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Characterization, potential and conservation of Pisang Kates (*Musa* cv. ABB), a unique local banana cultivar from Pasuruan, East Java, Indonesia

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Abstract. *Hapsari L, Damaiyani J, Yulistyarini T, Auliya I, Gusmiati LH, Zaro RM. 2022. Characterization, potential and conservation of Pisang Kates (*Musa *cv. ABB), a unique local banana cultivar from Pasuruan, East Java, Indonesia. Biodiversitas 23: 3521-3532.* Characterization of local banana (*Musa* spp.) germplasm is fundamental for identification and provides information on traits that support their sustainable conservation and optimum utilization. This study aims to characterize and discuss the potential and conservation of Pisang Kates, a local cultivar originating from Tutur, Pasuruan, East Java, Indonesia. The observed characteristics include morphology, molecular, agronomy and yield performance, leaf anatomy and stomata, pollen morphology and viability, fruit total soluble solids and nutrients. Results showed that Pisang Kates has a unique fruit shape ovoid to globose, weight of 40-150 g, appear solitary and randomly attached directly to peduncle, like papaya. It was molecularly by Internal Transcribed Spacer barcode confirmed as ABB genotype. It is tolerant to drought and banana bunchy top virus, but susceptible to wilts. Leaf anatomy has three palisades and isodiametric polygonal epidermals. Leaf stomata is amphistomatic, tetracytic on adaxial, and paracytic on abaxial. Pollen unit is monad, very large, subspheroidal, monocolpate aperture, granulum ornamentation, and low viability. Fruit moderately sweet with nutrient contents equal to dessert banana. Pisang Kates has potential as one serving consumer size fruit, ornamental fruit plant, female parent for breeding, and pioneer plant for rehabilitation programs. Both ex-situ and on-farm conservation strategies of this valuable local banana genetic resource are encouraged.

Keywords: Banana, characterization, conservation, *Musa,* potential

INTRODUCTION

Southeast Asia is the primary origin and diversity center of bananas (*Musa* spp., Musaceae), while secondary center is found in Africa (Oso et al. 2017). Cultivated bananas are the fourth most significant global crop with great socio-economic, health, and food security, especially in developing countries (Alemu 2017). They are thought to have resulted from a complex evolution and domestication history of wild bananas, particularly from two wild diploids of *M. acuminata* (AA) and *M. balbisiana* (BB) (Singh et al. 2021). According to archaeological and linguistic studies, the cultivated banana was first domesticated by farmers in Southeast Asia about 7,000 years ago, and then spread by travelers movement worldwide (De Langhe et al. 2009). To date, approximately 60 wild banana species and more than 1,000 banana cultivars and landraces have been recognized (Li and Ge 2017).

About 12 out of 66 described species of wild bananas were recorded (Iskandar et al. 2018; Hastuti et al. 2019) with more than 200 local banana cultivars in Indonesia (Nasution and Yamada 2001). Furthermore, approximately 79 local banana cultivars were documented during the ethnobotanical study in six districts of East Java (Hapsari et al. 2017). However, threats posed by habitat destruction and the replacement or loss of local banana cultivars intensify the urgency for collection and conservation efforts (MusaNet 2016). Conservation efforts with three complementary methods including in-situ, on-farm, and ex-situ of diverse wild species and varieties of bananas are necessary for safeguarding their adaptability and resilience to biotic and abiotic stresses, also available as source of genes material for further banana breeding programs (Van den Houwe et al. 2020).

The available genetic diversity of banana germplasm in the genebanks is an important asset that needs to be fully characterized. Characterization is a fundamental step in germplasm collection management for identification and providing information on traits that support their sustainable conservation strategy and optimum utilization (Engels and Visser 2003; Suryani and Owbel 2019; Dwivany et al. 2021). Characterization is the description of plant germplasm which determines the expression of highly heritable characters. A holistic approach through numerous research activities is suggested including morphotaxonomy, molecular genotyping, cytological, phenotyping, genomic and bioinformatic, nutritional traits, etc. (Christelová et al. 2017; Van den Houwe et al. 2020). The characterization should be carried out as soon as possible to add value to the collection. However, it is indeed very time consuming and expensive, therefore is often delayed or performed during regeneration to reduce costs (CGIAR 2022).

Purwodadi Botanic Garden (PBG) located in Purwodadi, Pasuruan, East Java has conserved many banana genetic resources, particularly from Eastern Indonesia. At least 103 collection numbers and 197 specimens consisting of eight wild banana species and 95 banana cultivars were collected (Hapsari 2014). Specifically, Pisang Kates is one of the local banana cultivar collections of PBG which is popular for its unique inflorescence and fruit appearance. It is considered a valuable banana collection that needs to be fully characterized. Without characterization, conservation and use will be pointless as the value of the material cannot be known and the information cannot be accessed or shared for further purposes (MusaNet 2016).

Hence, this study is the first to scientifically report the complete characterization of Pisang Kates including morphology, molecular, agronomy and yield performance, leaf anatomy and stomata, pollen morphology and viability, fruit total soluble solids and nutrients. In addition, we also discuss the potential utilization and conservation strategies based on the observed characteristic results. The results from this study may become basic information for broader and further studies on conservation and sustainable utilization of Pisang Kates in Indonesia.

MATERIALS AND METHODS

Plant materials and study site

Plant materials used in this study were five mature living specimens from Pisang Kates collection of Purwodadi Botanic Garden in Pasuruan, East Java, Indonesia. The plant specimens were originally collected from Tutur Village, Tutur Sub-district, Pasuruan District, East Java Province, Indonesia (Figure 1). It was first collected in 1972 and recollected in 2010 with registration number P1972051 and P2010091, respectively. The plant specimens were planted in the garden at Vak XXIV.D. number 97-ab (Primary source from PBG Registration Unit).

Procedures

Morphology characterization

The complete morphological characterization of the plant was carried out using a standardized descriptor for bananas (*Musa* spp.) from IPGRI-INIBAP/CIRAD (1996). Observations were made on both qualitative and quantitative characters in the vegetative and generative plant organs.

