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Research Article

Morphological Characterization and Seed Germination Study of Wild Banana *Musa acuminata* var. *flava* (Ridl.) Nasution

Trimanto[1]*, Elga Renjana[1], Dewi Ayu Lestari[1], Elok Rifqi Firdiana[1], Shofiyatul Mas'udah[1], Apriyono Rahadiantoro[1], Linda Wige Ningrum[1], Lia Hapsari[1]*

1) Purwodadi Botanic Garden – Research Center for Plant Conservation and Botanic Gardens, National Research and Innovation Agency (BRIN), Jl. Surabaya – Malang Km 65, Purwodadi, Pasuruan, East Java, 67163, Indonesia

* Corresponding author, email: triman.bios08@gmail.com; hapsari.lia@gmail.com

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ABSTRACT

Wild bananas provide important genetic materials for further banana improvement, therefore they need to be conserved and studied. This study aimed to describe morphological characteristics of plant and seed and also to study the seed germination of wild banana *M. acuminata* var. *flava* (Ridl.) Nasution. The morphological characteristics were observed descriptively by referring to the descriptor for banana. The internal and external morphology of the seeds were observed using a digital microscope. The germination testing was carried out by a completely randomized design, using fresh seeds extracted from a bunch of fruits with two ripeness levels *i.e.* fully-ripe (yellow peel) and under-ripe (green-yellow peel). The data resulted was then analyzed using an independent t-test. The results showed that *M. acuminata* var. *flava* is characterized as a perennial herb; pseudostem height ≥ 3 m; male bud like a top with prominent green-yellow bracts; fruit curved and tasted mild-sweet when ripe. The seed is angular with wrinkled surface, and dark brown-black color when ripe. The longitudinal section showed parts of the seeds comprising the seed coat, outer and inner integument, embryo, endosperm, chalazal mass, micropyle cap and channel. The seeds are classified as orthodox, with hypogeal type and gradual germination pattern. The seeds extracted from fully-ripe fruit germinated faster with higher germination percentage and growth variables (root number and plant height). Thus, it is suggested to use physiologically mature seeds (seeds from fully-ripe fruits) which should be separated from the seeds of under-ripe fruits to lower the heterogeneity.

INTRODUCTION

Banana is an important cash crop worldwide; ranked next to rice, wheat, and maize. The monocotyledon Musaceae family includes three genera *i.e. Ensete*, *Musella* and *Musa* in which most edible bananas belong to the genus *Musa* (Perrier et al. 2009). It consists of approximately 70 wild species and 500 cultivars (Hakkinen & Vare 2008; Valmayor et al. 2002). In particular, in Indonesia, being part of the center of banana diversity; not less than 12 wild species and 325 cultivars have been found (Nasution & Yamada 2001). However, the evolution of wild seeded to seedless edible bananas is a complex pro-

cesses involving multiple stages and separated by time (over centuries and/or millennials and places) (Perrier et al. 2009; Langhe et al. 2009; Hapsari et al. 2018).

Most of the current banana cultivars were putatively the results of hybridization between two major wild banana species of *Musa acuminata* (A genome) and *M. balbisiana* (B genome); and few were of *M. schizocarpa* (S genome) and *M. textilis* (T genome) (Perrier et al. 2009; Langhe et al. 2009). The diversity of banana crop wild relatives (especially the putative progenitors) is important to ensure the future of modern bananas. Conservation and collection of crop wild relatives through seed is a suitable strategy to conserve germplasm in gene bank because they are the reservoir of traits and genes required to face the emerging abiotic and biotic stresses (Singh et al. 2021). Nonetheless, they have been under considerable threats due to habitat destruction, fragmentation and conversion of tropical forests, and other anthropogenic disturbances. Therefore, it is important to prioritize the collection, effective conservation, improving the availability, and providing related studies of wild bananas for use in further banana improvement (Heslop-Harrison 2011; Ford-Lloyd et al. 2011; Hapsari et al. 2020).

Two major wild species *M. acuminata* mostly grow in tropical rainforests in Southeast Asia, while *M. balbisiana* is native to monsoon climates in Southeast Asia and South Asia (Ploetz et al. 2007). In Indonesia, wild bananas can be found in Java, Borneo, Mollucas, Papua, Sulawesi and Sumatra (Sulistyaningsih et al. 2014). As an *ex-situ* conservation institution, Purwodadi Botanic Garden (PBG) which is located in Pasuruan (East Java) has been conserving various species of bananas, mostly from Eastern Indonesia. At least 103 accessions and 197 specimens, consisting of eight wild species and 95 cultivars, have been successfully collected (Hapsari 2014). Furthermore, recent publication reported that PBG has collected wild bananas comprised of three accessions of *Ensete glaucum*, three accessions of *M. balbisiana* and seven accessions of *M. acuminata* variety (Hapsari et al. 2020).

The wild species *M. acuminata* is considered to be the most important genetic material contributor for cultivated bananas (Martin et al. 2020). However, this species is thought to be a species complex; possible continuous variation and phenotypic plasticity due to environmental modifications and adaptations have made this species as a taxonomically difficult group. About fifteen varieties of *M. acuminata* have been stated by Nasution (1991) in the Memoirs of Tokyo University of Agriculture XXXII. The descriptions and key identification of *M. acuminata* varieties have been provided. However, the distinguishing characters among varieties remained confusing due to high variations, especially in the wild populations of Indonesia.

