 µm. Studying the genomic content of chromosomes using a flow cytometer could help elucidate the connection between the larger pollen size and the sterility.

Based on staining tests using orcein dye, the viability percentage was quite high; the pollen from this sterile accession still cannot produce seeds when pollinated on fertile flowers. Thus, a viability experiment using orcein dye may serve as an indication of fertility and pollen functionality (Soares *et al*., 2008). However, the pollination confirmation in bananas seems crucial to demonstrate the ability to pollinate and produce the seeds. Therefore, the var. *microcarpa* accession cannot be effective for breeding purposes.

In this research, the tetrad formations from the sterile *M. acuminata* var. *microcarpa* bananas emerged. The tetrad had various configurations, though the most common form was tetragonal, which was the common tetrad formation. We suspect that the tetrad configuration in this sterile accession could be effects of the abnormal chromosome's distribution at the end of meiosis due to chromosome translocations. Unfortunately, we were unable to observe the cells in the anaphase stage with visible chromosomes to confirm this hypothesis.

Zhang *et al*. (2008) also reported similar variations in the tetrad configurations in *Larix leptolepis* Gord plants. Their results indicate the cytokinesis timing, simultaneously or successively, influenced the tetrad configuration. Simultaneous cytokinesis results in the direct formation of the callose wall after meiosis-II division and produces the dominant tetrahedral tetrad formation. Meanwhile, successive cytokinesis results in the callose wall forming after meiosis I and produces tetragonal, linear, T-shaped, and rhomboid tetrad formations. However, a tetrad configuration primarily occurs due to a spindle fiber orientation, especially during meiosis II.

Pollen grains originate from the meiosis in pollen mother cells, emerging as a single grain (monad) after passing through the tetrad phase. Generally, normal pollen is spherical, with a wall on the outside. This study reports for the first time the presence of pollen grains with still-existing septa inside the pollen in the sterile *M. acuminata* var*. microcarpa* accession LIPI-464. The observed pollen had a kind of inner wall dividing the pollen into two. The presence of cell walls in this pollen was due to the incomplete shedding of the septum cell walls during tetrad formation, with the same condition also observed in *Arabidopsis thaliana* mutants in *qrt1* and *qrt2* genes responsible for pectin shedding on tetrad septa (Rhee and Somerville, 1998). Until now, no study existed regarding the presence of these septa and the level of sterility in bananas.

Flow cytometry is a rapid and accurate method used to determine the ploidy level. Dolezel *et al*. (1994) developed methods for flow cytometry in banana plants using propidium iodide for DNA staining and intercalation, allowing for the quantification of the DNA content (Crissman and Steinkamp, 1990). In this study, the ploidy level of the sterile *M. acuminata* var. *microcarpa* accession was remarkable to be diploid (2X) using flow cytometry. This is indicative in the histogram for the sterile accession at channel 200, which was equal to the histogram of the diploid control (*M. acuminata* var. *malaccensis*). These results confirmed that diploidy does not necessarily lead to sterility in bananas.

Pollen germination rate can be a favorable indicator of a plant's fertility level. Pollen tube growth occurs when pollen lands on the receptive surface of the female flower (stigma). The pollen tubes will carry generative cell nuclei to fertilize egg cells and cause fertilization. In this sterile accession, pollen germination occurred less frequently compared with the *M. acuminata* var. *malaccensis.* This lower germination rates suggests that the sterility mechanism also impairs the ability of pollen grains to germinate.

CONCLUSIONS

Based on the results, the wild banana (*M. acuminata* var. *microcarpa*) accession, LIPI-464, proved sterile, which cannot be effective as a source of pollen and as a female parent. The morphological traits of this accession were similar to those of wild *M. acuminata* in general, except for the seedless fruit. Chromosome analysis during meiosis metaphase is an important part of this study, with the observation of trivalent and univalent chromosomes, which can be an indication of the sterility in this accession of bananas.

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