Molecular characterization

Molecular characterization was performed using Internal Transcribed Spacer (ITS) barcode with primer pairs of ITSL (5’-TCG TAA CAA GGT TTC CGT AGG TG-3’) and ITS4 (5’-TCC TCC GCT TAT TGA TAT GC-3’) (Nwakanma et al. 2003). DNA isolation, PCR, and electrophoresis were conducted at Plant Physiology, Tissue Culture and Microtechnique Laboratory of Biology Department, Brawijaya University (Malang, East Java, Indonesia). The whole genome DNA was isolated from fresh cigar leaf sample using DNA Purification Kit (Promega Wizard®) following the manufacturer’s protocols for plant. PCR amplification was conducted in a 30 µL volume contains 15 µL of DreamTaq Green PCR Master Mix (Thermo Scientific), 3 µL of 5 pmol each of forward and reverse primers, 3 µL of DNA template (5-25 µg/mL) and 6 µL of nuclease-free water. PCR thermal cycle program consists of initial denaturation at 95°C for 3 minutes; followed by 25 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 53ºC, and extension for 30 seconds at 72ºC. Final extension was carried out for 7 minutes at 72ºC. PCR product was then visualized in 1.5% agarose gel electrophoresis in TBE buffer with 2 µL Ethidium bromide for 50 minutes at 50 volts and documented with UV transilluminator. DNA ladder 100 bp was used to determine the size of PCR product. Later, the PCR product was purified and sequenced at 1st BASE Laboratory (Selangor, Malaysia) using Sanger method in ABI PRISM 3730xl Genetic Analyzer. Sequences evaluation was conducted in Seqscanner v.10. The sequence homology was detected using web BLAST in NCBI GenBank. Nucleotide variation was analyzed with Bioedit 7.0.5.3 and DnaSP 6.12.03. The phylogenetic tree was performed with some banana cultivars of ABB group reported by Hapsari et al. (2018) using Neighbour Joining method in MEGA 7.0.26.

Agronomy and yield performance observation

The agronomy and yield-related performances observed include flowering time, fruit/bunch production and productivity, resistance to pest/diseases, and environmental stresses. The characterization was following the guideline of variety registration for horticulture commodity on bananas from the Ministry of Agriculture Republic of Indonesia (Decree 700/Kpts/OT.320/D/12/2011).

Leaf anatomy, epidermal cells, and stomata

Leaf anatomy, epidermal cells, and stomata observations were conducted in the Plant Taxonomy Laboratory of Biology Department, Brawijaya University. The leaf samples were taken from the middle part of the leaf blade near to petiole. Stomata profiles were observed using replica method with clear nail polish (Auliya et al. 2019). Meanwhile, leaf anatomy method was conducted using cross-sectioning slice leaf following Harijati et al. (2013). The sample preparat slides of leaf anatomy were observed using a light microscope (LM) at 400× magnification whilst the stomata observation at 100× and 400× magnifications, with five randomly selected replicates of the microscope field of view.

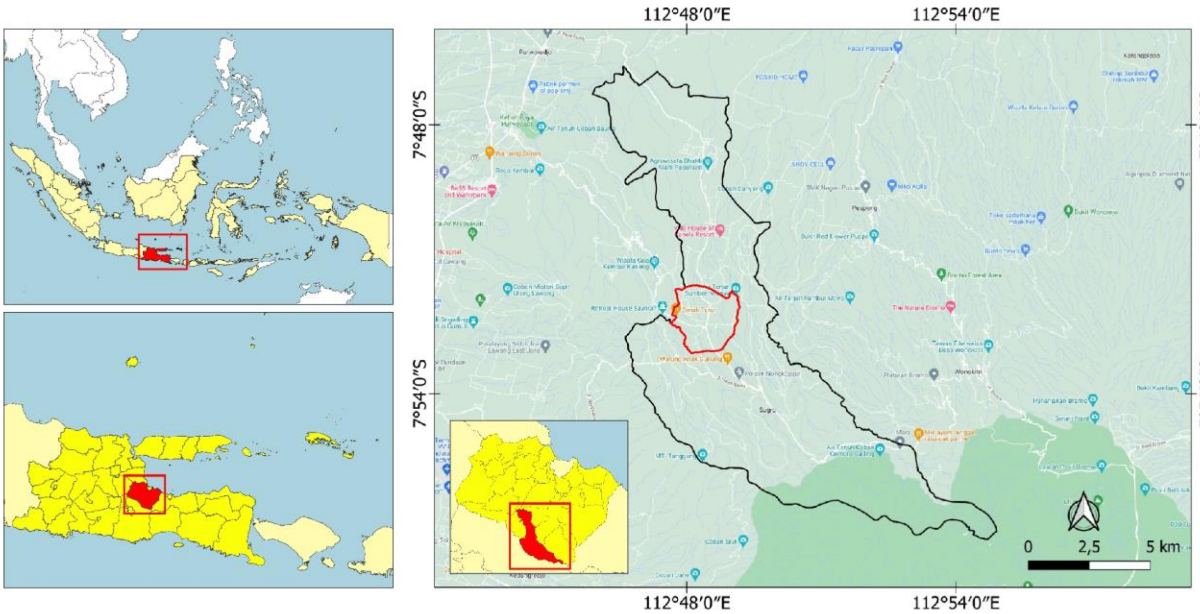

Figure 1. Source of Pisang Kates specimens in Tutur Village (red line), Tutur Sub-district, Pasuruan District, East Java Province, Indonesia

Pollen morphology and viability test

Fresh pollens were taken from male flowers at anthesis time between 7.00 to 9.00 am. Pollen morphology was observed using a self-modification solution of 10% methylene blue (Methylthioninium chloride). Descriptive analysis was performed on the pollen morphology according to Kapp (1969), Hesse et al. (2009), and Halbritter et al. (2018). Meanwhile, the pollen viability was determined using a solution of 1% TTC (2,3,5-Triphenyl tetrazolium chloride) (Beyhan and Serdar 2008). The sample preparat slides of both pollen morphology and viability test were observed using a LM at 400× magnification, with five randomly selected replicates of the microscope field of view.

Fruit total soluble solids (°Brix)

Determination of total soluble solids (TSS) of the fruit was carried out by destructive method using analog portable hand refractometer ATC. The measurement was done by dripping the liquid mature banana fruit extract on the detector. The refractometer was calibrated before use to 0% with sterile distilled water (Nurfazizah et al. 2019). Fruit TSS analyses were conducted in five replications.

Fruit nutrient analysis

Fruit nutrient analyses of mature banana pulp were conducted in the Laboratory of Testing for Food Quality and Food Safety of Agricultural Product Technology Department, Brawijaya University. Parameters tested include proximate contents comprised of protein, fat, water, ashes, and carbohydrates; total sugar; vitamin C; and Kalium (K). Protein was determined by Kjedahl method. Fat was analyzed by Soxhlet method. Water was analyzed using a gravimetric oven (105°C). Ash was analyzed using a gravimetric oven furnish (600°C). Carbohydrate was calculated by the difference between fat, protein, ash and moisture. Total sugar was estimated by anthrone method. Vitamin C was determined by iodine titrate ion method. K was determined by atomic absorption spectrometry method. Fruit nutrient testing was carried out in duplo reactions following Indonesian national standards for food and beverages testing SNI 01-2891-1992 refers to AOAC (2012).

Data analysis

The data on the characterization of Pisang Kates obtained from each parameter observed was compiled and analyzed descriptively. The observed characteristic data results were then compared to some previous studies on its closely related species and banana cultivars for a more comprehensive discussion and conclusion. In addition, the potential utilization and conservation strategies of Pisang Kates were also discussed by referring to related references.