Wild banana *M. acuminata* var. *flava* is one of variety of *M. acuminata*, a status novus (STAT. NOV) or got a new rank given by Nasution (1991). It was firstly described by Ridley as *Musa flava* Ridl. (Trans. Linn. Soc. 2,3: 385-386, 1888-1894 et Ridl., F. Mal. Pen. 4: 294; Anon. Kew. Bull. 92: 249, 1894. – Type: Ridley s.n., Pulau Tijam, on Pahang River, Pahang (SING n.v.). Ac-

cording to Nasution (1991), it was generally characterized by a small to medium clump, tall and slender pseudostems, purplish brown blotching without wax. Leaf blade lanceolate, long petiole, purplish brown blotching, with erect margins. Inflorescence horizontal then pendulous, peduncle thinly pubescent, 10-12 hands per bunch, 12-21 fruits per hand. Male bud ovoid, greenish yellow or yellow in color. Fruits medium, pericarp thin, pulp yellowish. Seeds many, irregularly angular, not smooth, and black when ripe.

Related studies of wild bananas for use in further improvement of bananas are essential due to the recent global threat to cultivated bananas. Diploid wild banana produces fertile and viable seeds which are preferable as genetic material for breeding purposes because they provides more variability and any possible desired traits. Banana seeds may vary in size, shape and color, and also germination rate, depending on the species and varieties (Vineesh et al. 2015). Seed gene banks for bananas are applicable for seed-producing diploid wild *Musa* species, not for cultivar bananas. *Ex-situ* seed conservation of diploid wild banana, especially collected from wild population, brings impact on the seed collection quality (Sipen et al. 2011; Kallow et al. 2020). Furthermore ex-situ seeds conservation of wild bananas is constrained by critical knowledge gaps in their germination ecology, behavior, and also storage which need to be addressed (Kallow et al. 2020; Kallow et al. 2021).

The seed studies of some varieties of wild *M. acuminata* have been reported but still limited. One of wild bananas that has been studied its propagation method is *Musa acuminata* var. *sumatrana* (Roostika et al. 2019). Hence this study aimed to describe the plant and seed morphological characteristics of wild banana *M. acuminata* var. *flava* cultivated at PBG, and also to study the seed germination from two different fruit ripeness levels to evaluate the seed physiological maturity. A complete morphological observation on *M. acuminata* var. *flava* is important for better identification of this variety. The study of banana germination by seeds is still limited because vegetative propagation is more common in banana. Information on efficiency of seed germination is required for plant propagation and breeding programs. In addition, no previous study of seed germination particularly on *M. acuminata* var. *flava* is reported.

MATERIALS AND METHODS

Materials

Plant material used in this study is the wild banana species living collection of Purwodadi Botanic Garden *i.e. Musa acuminata* var. *flava* located at the nursery (previously located at plot XXIV.E.40-a). It was originated from wild populations in Krawak Protected Forest of Tuban, East Java (Lestarini et al. 2012). Morphological observation was conducted directly to the living collection in September 2020. A mature bunch of fruits was harvested from a single plant. The fruits were then divided in two categories *i.e.* fully-ripe ones with yellow peel and under-ripe ones with green-yellow peel (Figure 1). Later, the seeds

from both categories were extracted for further testing (September to November 2020).

A B

Figure 1. Fruit maturity categories: A. fully-ripe (yellow peel), B. under-ripe (green-yellow peel).

Methods

Morphological characterization

Plant morphological characterization was performed by referring to the banana descriptor (IPGRI-INIBAP/CIRAD 1996). All parts of the plant were documented using a digital camera, while the documentation of the seeds was carried out using a digital microscope (Dino-Lite AM3113T). External morphological characterization of seeds was conducted on quantitative characters (length, width, thickness and weight) and qualitative characters (shape, color and texture) while internal morphological characterization was conducted on seed coat, endosperm and other internal seed parts (Kallow et al. 2021).

Seed moisture content and germination testing

1. Seed extraction. Seeds were hand-extracted from the ripe fruits in the laboratory, and the seeds were washed thoroughly using tap water and sieves, until all flesh was removed. Meanwhile the under-ripe fruits were left in the laboratory at room temperature to ripen until they began to yellow and soften and then seeds were extracted following the steps described above (Kallow et al. 2021). After extraction prior to air-drying, the seeds were soaked in water for a while in which the floating seeds were discarded and only the submerged seeds were used for further testing.
2. Measurement of seed moisture content. It was started by weighing the fresh weight of 25 seeds using an analytical scale (Mettler Toledo), then they were dried using a drying oven laboratory (Finco Inc Ovin 30) at 100°C for 18 hours and their dry weight was determined. Measurements were carried out in three replications for each ripeness level. Seed moisture content was calculated using the following formula:

$$\text{Seed moisture content} = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Fresh Weight}} \text{ x } 100\%$$

Furthermore, seed storage behavior was classified as referred to (Kallow et al. 2020). Seeds with moisture content of less than 22% are predicted to have orthodox storage behavior; seeds with higher moisture content (>40%) are predicted to be recalcitrant; while seeds in-between those limits (22% - 40%) are predicted to be intermediate.

