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Phenological Development of Rambai (*Baccaureamotleyana*(Müll.Arg.) Müll.Arg.) Flower and Fruit

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Abstract
Rambai (Baccaureamotleyana(Müll.Arg.) Müll.Arg.) is an underutilized fruit tree from Indonesia.The growth and developmental stages of the rambai fruit are not well characterized.This study aimed to investigate the flowering phenology and fruit development of rambai at the Mekarsari Fruit Garden, Bogor (Indonesia),fromJune 2019 to July 2020.Four trees were used in this study, and ten panicles from each tree were labeled as samples.The results of the study revealed that therambai fruit undergoes five stages of phenological development: flower initiation, flower differentiation, anthesis, fruit development, and fruit ripening, which occurred at 0, 14, 21, 91, and 117 days after panicle shoot appearance, respectively.The ripening stage required the longest duration (117 days after the appearance of the panicle shoot) and largest thermal units (2108.5°C days).The fruit is predicted to be physiologically ripe at 14 weeks after anthesis, and the peel and fruit turned yellowish-green and became softthen.After ripening, the fruit had the highest level of sweetness, with total soluble solids up to 12–18 °Brix and a total titratable acid contentof 3.5–5%.

Keywords: arillode, day degree, panicle shoot, thermal unit

Introduction

Indonesia hosts several tropical rainforests in the Indo–Malaya region and has diverse plant genetic resources, including woody plants, medicinal plants, and fruit trees.However, many fruit trees,including underutilized species such as Menteng (*Baccaurearacemosa*), rambai (*Baccaureamotleyana*), and gandaria (*Bouea macrophylla* Griffith), have not been widely cultivated (Gunawan et al., 2016).Rambai (*B.motleyana*) belongs to the family of *Phyllanthaceae* and is native to Sumatra, Kalimantan, and Java;itis widely cultivated in the Malay Peninsula, Thailand, and the Philippines (Subhadrabandhu 2001).In Kalimantan, rambai is one of the non-timber forest products (NTFPs) consumed as a table fruit (Subhadrabandhu, 2001).

Rambaigrows in primary and secondary lowlands, shrubs, and riparian forests and is cultivated in home gardens (Lim, 2012).The rambai fruit contains 64 kcal energy, 1.6 g carbohydrate, 0.4 g protein, 0.4 g fat, 1.0 g food fiber, 83.7 g water, and 0.2 g ash per 100 grams.The fruit also contains functional nutrients such as vitamins (B1, B2, and C) and minerals (Ca, P, Fe, Na, and K) (Siong et al., 1997). Moreover, rambai contains phenols, flavonoids, and antioxidants as secondary metabolites, including decanoic acid, 1-Decene, methyl salicylate, and stearyl alcohol-specific secondary metabolites (Mokhtar et al., 2014; Halim et al., 2019).In addition to the nutrient content oframbaiarillode, the fruit peel also contains phenolic compounds and antioxidantsin the amounts of 51–82.4 (µg/mL) and 43–75 (µg/mL), respectively (Ismail et al., 2012).

The best fruit quantity and qualityare obtained during harvest because of the high concentration of secondary metabolites at that stage.However, information on the growth and development stages of rambai fruit is not available except forthe harvesting period.Climate factors such as temperature and precipitation affect crop production.Different climates and seasonal patterns influence crop productivity (Schlenker et al., 2007).Phenology is a general concept used to study the relationship between plant development and climate factors.Phenological studies elucidate the interaction between plants and environmental conditions and their correlation with plant morphology (Awachare and Urpeti, 2020).Previously, tropical fruit phenology has been studied in rambutan, mangosteen, and duku (Lizawati et al., 2013).However, a phenological study of the rambai fruit has not yet been conducted.Therefore, this study aimed to investigate the phenology and fruit development of the rambaitree.

Material and Methods

This study was conducted from June 2019 to July 2020.The research was conducted at Mekarsari Fruit Garden, Cileungsi, Bogor (6°25'14.1", 106°59'08.4"; 70 m above sea level).Further analysis was conducted at the post-harvest laboratory, Department of Agronomy and Horticulture, and the soil laboratory, Department of Soil and Environmental Resources, Faculty of Agriculture, IPB University.