RESULTS AND DISCUSSION

Morphology characteristics

The complete morphological observation of Pisang Kates from this study is presented in Figure 2. Plant general appearance is leaf habit drooping with leaves normal or not overlapped. Pseudostem height 2.1-2.9 m, normal to robust aspect, 20-40 cm in diameter, yellow-green, shiny with few waxes, predominant underlying light green with pink-purple pigmentation, sap milky and moderately waxy on leaf sheaths. Plant suckers 3-5, height between 1/4 and 3/4 of the parent plant, position close to parent (vertical growth). Leaf petiole medium length 51 to 70 cm (average 57.21±5.87 cm), green with small dark brown blotches at the base, canal shape straight with erect margins. Petiole margins narrow (≤1 cm), dry, not winged, and clasping the pseudostem. Leaf blade length 171 to 220 cm, width ≤70 cm, and high ratio ≥3. Leaf shape at base both sides rounded, upper surface green and dull, color lower surface green-yellow and dull, moderately waxy. Leaf midrib dorsal and ventral surface light green, with insertion point of blades on petiole symmetric.

Pisang Kates has a short peduncle (length ≤50 cm) with 2 empty nodes, light green and hairless. Bunch position hanging at angle 45°, shape asymmetric, appearance lax. All fruits are formed from female flowers, with solitary position, pedicel directly attached to peduncle (not forming a hand or *sisir* in Indonesian language). Rachis type present and male bud persistent, appearance bare, with position falling vertically. Male bud type normal (present), shape lanceolate with length 18.6±1.27 cm and 6.05±0.64 cm in diameter at harvest. Bract base shape lanceolate, large shoulder, apex pointed, young bracts slightly overlap, external face red-purple, internal face red, apex tinted with yellow, without discolored lines on the external face (homogenous) and very waxy. Bract behavior revolute (rolling), lifting two or more at a time, and left a very prominent scar on rachis. Male flower has compound tepal basic, yellow with pink pigmentation, and yellow lobe. Free tepal fan-shaped, cream, and apex triangular. Anthers five, exerted, yellow with yellow filament and cream pollen sac. Style shape straight, basic color yellow without pigmentation, inserted. Ovary shape straight, basic color yellow with red-purple pigmentation, stigma yellow. The dominant color of male flower is yellow. Arrangement of ovules four-rowed.

The fruits appear solitary and attached to peduncle, not forming a hand. The fruit position curved upward (obliquely, at a 45°angle upward) to peduncle, number of fruits per bunch 4-15. The fruits are comprised of single fruit and double fused fruits. Number of single fruits per bunch 3-9 and double fused fruits 1-6. Weight of a single fruit 43-89.5 g and a double fused fruit 95-133 g. Pedicel surface hairless, length medium 11 to 20 mm, width >10 mm. Fruit shape ovoid to globose, transverse section rounded, apex blunt-tipped to rounded with persistent flower relicts. Immature fruit peel is light green, yellow when mature, waxy. Fruit peel thick ≥3 mm, without cracks. Pulp before maturity white-cream and at maturity cream-yellow. Fruits do not easily fall from peduncle (persistent). Flesh texture firm, predominant taste sweet and acidic (apple-like), and seed absent.

Molecular characteristics

The whole genome DNA of Pisang Kates was easily amplified by ITS primers resulted an amplicon at approximately 600-700 bp. The Sanger sequencing resulted 658 nucleotides (Figure 3). NCBI BLASTing showed that the sequences were homolog with ITS sequences of *M. balbisiana* and ABB group cultivars with similarities of 93-95%. Nucleotide composition analysis showed that Pisang Kates was high in GC content (62.2%) thus leading to high levels of mutations. Neighbour-Joining phylogenetic analysis with other local banana cultivars ABB group from previous study (Hapsari et al. 2018) showed that Pisang Kates was clustered in sub-group II and closely related to Pisang Kepok Ebung, Pisang Raja Wesi and Pisang Gajih Bali supported by strong bootstrap (Fig. 4). Furthermore, the ITS sequences of Pisang Kates from this study has been deposited to NCBI Genbank with accession number KT696447 as an open access source for broader study.

Agronomy and yield performance

The agronomic performance of Pisang Kates is considered easy to maintain with minimum agricultural practices. The plant is relatively tolerant to water shortage conditions but needs sufficient water for maximum production. Like most bananas from the ABB group, Pisang Kates is susceptible to wilt diseases, but is likely resistant to banana bunchy top virus (BBTV). The flowering time of Pisang Kates is about 10-11 months after planting and can be harvested approximately 4-5 months after flowering. Unlike the common bananas which produced hands, Pisang Kates produced solitary fruits attached to peduncle about 4-15 fruits per bunch, therefore fruit productivity is considered very low. The single fruit weight on average 67.79±19.99 g/fruit and double fused fruit weight on average 121.30±16.39 g/fruit. Based on our observation, a single fruit can weigh up to 150 g. Fruit bunch weight on average 1,148.65±755.13 kg/bunch. the expected productivity per ha with planting pattern 2 m x 2 m is about 2.87 tons/ha. However, fruit production and productivity can be increased by optimizing nutrient requirements and cultivation methods.

Leaf anatomy, epidermal cells, and stomata characteristics

The leaf anatomy transverse sections characteristic of Pisang Kates is shown in Figure 5.A. It consisted of adaxial and abaxial epidermis, adaxial and abaxial hypodermis, palisade tissue, spongy tissue, sclerenchyma, bundle sheath, phloem, xylem, and air space. The adaxial and abaxial epidermis are comprised of a single layer of simple and small size cells. Meanwhile, the hypodermis is comprised of two layers of cells on the adaxial and abaxial, but smaller and flatter on the abaxial. The adaxial hypodermis cell layer size is larger than the epidermis layer. Two size groups of vascular bundles are observed, large and small located between the mesophyll cells. Palisade and spongy tissues also form the mesophyll. The palisade tissue is composed of three layers of cells while the spongy tissue consists of irregularly shaped cells.

The LM observation of leaf epidermal cells and stoma characteristics are shown in Figure 5.B-E. The epidermal cells on the adaxial and abaxial surface of Pisang Kates leaf showed relatively the same shape which is predominantly polygonal. They are arranged isodiametrically, characterized by straight to curved anticlinal walls. Furthermore, Pisang Kates stomata are distributed on both adaxial and abaxial surfaces, thus classified as amphistomatic. They are regularly arranged, particularly at the abaxial side (Figure 5.C). Interestingly, there are variations in terms of type and density of stomata between adaxial and abaxial. Stomata type on adaxial is tetracytic with a low density of 30.64 per mm^2 (Figure 5.D).