3. Sowing the seeds. The seeds were sterilized before sowing by immersing them in a 10% chlorox solution (active substance NaOCl 5.25%) for 10 minutes. The experiment used a completely randomized design (CRD) with two treatments (two seed categories *i.e.* extracted from fully-ripe fruits and under-ripe fruits) and three replications for each treatment. The seeds were sown on plastic seed tray with moist straw paper media. After being sown, the seed trays were then covered with black plastic to keep them moist (regular watering was carried out when the media started to dry). Maintaining the air circulation was conducted by opening the plastic cover during observation in order to prevent the seeds from rotting.
4. Observation of germination. The seed germination variables, the number of seeds germinated, pattern and type of germination, were observed every day. Whereas to observe the growth variables *i.e.* plant height and number of roots; seven seedlings were taken randomly at 60 days after sowing (DAS). The percentage of seed germination was determined using the following formula (Sutopo 2010; Muschick et al. 2010; Darmayanti et al. 2017):

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds sown}} \text{ x } 100\%$$

Data Analysis

The morphological characterization was described qualitatively, while the seed moisture content, seed germination, and growth variables data were analyzed quantitatively. Quantitative data were compiled and analyzed using Microsoft Excel 2016 to figure out the data characteristics and distribution. Later, the data normality was tested using Shapiro-Wilk ensued by the homogeneity test using Lavene. Independent t-test was conducted using SPSS 16.0 to determine the statistical significant difference between the means of the two fruit ripeness levels on the seed moisture content, seed germination and growth variables.

RESULTS AND DISCUSSION

Plant morphological characteristics of *M. acuminata* var. *flava.*

The detailed morphological characterization of both the plant and the seeds of *M. acuminata* var. *flava* from this study `are presented below (Figure 2, 3 & 4). The plant habitus of *M. acuminata* var. *flava* is perennial herbaceous plant, leaf habit intermediate, small to medium clump, number of suckers 2-5, close to parent (vertical growth). ***Pseudostem*** height ≥ 3 m, slender to normal circumference, light green, shiny (not waxy), predominant underlying watery

green with purple pigmentation, milky sap, very little or no visible sign of wax on leaf sheaths. ***Petiole*** black-purple blotches, large blotches at petiole base, length 53-70 cm, width 2.5-3 cm, green, the third petiole canal leaf wide with erect margins (not clasping), margin width ≤ 1 cm and dried. ***Leaf blade*** length 185-205 cm, width 45-50 cm, upper surface green and shiny, lower surface medium green and dull, moderately waxy, the symmetric insertion point of blades on the petiole, very corrugated, green midrib dorsal surface, light green midrib ventral surface, green cigar leaf dorsal surface, leaves of water suckers without blotches. ***Peduncle*** length ≤ 30 cm, width ≤ 6 cm, green, slightly hairy. ***Bunch*** position horizontal, spiral to asymmetric shape, compact appearance. ***Rachis*** type present, horizontal position with some neutral flowers (one to few hands only, the stalk is bare below. ***Male bud*** present, like a top, diameter 6-7 cm, length 8-9 cm; ***bract*** base shape large shoulder, length 15.7 cm, width 7.5 cm, pointed old bracts overlap at the apex of bud, green-yellow external face, green-yellow internal face, color homogenous green-yellow, without discolored lines, lanceolate shape, lifting two or more at a time, revolute behaviour before falling, very few waxes, moderate grooving; bract scars on rachis very prominent. Female flowers not observed. ***Male flowers*** small, 17-18 flowers per hand; ***compound tepal*** cream, rust colored spots, yellow lobe; ***free tepal*** tinted with yellow, oblong, corrugated (several folding under apex), apex thread-like; ***anthers*** exserted, cream to yellow, 2.1 cm; ***filament*** cream, 1 cm; ***pollen*** brown/rusty brown; ***style*** cream, without pigmentation, same level exserted, straight; ***stigma*** pale orange; ***ovary*** cream, very little or no visible sign of pigmentation, male flower cream, two arrangement of ovules. ***Fruit*** biseriate, 10-12 hands per bunch, position curved upward, ≥17 fruits per hand, length 13 - 15 cm, 1.5-2.0 cm in diameter, curved (sharp curve) shape, rounded transverse section, bottle necked apex, without any floral relicts at apex; ***pedicel*** length 11-15 mm, width 5 to 10 mm, hairless surface, deciduous at maturity (fruits fall from hand); ***peel*** light green when immature, yellow when mature, two or less thickness, cracked at maturity; ***pulp*** cream before maturity, soft flesh texture, mild to sweet taste. ***Seeds*** numerous 20-170 seeds per fruit, wrinkled surface, angular (more or less pyramidal), dark brown to black when ripe.

The habitat of *M. acuminata* var *flava* is at open places, along the rivers or roads, 300-600 m above sea level (Nasution 1991), with distribution reported in Central Borneo and the Malay Peninsula. Even though the specimen examined in this study was originated from Krawak Protected Forest of Tuban (East Java) which considered far from its original type species, but the morphological characterization results matched very well to the description of *M. acuminata* var. *flava*, particularly for the green-yellow male bud color. There were some morphological variations observed, however it may possibly due to continuous variation and phenotypic plasticity of environmental modifications. It is locally named by East Javanese as the *pisang jantung kuning* (yellow bud banana), and they utilize its leaves for food wrapping. All plant parts are also used as fodder for wild animals and cattle (Hapsari 2014).