The soil where the rambai tree grew was acidic (pH 4.74) and had a low soil fertility status according to the technical guidelines for evaluating soil fertility by Puslittanah (1995).The soil in the research location contains organic C content of 1.69%, total N content of 0.16%, C/N ratio of 10.56, P content of 3.65 ppm, Ca content of 4.79 cmol/kg, Mg content of 0.58 cmol/kg, K content of 0.2 cmol/kg, Na content of 0.05 cmol/kg, cation-exchange capacity 18.2 cmol/kg, and alkaline saturation of 30.85%.

Observation of Flower and Fruit Phenology

Phenological observations began with the measurements of microclimate conditions conducted daily using an Encomotion data logger that records temperature, humidity, and rainfall.The results of the daily temperature measurements were used to calculate the heat units.The heat unit was calculated as the growing day degree (GDD)(Parthasaranthi et al., 2013).

Flowers and fruits on four trees were observed; ten panicles were obtained from each tree as samples.The panicles were selected from four cardinal directions.Measurements were performed regularly every 14 days, including panicle diameter, length, width, number of flowers, and fruits.In addition, the nutrient status of the leaves was determined every 30 days (from July 04, 2019, to February 04, 2020) to evaluate the nutrient dynamics in leaves at each phenological development phase.The flower phenology and development stages of the rambai fruit were divided into five stages as follows: flower primordia, marked by the emergence of panicle shoots on the branch (FP); panicle elongation, marked by an increase in flower panicles and flower buds (PE); anthesis, marked by 90% of the flower's full bloom (AN); fruit development until seed hardening (SH); and physiological maturity,indicated as the stage when the fruit reached the best physical and chemical quality (MF).

Observation of the Physical and Chemical Characteristics of Fruits

The physical characteristics of the fruits were observed, and the chemical characteristics were determined after separating the arils and seeds.The physical characteristics were fruit length, diameter, aril weight, seed weight, edible portion, and fruit softness level.The fruit softness level was measured using a penetrometer (model H-2529 from Paul N. Gardner, BYK Instruments Company) with 50 g and a retention time of 5 s at a depth of 10 mm.Measurements were made at the fruit's base, middle, and tip and recorded the average value (Chen et al., 2017).

The chemical characteristics were the total dissolved solids (TSS) and total titrated acid (TTA).TSS measurements were performed using a digital refractometer, which produces a value in Brix units.TTA was measured using the acid titration method using NaOH and phenolphthalein indicator (Chen et al., 2017).

Results and Discussions

Microclimatic Conditions of the Rambai Growing

The daily air temperature at the research location during the field research ranged from 18 to 35 °C, regarding the base temperature of the mango in Zagade and Pujari (2014); the lowest temperature was still above the baseline temperature (T_0) of plants that were in their flowering and fruit ripening stages (10 °C).The daily humidity was between 70% and 95%, and rainfall was 0 and 150 mm/day.Therefore, daily temperature measurements were used to calculate the heat unit, indicating that each flowering phenology phase and fruit development requires a different duration and heat unit (Table 1).

Flower Phenology and Fruit Development ofRambai

The flowering phase begins with the emergence of panicle buds and develops into the following stages: mature panicles, followed by anthesis, fruit development until seed hardening, and then fruit ripening until physiological maturity (Figure 1).There was a change in the nutrient status of the leaves; for example, the C/N ratio at the flowering and fruiting stages was higher than that after the fruit was ripe.Changes also occurred in C, N, P, K, and B nutrients (Table 2).Due toplant metabolic changes,leaf nutrient dynamics occur to provide assimilates and nutrientsdistributed to the flower or fruit parts.

Flower Primordia

The flower primordia phase is the initiation or beginning of the flowering period and is marked by the appearance of flower panicles on the tree branch,which develops into one, two, or three flower panicles (Figure 2).The climatic conditions in this phase were still the dry season, with rainfall of less than 100 mm, humidity of 70–80%, and temperatures ranging from 18 to 35 °C.However, the leaf C/N ratio in this phase was higher than that after fruiting, indicating that carbon translocation from leaves to flowers occurred (Table 2).Upreti et al. (2013) stated that the increased C/N ratio in leaves at the beginning of flowering induction resulted from the increased availability of carbohydrates to support flower formation.