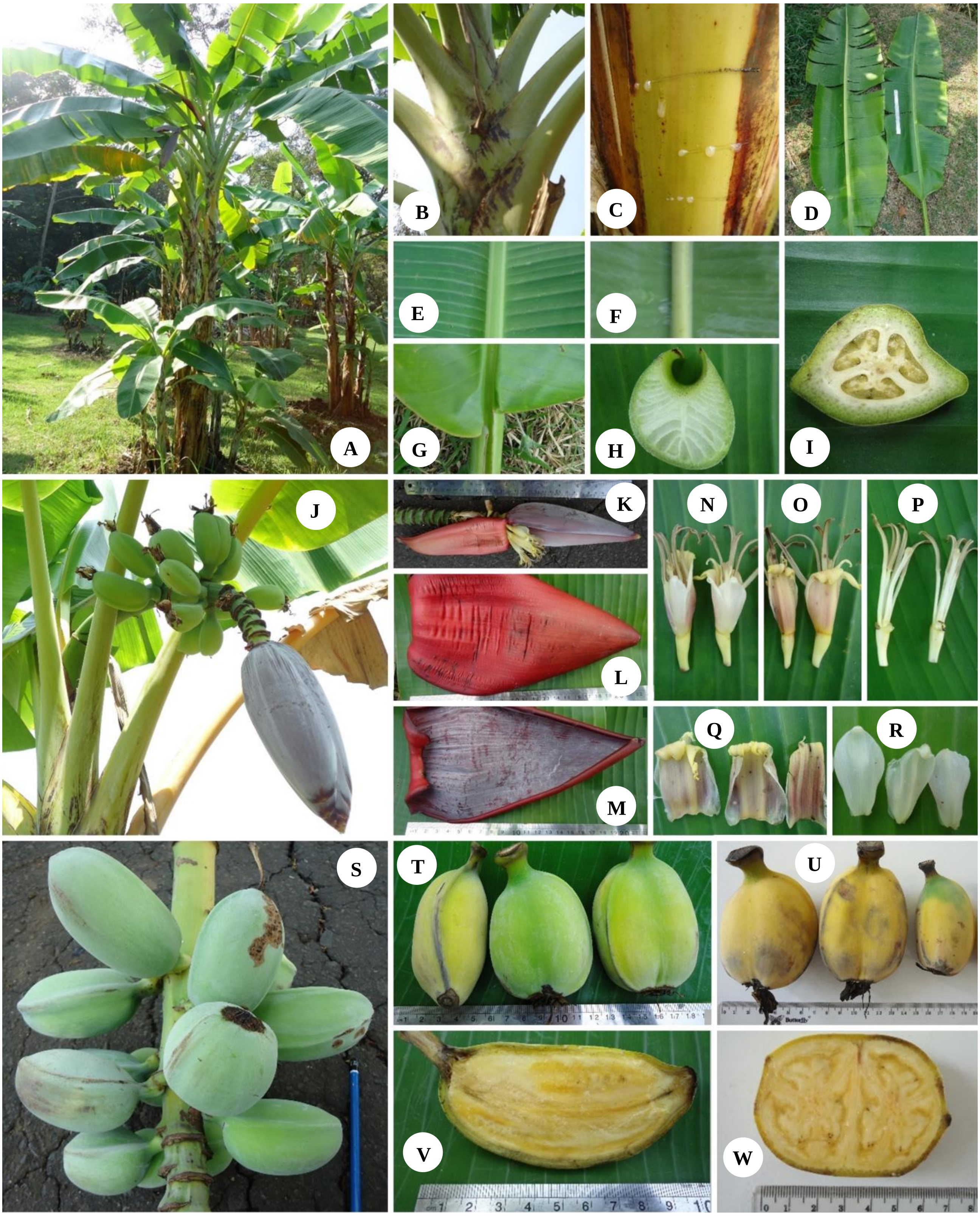

Figure 2. Morphological observation of Pisang Kates: A. Plant clumps, B. Petioles base insertion, C. Pseudostem sap, D. Leaves, E. Upper leaf surface, F. Lower leaf surface, G. Leaf base, H. Petiole canal shape, I. Ovule arrangement, J. Inflorescence, K. Male bud, L. Internal bract, M. External bract, N. Male flowers front view, O. Male flowers back view, P. Style and filaments, Q. Compound tepal, R. Free tepal, S. Infructescence, T. Immature fruits, U. Mature fruits, V. Longitudinal section of single fruit, and W. Cross section of double fused fruits

>Pisang Kates_ KT696447

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1    ACATGCGGAG  TACTGTCAGA  CACTGACGAG  GACGACCGTG  AATGCGTCAC  50
51   GATTGCTCGT  CGGGCTCGTC  CCGACAACAC  CCCGATGTCC  ATCCCCCCTC  100
101  CGGTGGGACG  ACTGAGGGGA  TGAAATACCA  ACCCCGGGGC  GGAGAGCGCC  150
151  CAGGGACACG  AACATCCGAA  TCCGAGGGGC  TCTCTGCATG  GAGGAGGATA  200
201  CAATTCCCAC  GGTGACCCCC  TTGGATGACT  CTCGGCAACG  GATATCTCGG  250
251  CTCTCGCATC  TATGAAAAAA  GTAGCGAAAT  GCGATACCTG  GTGTGAATTG  300
301  CAAATCCCGT  GAACCCTCGA  GTCTTTGAAC  GCAAGTTGCG  CCCGAGGCCC  350
351  TCCGGCTAAG  GGCACGCCTG  CCTGGGCGTC  ACGCTTTCGA  CGCTTCGCCG  400
401  ATGCCCCCCT  CGGGGGGGGT  GGAGGCGTGT  GCGGAGGATG  GCCCCCCGTG  450
451  CCCAAGGGTG  CGGTTGGCCA  AAAAGCGGGC  CCTCGGTGGT  TGTCAAACAC  500
501  CACGCGTGGT  GGATGCCTTG  TGCGAGCCAT  ATGTCGTGCC  TTCGGGACCC  550
551  GGGAGAGGCC  TCGAGGACCC  AAGTCGTGGT  GCGAGTCTAT  GCCACGGAGC  600
601  GCGACCCCGG  GTCAGGTGGG  GCTACCCGCT  GAGTTAAGCA  TCTCATAAGC  650
651  GGGAAGAA                                                 658
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Figure 3. ITS DNA sequences data of Pisang Kates