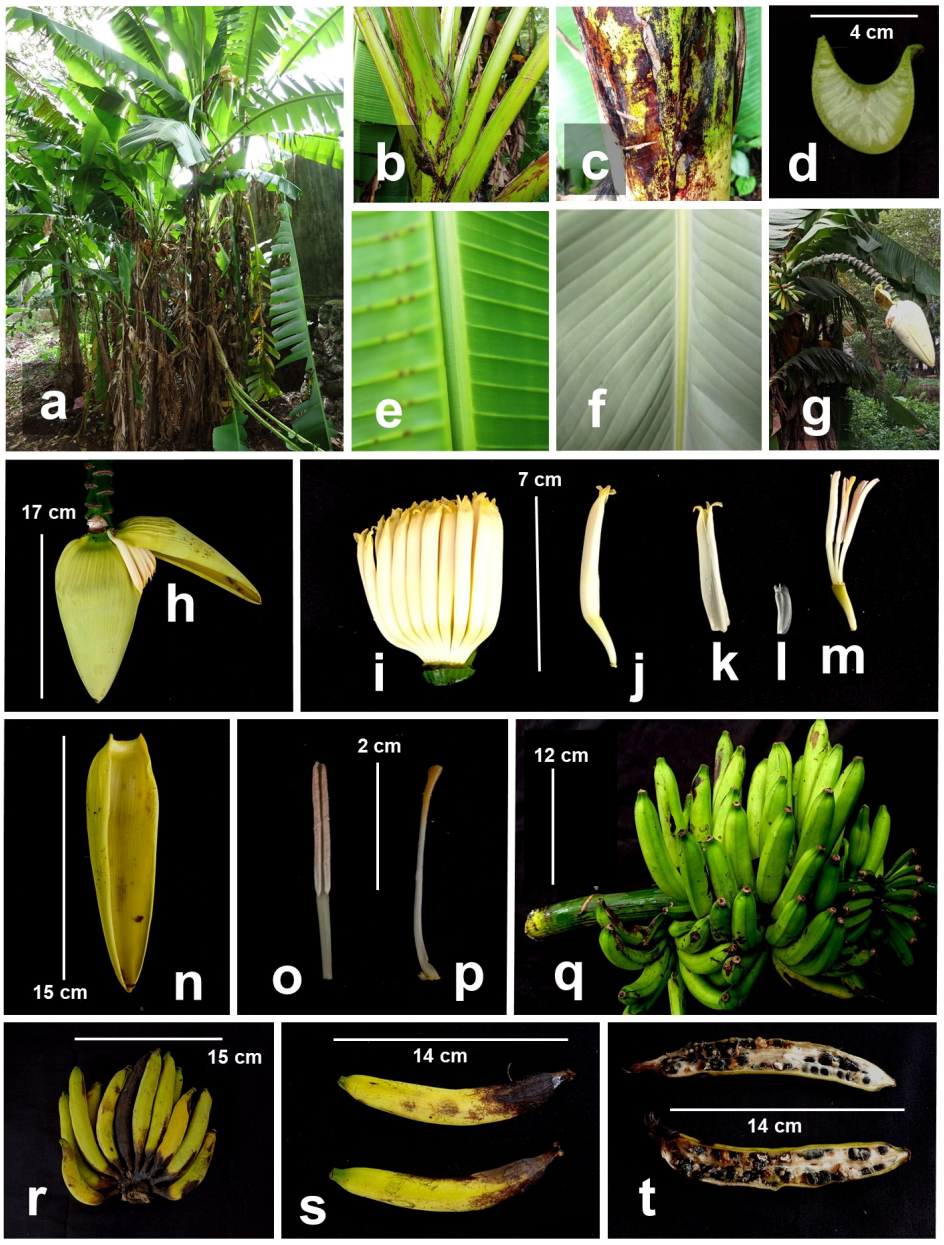

Figure 2. Plant morphological characteristics of *M. acuminata* var. *flava*. a. plant general appearance, b. petiole base arrangement, c. pseudostem blotches, d. cross-section of petiole canal, e. leaf adaxial surface, f. leaf abaxial surface, g. inflorescence, h. male bud, i. male flowers, j. ovary and compound tepal, k. compound tepal, l. free tepal, m. ovary, style and filament, n. male bract, o. filament and anther, p. style and stigma, q. a bunch of fruits, r. a hand of fruits, s. mature fruits, t. longitudinal section of fruit.

Morphological characteristic studies of *M. acuminata* var. *flava* are useful for determining morphological variations of wild bananas in Indonesia, although other studies have shown that various *M. acuminata* have been identified but currently do not have economic value for the community (Hastuti et al. 2019). Neglected wild bananas will be a threat to the existence and reduction of banana gene variation in nature. Conservation is a strategy to saving wild bananas before they become extinct. Wild bananas are very valuable for future breeding programs. One of the important activities in plant breeding is selecting phenotypes that have the desired morphological characters. Pheno-

type diversity can be influenced by environmental factors while morphological characters are expressions of genetic and environmental factors. The diversity of *M. acuminata* from Indonesia confirms that this species are genetically diverse (Poerba et al. 2019).