Panicle Elongation

Panicle elongation and flower buds required seven days or a heat unit of 252.15 °C days after the appearance of panicle shoots.This was marked by an increase in panicle size, flower bud size, and flower number in each panicle (Table 3).The flower parts, such as the stalk, ovary, ovule, and petals, were visible (Figure 3).

Flower enlargement is caused by photosynthate translocation from the leaf tissue (source) to the flower organ (sink).This is corroborated by the fact that the C content in the leaves was lower during the flowering and fruit development stagesthanduringthe post-fruiting phase(Table 2).Moreover, the C content of leaves in the flowering and fruit development stages tended to be constant, indicating that the rate of photosynthesis and C formation was stable.Mastur (2015) explained that when entering the flowering phase, the leaves provide photosynthates for the sink parts of plants, such as the flowers and fruits.

Anthesis

The anthesis period of rambai flowers takes 21 days or a heat unit of 372.40 °C days after the emergence of panicle shoots.The flower parts were visible at this phase, starting from the petals, ovaries, ovules, stigma, and anther (Figure 4).In addition, the flowers also increased in size, and theloss of flowers occurred (Table 3).The C/N ratio of leaves and C, P, and K decreased during the flowering period.This was caused by the translocation of these nutrients from the leaves to the flowers.Similar conditions occurred in mangosteen; the nutrients in the leaves decreased at the trubus and generative stages, caused by the translocation of mineral and photosynthate nutrients from mature the leaves to other younger organs such as flowers and fruits (Liferdi and Poerwanto, 2011).However, there is an increase in N and B elements,which support photosynthesis in leaves and maximize the production of assimilates to flow into the flower.

Fruit Development

The formation and development of fruit begin after the flower has finished anthesis, and fruit set occurs after the fruit experiences seed hardening.This phase required the longest duration of 97 days and the largest heat unit of 1602.40 °C days after the appearance of panicle shoots.TheB content of the leaves at this phase was lower than that at the flowering and post-fruiting stages during the fruit development period.This is thought to occur because the B element in the leaves is used for flower and fruit enlargement.The B content in plants can transport assimilates from photosynthesis so that adequate boron can optimally support plant growth (Ai, 2012).The research results by Wirawan et al. (2016) showed that B contents of 0.5% to 0.16% cause the most optimal assimilate filling in the banana variant raja bulu, producing harder fruitswith longer shelf life.There was an increase in fruit length, diameter, number of fruits, and weight during this period.However, the maximum fruit size and number of fruits gained at 14 MSA decreased afterward (Figure 5).In addition, there was a change in the fruit's skin from green to yellowish-green and yellow (Figure 6).

At 10 WAA, the fruit was predicted to have completed seed development and had undergone seed hardening, as indicated by the cessation of seed weight gain (Figure 7b).After the seeds were fully developed, assimilateswere translocated to the seed coat/arillode,which caused the arillode weight to increase rapidly to 14 WAA.The decrease in fruit number occurred after the fruit was physiologically mature.During this phase, the rate of assimilate translocation to the fruit was thought to have decreased.Moreover, the fruit began to experience senescence, which reduced the turgor pressure on the petiole, making it easier for the fruit to fall from the panicle.

However, the reduction in arillode weight was caused by water loss in the fruit.Nurhayati (2004) reported that rambutan fruit (*Nephelium lappaceum*) had higher water evaporation at older maturity levels.The difference in water accumulation and water evaporation ratio caused this condition.This ratio was higher in green fruit than in red fruit.

Fruit Ripening

Thefruit peel color changed from green to yellow during the fruit ripening. In addition, the red color of the arillode also faded with fruit maturity (Figure 8).Similar occurrences have been reported for similar fruits, such as *B. sapida* and *B. ramiflora* (Gunawan et al., 2016; Pradhan et al., 2015).The change in peel color was thought to occur due to chlorophyll degradation, while the color change inarillode was thought to be caused by changes in secondary metabolites, such as anthocyanins and carotenes.According to Mokhtar et al. (2015), the phenolic content in ripe rambai fruit is lower than that in raw fruit; the phenolic content of raw, physiologically ripe (mature), and ripe fruit was 97.23 mg/100 g, 63.90 mg/100 g, and 79.57 mg/100 g, respectively.