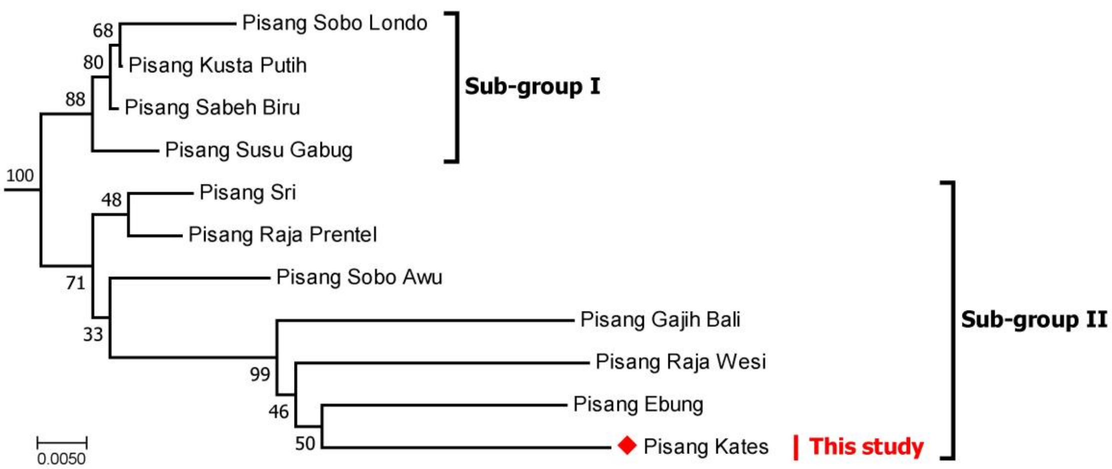

Figure 4.. NJ phylogenetic tree of Pisang Kates with other bananas ABB group from East Java, Indonesia

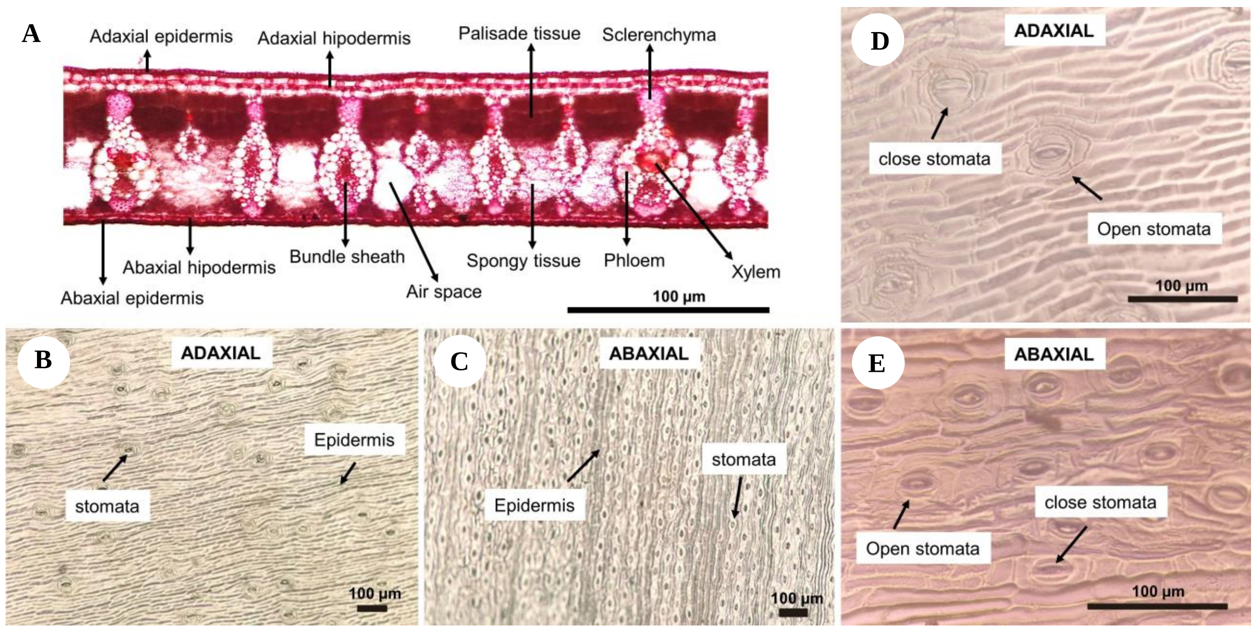

Figure 5. Leaf anatomy and stomata observation of Pisang Kates. A. Anatomy at transverse section, B. Adaxial surface replica, C. Abaxial surface replica, D. Tetracytic stomata on adaxial, E. Paracytic stomata on abaxial

Whereas, abundant paracytic stomata are present on the abaxial with a high density of 153.32 per mm^2 (Figure 5.E). A tetracytic stomata is characterized by the presence of an encircling ring (rosette) of four or more subsidiary cells. Whilst, a paracytic stomata is characterized by the presence of one or more pairs of lateral subsidiary cells oriented parallel with the guard cells (Rudall et al. 2013). Comparison of stomata aperture size showed that the opening aperture on the abaxial is wider than adaxial, i.e. 25.64 µm x 11.84 µm and 25.69 µm x 7.83 µm, respectively. The stomata on both adaxial and abaxial are surrounded by epidermal cells known as guard cells in kidney-like shape.

Pollen morphology and viability characteristics

The 10% methylene blue solution successfully stained the pollen grains of Pisang Kates for identification purposes. The pollen morphology of Pisang Kates is illustrated with several LM photographs in Fig. 6. The dispersal unit of pollen grain is monad. It means dispersal unit consisting of a single pollen grain. According to the pollen size classification by Hesse et al. (2009), it is categorized as very large (>100 µm). The average length of the polar axis (P) and equatorial axis (E) are 122.46 µm and 122.84 µm, respectively. The P/E ratio is 1.00, therefore it is identified as subspheroidal shape (P/E ratio 0.75-1.33). The outline in the polar view in wet condition is mostly circular. The exine ornamentation is granulum, characterized by structure or sculpture elements of different sizes and shapes, smaller than 1 µm. The pollen aperture is monocolpate, characterized by a single furrow on one side (Figure 6.B). Furthermore, it was able to germinate as indicated by the appearance of a pollen tube (Figure 6.C). However, the pollen viability is considered low at only 14.55±7.19%. The viable pollen is reddish and non-viable pollens remain colorless in 1% TTC test (Damaiyani and Hapsari 2018).

Fruit total soluble solid (ºBrix)

The results of TSS of mature fruits of Pisang Kates ranged from 17 to 23 °Brix. When a Brix refractometer is calibrated using the brix scale, one degree of brix (°Brix) is a rough measure equivalent to 1% total sugar by mass (Wilson 2021). Mature fruit of Pisang Kates is considered to have rough total sugar approximately an average of 20.17±2.79 g per 100 g of edible pulp.

Fruit nutrient characteristics

Nutrient analysis showed that 100 g of mature fruit pulp of Pisang Kates contains 25.82 g carbohydrates, 1.86 g protein, 0.03 g fat, 71.4 g water, 0.89 g ash, 17.59 g total sugar, 27.87 mg vitamin C, and 146.34 mg Kalium. The equivalent values of 1 g of carbohydrate, protein, and total sugar provide 4 calories, whilst 1 g of fat provides 9 calories (USDHHS and USDA 2015). Therefore, 100 g edible portion of Pisang Kates provides approximately 181.35 calories. Fruit nutrients of Pisang Kates compared to other local banana cultivars reported by Hapsari and Lestari (2016) including Pisang Berlin (AA), Ambon Hijau (AAA), Raja Bandung (ABB) and Kepok (ABB) is presented in Figure 7.