Musa acuminata var. *flava* is a diploid banana. Based on the results of morphological characterization, the fruits of *M. acuminata* var. *flava* are quite good in quality with 10-12 hands per bunch, curved position upwards, 17 fruits per hand, 13-15 cm in length and 1.5-2.0 cm in diameter. The strategy of crossing diploid banana with good agronomic qualities with a triploid banana that has disease-resistant will produce a diploid hybrid with agronomic advantages, such as resistance to pests and diseases (Pedraza et al. 2005). Conventional sexual hybridization is often applied in most cultivated banana. Banana breeding efforts are focused on increasing selected wild diploid, semi -partenocarpic and parthenocarpic male parents (Sipen et al. 2011).

Seed morphological characteristics of *M. acuminata* var. *flava*

The seeds of *M. acuminata* var. *flava* weight was around 0.038 ± 0.001 g (mean ± standard deviation). The external morphological of the seeds comprised the seed coat (testa) and micropyle. They were angular in shape, dark brown to black in color, with size 2.19 ± 0.15 mm in diameter and 3.88 ± 0.10 mm in thickness (Figure 3a). The seed size is a plastic characteristic that can be altered within populations, individual plants, inflorescences and even in fruits due to environmental conditions in ripening, genetic factors, pollination rate, availability of nutrients, water, light and position of the fruit on the plant (Kaiser et al. 2016). Furthermore the seed had thick and hard coat with thickness of 0.46 ± 0.43 mm. The micropyle is located a the center of the seed, with size of 0.78 ± 0.03 mm (Figure 3b). The thick hard seed coat in wild bananas prevents the oxygen and water that are essential for germination from entering the seeds which subsequently leads to limiting factors of germination. However, it is still considered as water permeable, thus the process of water imbibition may occur (Kallow et al. 2021).

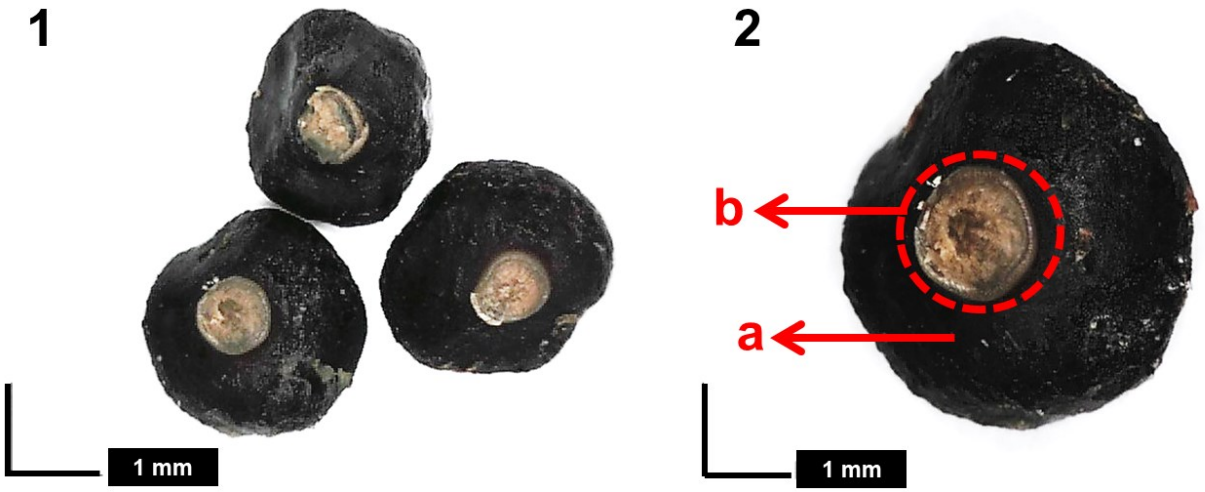

Figure 3. External seed morphology of *M. acuminata* var. *flava*. 1. seeds, 2a. seed coat, 2b. micropyle.

The longitudinal section of the seed showed the seed coat, outer and inner integument, embryo, endosperm, chalazal mass, micropyle cap and channel (Figure 4). Detail observation showed that the seed has two layers of integument (the outer multiple layers and the thin inner layer) which protect the seed during maturation, dispersal and dormancy (Silva et al. 2019). Furthermore, the seed has two chambers within the double-layered of the integument. The first chamber, which was larger, contained the embryo and endosperm. The embryo was small and undifferentiated, measuring 0.31 x 0.32 mm, and the embryonic axis extended to the micropyle collar. The embryo was surrounded by white flour-like (endosperm) served as food reserves for the embryo, with a size approximately of 1.04 x 0.35 mm. At the top of the embryo, there was the micropyle. The micropyle is where the shoot appears when seed germinated. The micropylar part of the seed coat develops into an operculum (a lid-like structure). During germination this lid is later displaced by the elongating radical-hypocotyl axis (Vineesh et al. 2015). In the second chamber, there was a brown chalazal mass measuring 2.65 x 1.57 mm, located at the basal part of the inner seed (Figure 4). The morphology and seed mass of *M. acuminata* var. *flava* observed in this study are in accordance to previous reports on other varieties and subspecies of *M. acuminata* (Puteh et al. 2011; Vineesh et al. 2015; Kallow et al. 2020) and also other wild *Musa* species (Burgos-Hernández et al. 2014; Bohra et al. 2020).