Discussions

In general, the plant appearance of Pisang Kates is similar to other banana cultivars from ABB group, such as Pisang Kepok and Pisang Awak. They have normal to robust pseudostem, drooping leaves and waxy, incurved petiolar canal, fewer blotches in petiole base, both sides rounded at the leaf base, internal bract homogenous red, and four-rowed ovules (Sukartini 2007; Sunandar and Kahar 2017; Gusmiati et al. 2018). However, Pisang Kates has unique inflorescence and fruit characteristics which become the distinguishing characteristics. The fruit fingers of Pisang Kates appear solitary and randomly attached directly to peduncle, not forming a hand like in common bananas. The fruit shape is ovoid to globose with blunt-tipped to rounded (Figure 2.S-T-U). The fruit shape resembles papaya fruit, so it is called Pisang Kates by local people ("*Kates*" meaning "papaya" in Javanese language). Hence, it also can be referred to as a papaya-like banana.

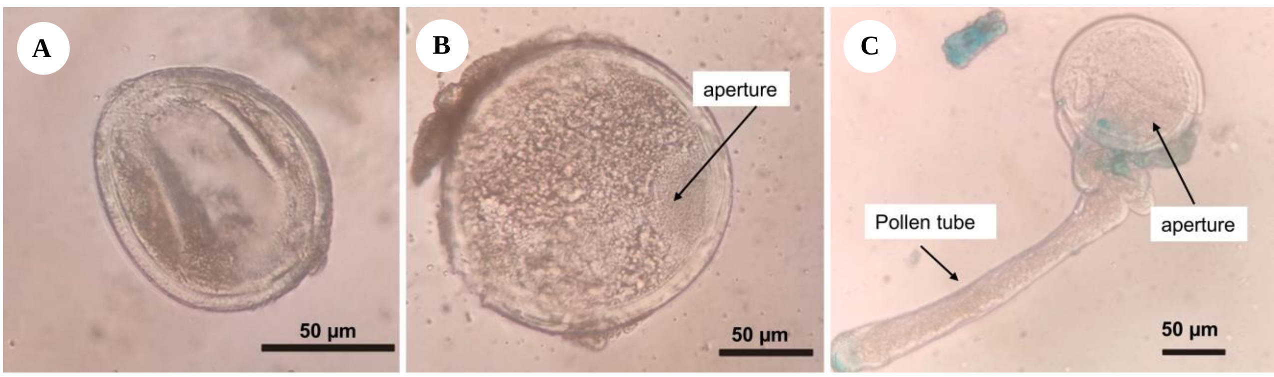

Figure 6. Pollen morphology observation of Pisang Kates. A. Pollen in dry condition, B. Pollen in wet condition, C. Pollen germination

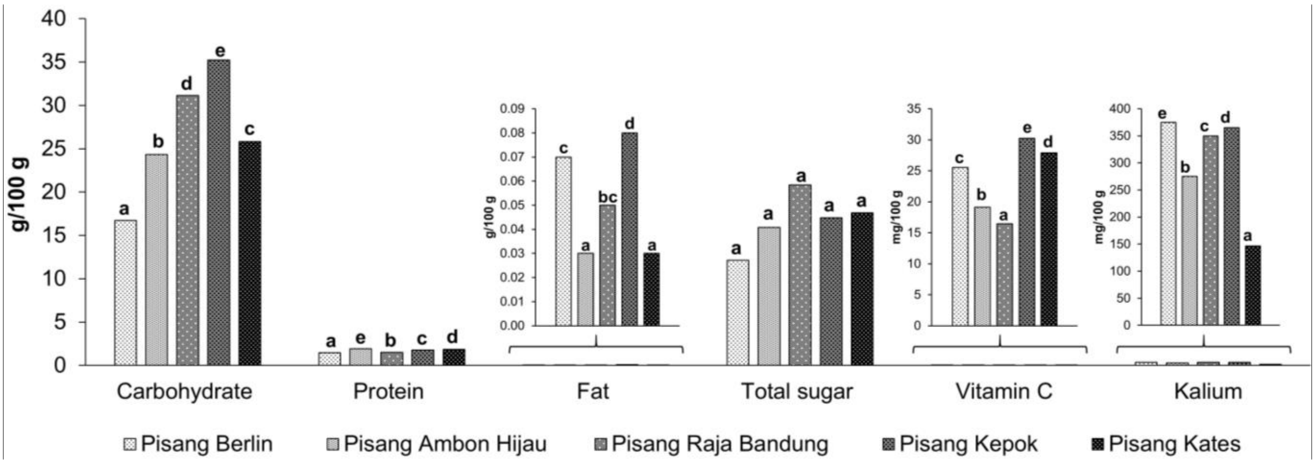

Figure 7. Fruit nutrients comparison of Pisang Kates to other local banana cultivars from East Java. Remark: Values on the bar with the same alphabet were not significantly different based on Tukey test 95%

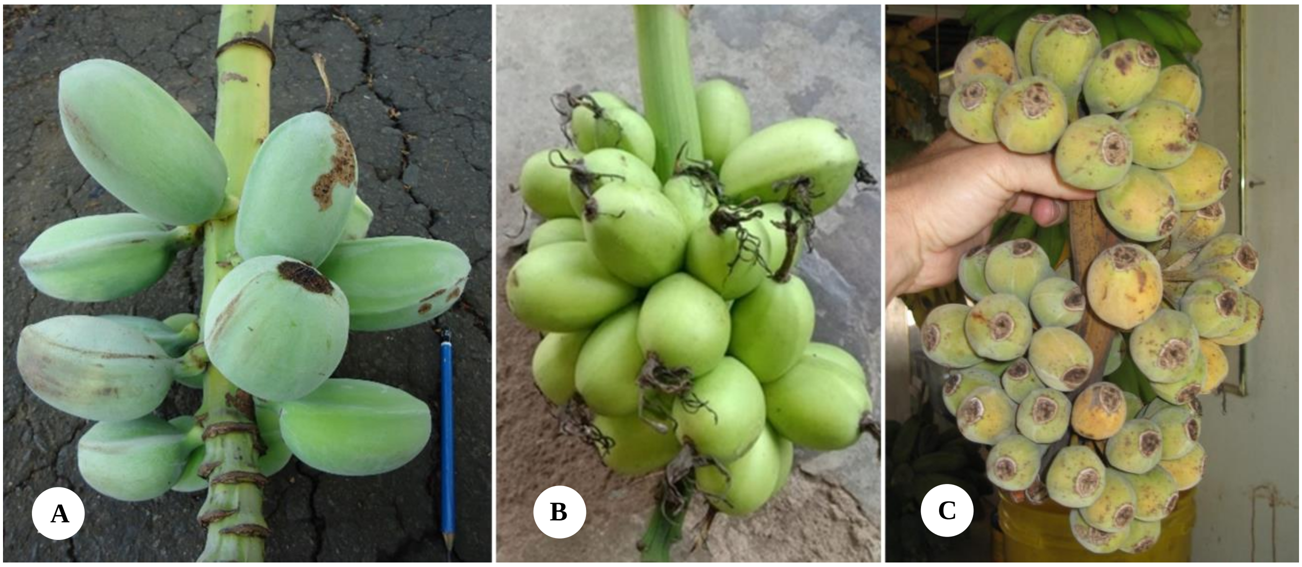

Figure 8. Fruit characteristics comparison of A. Pisang Kates, B. Pisang Telor (Torowiro, Lampung), C. Pitogo (www.bananas.org)