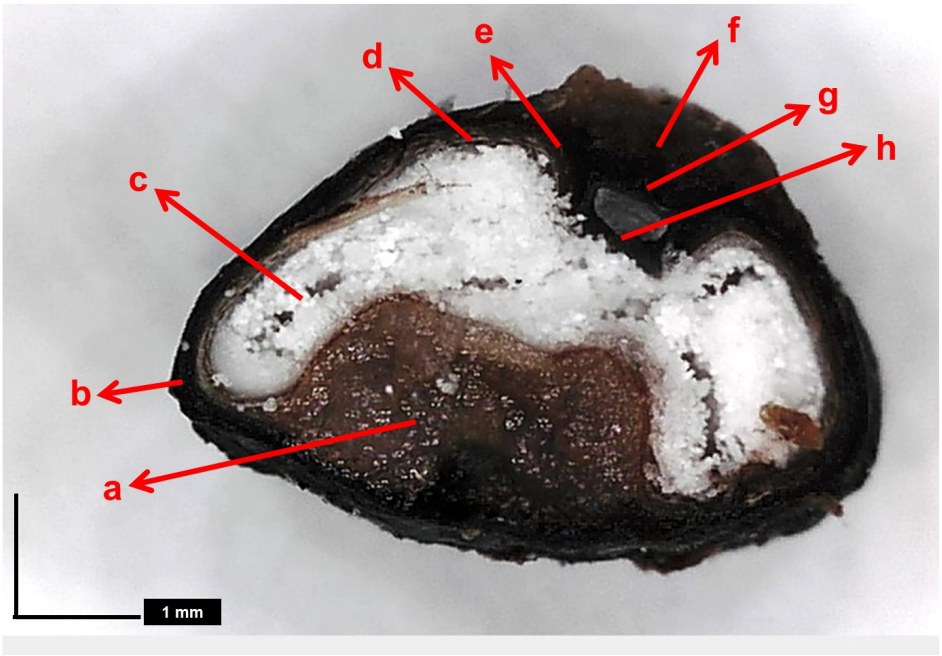

Figure 4. Longitudinal section of *M. acuminata* var. *flava* seed. a. chalazal mass, b. seed coat, c. endosperm, d. inner integument, e. outer integument, f. micropyle cap, g. micropyle channel, h. embryo.

Viability and quality of seeds can be determined by observing their morphological characters. Full mature of seeds are characterized by a darker color of the coat which indicated good seed development. The character of a healthy embryo is characterized by a compact mass in the seed (Figure 4H).

The morphological character of the embryo can be used as a measure of seed viability. Immature seeds show increased air space in the endosperm on drying since there are greater loss of structure during drying in embryos from less mature seeds (Kallow et al 2020). Full mature of seeds are estimated to have good seed viability (Figure 4). Some of their characteristics include compact embryo, white endosperm, darker seed coat and intact chalazal mass. Good quality seeds are characterized by successful germination approximately two weeks after being sown. Seeds with full maturity are characterized by a more powdery endosperm and are harvested from larger fruits with a softer flesh texture.

Seed germination of *M. acuminata* var. *flava*

Fresh and dry weight of seeds from fully-ripe fruit was significantly lower than those from under-ripe fruit. However, the seed moisture content of both categories were not significantly different, but seeds from fully-ripe fruit were slightly higher (Table 1). The seeds moisture content of *M. acuminata* var. *flava* from this study were less than 22%, thus predicted to have orthodox storage behaviour. Wild banana seeds are generally known to be orthodox or long-term storage under very low moisture and sub-zero temperature. However, several studies have shown that some banana species have intermediate seeds, such as *Musa indandamanensis*, where the seed viability decreases over time, especially after three months of storage (Bohra et al. 2020). Meanwhile Kallow et al. (2020) reported that *M. balbisiana* is considered as orthodox and indicated that *M. acuminata* subsp. storage behavior was between orthodox and intermediate. To confirm the storage behavior of *M. acuminata* var. *flava* seeds, further studies are required.

Furthermore the seed germination study showed that seeds from fully-ripe fruit germinated faster with a higher germination percentage than seeds from under-ripe fruits (Table 1; Figure 5 & 6). Banana seeds are generally known to germinate after 20-21 DAS, either with special treatment or not (Burgos-Hernández et al. 2014). From this study, seeds from fully-ripe fruits initially germinated on the 15th DAS, while seeds from under-ripe fruit germinated three days later on the 18th DAS. More than 50% of seeds from fully-ripe fruits germinated (61.33% ± 2.32%), meanwhile the germination percentage of the seeds from under-ripe fruits were lower (37.33% ± 1.01%). Germination began with the appearance of white shoots through the micropyle, followed by the growth of radicles which then developed into fibrous roots. The roots and shoots showed their optimum growth on the 36th DAS. Seedlings from fully-ripe fruits also had significantly higher root numbers and plant height than seedlings from under-ripe fruits (Table 1). The first leaf of seedlings from the fully-ripe fruit appeared on the 43rd DAS, with the number of roots of 8.76 ± 0.36 on the 50th DAS.

Heterogeneity of seed maturity between and within bunches is considered as important factors for germination potential (Kallow et al. 2021). The level of fruit ripeness is related to physiological quality of seeds (Kaiser et al.

Table 1. Comparison of seed germination result of *M. acuminata* var. *flava* from fully-ripe fruits and under-ripe fruits at 50th DAS.

Observed characters		Seeds from fully-ripe fruits	Seeds from under-ripe fruits
Seed variable	Fresh weight of seeds (25 seeds) (g)	0.94 ± 0.02[a]	1.11 ± 0.38[b]
	Dry weight of seeds (25 seeds) (g)	0.82 ± 0.44[a]	0.97 ± 0.32[b]
	Moisture content of seeds (%)	14.61±1.99[a]	13.69±0.06[a]
Germination & growth variable	Germination percentage (%)	61.33 ± 2.32 [a]	37.33 ± 1.01 [a]
	Number of roots	8.76 ± 0.36[a]	6.62 ± 0.29[b]
	Plant height (cm)	6.65 ± 1.07[a]	3.74 ± 0.72[b]

Note: The same letter in the same line shows no significant difference with independent t-test.

2016; Villa et al. 2019). The results of this study showed that with the advance of the ripening process, seeds extracted from fully-ripe fruits (yellow peel) generated seedlings with higher percentage and faster germination, also more vigorous in growth variables. The seeds from yellow fruits are considered to be fully developed (physiologically mature) compared to the seeds from green-yellow fruits (under-ripe). When the seeds were not completely mature, they could germinate, but did not result in seedlings as vigorous as those harvested at the appropriate ripening time. It was also observed from the lower amount of reserves deposited (endosperms) which may cause a limiting factor for the development of seedlings (Moiwend et al. 2015; Villa et al. 2019). The level of fruit maturity was also reported to affect *in vitro* seed germination percentage of *M. ornata* (Dayarani et al. 2014).

The seed germination type of *M. acuminata* var. *flava* was observed as hypogeal. In this type of germination, the cotyledons remain below the germination media due to rapid elongation of epicotyl (part of the stem above the cotyledon), while the hypocotyl (part of the stem below the cotyledon) remains the same in length. Then, the epicotyl pushes the plumule above the germination media, and followed by the formation of leaves. Most of monocots species considered to have hypogeal germination type (Tillich 2007), including wild banana in this study. In addition, the seeds germinated gradually over time if not simultaneously. It was started to germinate on the 15th DAS and continued until the 27th DAS in almost all replications (Figure 5 & 6).

In the wild, banana seeds buried in the soil might survive for years and germinate due to disturbances, especially after logging in the forest (Chin 1996). The germination may be stimulated by micro-climate changes in relation to disturbance, such as sunlight (due to the opening of forest canopy), moisture regimes, temperature, as well as changes in the ecological community such as predators and dispersers (Kallow et al. 2020). The germination rate is also affected by genetic factors, pollination rate, population size, pollinators availability and environmental conditions during fruit ripening (Kaiser et al. 2016; Fidalgo et al. 2019).

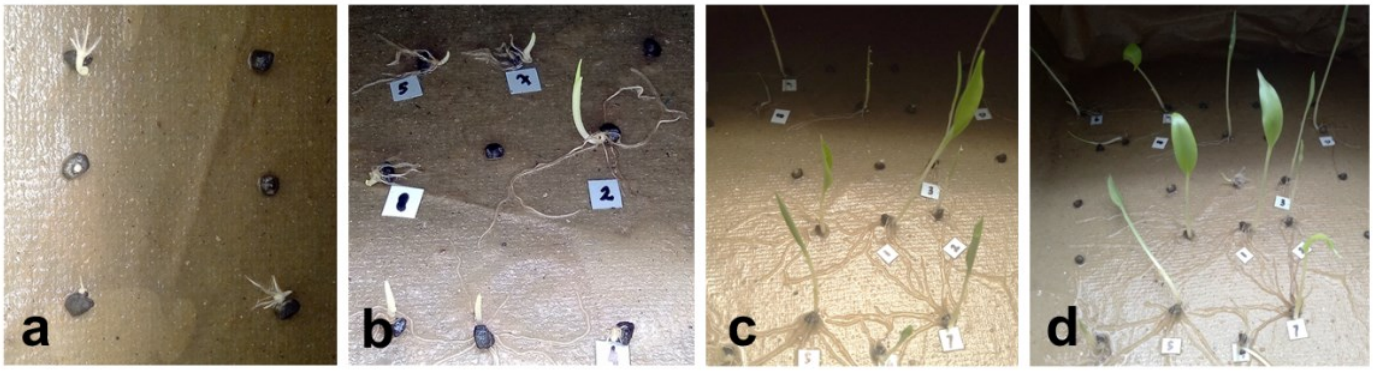

Figure 5. The germination of *M. acuminata* var. *flava* seeds extracted from fully-ripe fruits, a. 22nd DAS, b. 31st DAS, c. 43rd DAS, d. 50th DAS (DAS=Days After Sowing).