The fruits are comprised of single and double fused fruits (Figure 2.V-W) and are weighted 40 to 150 g per fruit. The mechanism of the fusion of two ovaries into a finger in Pisang Kates is suggested to be studied. The fruit weight and size can be increased by optimizing nutrient requirements and cultivation methods. The larger the size of the fruit, the more it resembles papaya. Specifically based on the fruit shape, Pisang Kates is quite similar to Pisang Telor from Sumatra (Lampung and Bengkulu) and Pitogo from the Phillippines, Hawaii, and the surrounding Pacific Islands (Valmayor et al. 2000). However, Pisang Telor and Pitogo fruits are form a hand meanwhile Pisang Kates is solitary (Figure 8). Pisang Telor was reported to have ±5 hands per bunch and ±15 fingers per hand (Miswarti et al. 2017).

The ITS phylogenetic analysis showed that Pisang Kates was clustered with other bananas of ABB group from East Java in subgroup II closely related to Pisang Kepok Ebung from Ponorogo, Pisang Raja Wesi from Ngawi, and Pisang Gajih Bali from Jember with high genetic similarity of 93.19-94.34% (Figure 4). Therefore, it was confirmed and identified as ABB group genome. The ITS barcode of the nuclear genome has proven to be superior in terms of detecting the inter/intra-species variations and classifying until cultivar level of bananas over the chloroplast genome (Lallawmawma et al. 2013; Hapsari et al. 2018; Meitha et al. 2020). Pisang Kates is revealed high in GC content (Figure 3), which is considered to have a higher rate of mutations. DNA sequences with higher GC content are hotspots of mutation, C base is often methylated and occurred errors during multiplication (Ubaidillah and Sutrisno 2009). Mutations are essential to evolution (Carlin 2011). Thus, Pisang Kates is presumably the result of mutation and adaptation, then conserved through vegetative propagation by farmers over a long period of time in Tutur, Pasuruan (Hapsari et al. 2018). The distribution of Pisang

Kates is reported by citizens of Indonesian banana community still be limited to Pasuruan and Malang. However, it may have been spread throughout East Java by farmers who are interested due to its uniqueness.

Agronomy performance characterization showed that Pisang Kates is relatively tolerant to water shortage condition (drought) and BBTV, but susceptible to wilt diseases caused by both bacterial and *Fusarium*. These results were in agreement with previous studies which indicated that bananas with ABB genomes are more tolerant to drought and other abiotic stresses than other genotypes (Ravi et al. 2013). Likewise, genotypes with one or two B genomes tend to be more tolerant to BBTV but susceptible to wilt disease (Niyongere et al. 2011; Hapsari and Masrum 2012). Good husbandry management of agricultural practices needs to be a concern for optimum fruit production of Pisang Kates, particularly for commercial purposes.

Pisang Kates is considered very low in productivity (2.87 tons/ha) compared to common bananas, so it is not suitable to be developed as a highly productive commercial banana fruit plantation. As for the commercial banana cultivars, fruit productivity at household scale plantations reached 20-25 tons/ha for Pisang Ambon (Yogyakarta Department of Agriculture 2009) and 35-40 tons/ha for Pisang Kepok (Rizal et al. 2015). It is also considered low productivity compared to local banana cultivar Pisang Talas (AAB) from East Kalimantan reached 16-23 tons/ha (Sunaryo et al. 2017). However, due to the uniqueness of the fruit shape and size, Pisang Kates has the potential to be promoted as a commercial one serving consumer size fruit by sorting and grading the fruit size at approximately 125-150 g. A standard serving of a medium banana for one consumer is about 150 g (ANHMRC 2016).

The leaf anatomy of Pisang Kates is similar to other bananas from previous studies (Harijati et al. 2013; Nugroho and Sumardi 2015; Sunandar and Kahar 2017, 2018) by the presence of a single layer on the adaxial and abaxial epidermis, two layers of hypodermis on adaxial and abaxial, spongy tissue, sclerenchyma, bundle sheath, phloem, xylem, and air space at the mesophyll. Further, the occurrence of 2-3 layers of hypodermal cells is typical to the Musaceae, in contrast with the closely related species *Heliconia* sp. which has only one hypodermal layer (Harijati et al. 2013). Pisang Kates has three layers of dense palisade cells slightly higher than previously reported in Pisang Kepok and Awak which have two layers (Sunandar and Kahar 2017, 2018). Palisade is the place where chloroplasts are most commonly found, which is related to its important function for photosynthesis (Gotoh et al. 2018). The number of layers, cell size, and density of palisade in plants reflect its environmental conditions. The leaves of plants in open habitats generally have a denser and higher number of palisade cells than in shaded habitats (Torre 2003; Nugroho and Sumardi 2015). Based on the leaf anatomy characteristics, Pisang Kates is thought to have adapted and is able to grow well in open habitats. It is potential as a pioneer plant for rehabilitation program of degraded lands.

Leaf stomata in plants have various characteristics in terms of density, size, and shape. The stomata is easily get affected by environmental conditions and their micromorphological characteristics are often used for plant classification (Razzaq et al. 2021). In general, bananas only have one type of stomata with mostly parasytic type (Oso et al. 2017) or anomocytic (Sumardi and Wulandari 2010; Auliya et al. 2019). However, Pisang Kates was found to have two types of stomata, i.e. tetracytic on adaxial and paracytic on abaxial which can be used as its distinguishing characters. Rudall et al. (2017) reported that both paracytic and tetracytic types can be found in Musaceae. In terms of stomata density and size, it was similar to other bananas in the ABB group such as Pisang Kluthuk Susu (Sumardi and Wulandari 2010), Kepok Ebung and Sobo Londo (Auliya et al. 2019). The stomata size is influenced by the genome group. Bananas with ABB genome have larger stomata size than AA and BB genome bananas (Auliya et al. 2019).

Studies related to pollen morphology in bananas are poorly reported. The pollen size of Pisang Kates is considered very large compared to other bananas from AA, AAA, BB, and even the same ABB genome groups (Sukkaewmanee 2019). It is found to have an aperture with monocolpate (or monosulcate) type while previous studies were reported to have a monocolporate type (Sukkaewmanee 2019) and inaperturate (Ghosh and Karmakar 2017). Pollen in monocot is more primitive than pollen in dicot, especially in their apertural forms. Simple apertural grains were frequently found such as monocolpate, porate, or even with ill–defined apertures (Perveen 2000). Further, pollen from monocot has a wide range of exine ornamentation patterns that can be used as distinguishing characters. Pisang Kates has a granulum ornamentation. Meanwhile, pollen ornamentation has been reported in *M. sapientum* as a psilate type (Ghosh and Karmakar 2017). Based on the morphological character and size of pollen, it is presumed that Pisang Kates belong to zoophily group (biotic pollination), whereby pollen is transferred by animals, particularly birds and bats, etc. The zoophyly is also reflected by the rough surface of the pollens (Stroo 2000). However, this assumption certainly needs further study.

The percentage of pollen viability of Pisang Kates is considered low (less than 30%). Low pollen viability was also recorded in other banana cultivars ABB group such as Pisang Kepok Ebung, Tlekung, Jambe, Raja Bandung and Raja Prentel (Damaiyani and Hapsari 2018). It may become a barrier factor in their natural reproductive biology and hybridization efforts. Meanwhile, diploid hybrids and landraces (AA) produced significantly more viable pollen than triploids and tetraploids, suggesting more successful crosses when using diploid accessions as male parents (Ssali et al. 2012). Thus, Pisang Kates is not suitable for a male parent (pollen donor), but still possible for a female parent. Further research is required to study the ability of female fertility (Krishnamoorthy and Kumar 2004). In addition, biotechnology approach is needed for further banana breeding efforts.

The mature fruit taste of Pisang Kates is sweet and acidic (apple-like), suitable as fresh dessert fruit than cooked. The total sugar is moderately sweet as indicated by the average TSS value using refractometer is 20.17 °Brix. This brix value is similar to Pisang Cavendish of 20.75 °Brix (Swara 2011), but lower than Pisang Telor of 25.20 °Brix (Miswarti et al. 2017) and Pisang Kepok Kuning of 29.88 °Brix (Fauziah et al. 2014). Furthermore, in comparison to other banana cultivars from East Java reported by Hapsari and Lestari (2016) showed that the nutrient content which comprised of carbohydrate, protein, fat, and total sugar of Pisang Kates are considered equal to dessert banana of Pisang Ambon Hijau, but lower than Pisang Raja Bandung and Pisang Kepok. Meanwhile, the vitamin C content is considered high almost equal to Pisang Kepok, but the Kalium content is the lowest (Fig. 7). In addition, the cream-yellow color of the mature pulp of Pisang Kates indicates the high content of carotenoids comprised of lutein, α-carotene, and β-carotene (Sunandar and Kurniasih 2019; Jodiawan et al. 2021). Further research to reveal the potential of Pisang Kates as a source of carotenoids should be conducted.

From the conservation perspective, cultivation of plant species by local community is part of the strategy. They build genuine conservation strategy of natural resources and environment for their sustainable living (Setyawan 2010). Hence, Pisang Kates should be promoted among collectors, hobbyists, and general community as a unique ornamental fruit plant in their home gardens, mixed farming systems and agroforests. Ex-situ conservation in botanic gardens is important as the last guard to prevent extinction through integrated conservation actions (Mounce et al. 2017). Nevertheless, on-farm cultivation takes part as a suitable conservation strategy for local banana cultivars so that they are not lost as shifted by commercial cultivars (MusaNet 2016; Hapsari et al. 2017).

Bananas in combination with tree crops are important components of home garden, mixed farming system, and agroforestry in order to rehabilitate and reforest landscape, also to meet the economic needs and food security of local communities (Hapsari et al. 2017; Ssebulime et al. 2017). Based on the anatomy and stomata characteristics in this study, Pisang Kates is potential as a pioneer plant for rehabilitation program due to its adaptability to drought and open habitats. When grown in perennial production systems, bananas also may serve as ground cover vegetation throughout the year, thus protecting the soil from rain and wind erosion (Rajarathnam and Ramteke 2011). In addition, banana plants were reported to store more carbon (0.98 tons/ha) than *Imperata* grassland (0.7 tons/ha) and cassava (0.5 tons/ha) (Hairiah 1997; Danarto and Hapsari 2015). Pisang Kates as ABB group is vigorous in agronomic performance thus potential contributed to high biomass and carbon stock (Danarto and Hapsari 2015).

Commonly, Pisang Kates is vegetatively propagated by suckers and corms. Due to the limited number of plant materials, in-vitro culture is recommended for large-scale propagation and conservation purposes. From plant conservation to development, plant tissue culture is a promising alternative for plant propagation as compared to conventional efforts (Tripathi et al. 2021). Management of agricultural practices remains a concern for long term survival and maintenance of the ex-situ collection of Pisang Kates although it is considered drought tolerant and BBTV resistant. Nonetheless, since it is susceptible to wilt disease, therefore, must be kept away from the infected source. Due to the threats, field ex-situ collections should be duplicated in more than one site (Engels and Visser 2003; Mounce et al. 2017) and an in-vitro genebank as a safety backup (Gueco et al. 2022). However, challenges for in-vitro conservation include the high cost, required specialized facilities and staff skills, also the possible appearance of somaclonal variation on stored materials (Gueco et al. 2022).

In conclusion, Pisang Kates is a valuable local banana genetic resource from Pasuruan, East Java, Indonesia with unique characteristics and high potential which needs to be conserved and utilized sustainably. Pisang Kates was characterized by its unique fruit shape that resembles a papaya fruit, fruit shape ovoid to globose weight of 40-150 g, appears solitary and randomly attached directly to peduncle. It was molecularly confirmed as ABB genotype by ITS sequencing. Due to the large fruit size, it is potential as a commercial one serving consumer size fruit. Based on leaf anatomy and stomata, it is adaptable to open habitats thus potential as a pioneer plant for rehabilitation programs. The fruit of Pisang Kates is moderately sweet and has high nutrient value approximately equal to dessert banana. It is potential to meet the economic needs and nutrient sufficiency of the local community. The pollen viability is low, not suitable for a male parent but still possible for a female parent for further breeding programs. On-farm cultivation as ornamental fruit plant in home garden, mixed farming system and agroforest by collectors, hobbyists, and the wider community is encouraged as part of a suitable conservation strategy. Meanwhile, for ex-situ conservation strategy the field collections should be duplicated in more than one site and ideally in an in vitro genebank as a safety backup to mitigate threats. The results of this study can be used as a basis for compiling a description of Pisang Kates to be registered as a local banana variety at the Ministry of Agriculture Republic of Indonesia in collaboration with the Pasuruan Government of East Java.

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