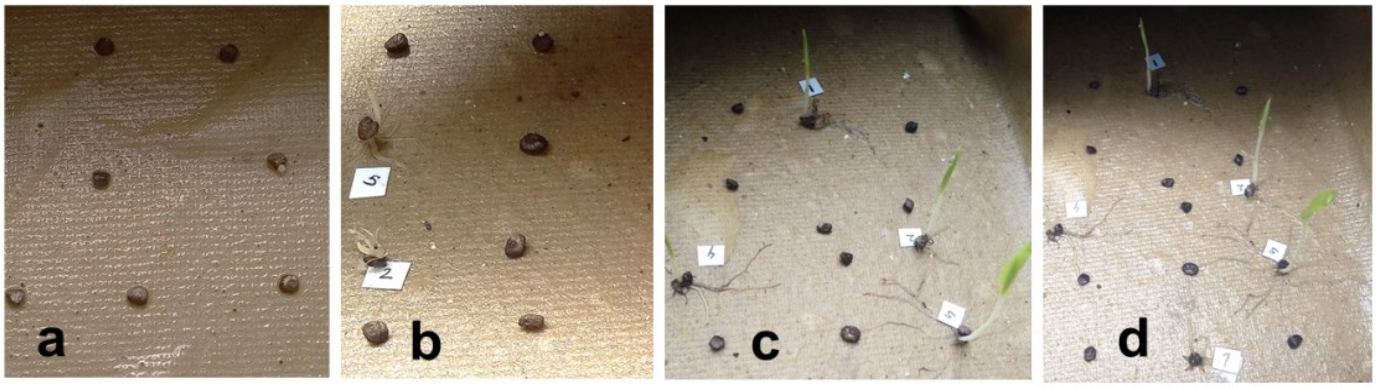

Figure 6. The germination of *M. acuminata* var. *flava* seeds extracted from under-ripe fruits. a. 22nd DAS, b. 31st DAS, c. 43rd DAS, d. 50th DAS (DAS=Days After Sowing).

Implications for further seed conservation efforts and studies of wild bananas

A complete plant morphological characterization is necessary specifically on core collection to confirm the species identity. Because the morphological characteristics of wild bananas are very varied, especially at the infraspecific level, subspecies of *M. acuminata* is also difficult to identify. Therefore the collectors must prioritize to conduct the detail morphological characterization on site preferably using full descriptor for bananas (IPGRI-INIBAP 1996) or minimum descriptor (at least). Considering the time limit during the collecting mission or fieldwork, it is also important to take plenty of photographic documentation of various plant parts, populations and surrounding habitats for further identification and supporting information. Molecular analysis is also required for more advanced study to confirm genetic fidelity by taking leaf samples of each distinctive specimen or population (Hapsari et al. 2020). In addition, since wild bananas can be both propagated generatively and vegetatively, collectors should collect both seeds and suckers to complement each other for *ex-situ* conservation effort.

In seed conservation efforts, physical variables are generally adopted to harvest forest seeds, such as change in fruits and seeds color, size, odor, presence of predators, dispersers and dehiscence of fruit as indicators of ripening (Kaiser et al. 2016). Generally in wild bananas, seeds from fruits of basal

hands are produced and matured first, so that when harvested they are already in a more advanced state compared to the distal hands. The banana fruits from basal hands are faster in changing peel color to yellow and cracking, also the pulp may be softened and rotting with some aromatic odor (Hapsari 2014; Kallow et al. 2020). However, obtaining perfectly mature bunch of fruits during collecting missions is very challenging. Whereas, collecting mature fruits from field germplasm collections is more manageable in term of harvest time. Thus, if possible, initial survey should be conducted to manage the readiness of the population for seed collection. For seed collection management, it is suggested to apply different accession numbers of each bunch from different individual plants. Furthermore, to lower the heterogeneity in further studies (as highlighted from this study), the seeds from fully-ripe fruits (yellow peel) are suggested to be separated from under-ripe fruits (green, green-yellow peel) within a bunch, or fruits from basal hands are separated from distal hands (at least). It is not recommended to bulk or mix up the seeds within a bunch of wild banana fruits.

CONCLUSION

The most prominent plant morphological characteristics of wild banana *M. acuminata var. flava* are the green-yellow male bud and bracts. The seeds are 20-170 seeds per fruit, wrinkled surface and dark brown-black when ripe. The seed had a thick and hard coat with a small embryo; and was classified as having orthodox storage behavior. Fresh seeds were germinated in two weeks after sowing, with hypogeal type and gradual germination pattern. The level of fruit ripeness was significantly affected the seed germination percentage, and growth variables *i.e.* plant height and number of roots. The seeds from fully-ripe fruit were considered more mature physiologically than the seeds from under-ripe fruits. When the seeds were not completely mature, they could germinate, but did not result in seedlings as vigorous as those harvested at the appropriate ripening time. For further conservation, storage, propagation, and related studies on seeds of wild bananas; it is suggested to clearly define and separate the seeds from the fully-ripe fruits out of the under-ripe fruits to lower the heterogeneity. Further studies on germination of periodically storage seeds of this species are required to confirm the behavior character of the storage. Germination studies by embryo culture method are also suggested in order to overcome the medium-low germination percentage of this species by conventional seed germination method.

AUTHORS CONTRIBUTION

All authors contributed equally from conceptualization, writing, review and editing of the final manuscript.

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CONFLICT OF INTEREST

The authors state no conflict of interest from this manuscript.

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