Changes in fruits' physical and chemical qualities were also observed (Figures 9 and 10).In the early stages of development, the edible portion tended to be low because of arillode enlargement and immature fruits.The highest edible portion (45 %) was obtained at 14 WAA.Rambai fruit firmness decreased due to pectin degradationin the fruit peel during ripening.Pradhan et al. (2015) reported that a similar occurrence happened to *B. sapida*, with increased fruit firmness at the beginning of development,which later decreased at maturity.

Total dissolved solids (TSS) increased with an increase in fruit maturity.The highest TSS value occurred at 14 WAA (15.53 °Brix)but began to decline after 16 WAA.An increase in TSS content is usually associated with increased sugar content in fruits.Nurhayati (2004) explained that a maximum TSS value is achieved if the substrate in complex sugar compounds is still available in large quantities and invertase enzyme activity is still high.When the fruit was ripe, the amount of starch and complex sugar compounds decreased;hence, simple sugar content decreased(resulting in a decrease in TSS value).A study that identified 18 varieties of *B. sapida* explained that the TSS in ripe fruit arils ranged from 14 to 18%, with a total sugar content of 1.7–3.8% (Akter 2019).

The TSS content was inversely related to the organic acid content (total titratable acid/TTA).The decrease in TTA value was caused by decreased metabolic activity in the fruit to produce organic acids.The acid components in fruits are secondary metabolites or byproducts of the cell metabolic cycle, such as malic acid, oxalic acid, and citric acid produced from the Krebs cycle (Rahman et al. 2015).The highest ATT value was 9%;it occurred at 2 MSA fruits and then gradually decreased to 3–5.5% at 14 WAA.Mokhtar et al. (2015) determined that the total organic acids in rambai fruit (*B. motleyana*) at three different maturity levels (i.e., young, mature, and ripe) were 56.11, 50.96, and 45.56 mg/mL, respectively.The organic acid content of *B. motleyana* was dominated by citric acid, followed by tartaric acid, malic acid, and oxalic acid.

Conclusions

The flowering phenology and fruit development oframbai (*B. motleyana*) can be divided into five stages: flower initiation, panicle elongation, anthesis, fruit development to seed hardening, and physiological maturity; the required heat units in these stages are 0, 252.15, 372.40, 1602.40, and 2108.50 °C days (GDD), respectively, after the panicle shoots appeared.Therefore, thebest harvest time is predicted at 14 WAA in Bogor or 2108.50 °C days after panicle shoots appear physically and when the fruit's skin turnsyellowish-green.After ripening, the fruit has the highest level of sweetness with a total dissolved solid value of up to 15.53 °Brix and a total titratable acid content of 3.25%.

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Table 1. Observation of flowering phenology stages, duration, and heat units in Rambai

Phenological phase	Duration (days)*	Heat unit (^{o}Cdays)*
FB – PE	7	252.15
PE – AN	21	372.40
AN – SH	97	1602.40
SH – PM	117	2108.50

Note: *The calculation is carried out since the panicle shoots appear
Flower primordia (FP), panicleelongation (PE), anthesis(AN), Seedhardening(SH), physiologically mature (PM)

Table 2 Analysis of leaf nutrient status in Rambai during flower and fruit development.

Observation time (days)	0*	30	60	90	120	150	180	210
Phenological stages	Flower primordia - *anthesis*		*Seed hardening*			Physiologicall y mature	Post fruit	
C/N Ratio	37.73	36.54	35.76	42.19	34.14	30.60	31.34	33.10
C (%)	49.80	48.97	49.35	49.36	49.51	51.4	51.71	51.64
N (%)	1.32	1.34	1.38	1.17	1.45	1.68	1.65	1.56
P (%)	0.16	0.11	0.11	0.24	0.10	0.36	0.23	0.17
K (%)	1.59	1.14	0.74	0.84	0.82	0.71	0.86	0.57
B (ppm)	18.32	19.20	15.67	13.02	12.14	77.24	72.38	82.11

Note: *0 = First observation on 04 July 2019

Table 3 Observation of panicle and anthesis development in Rambai

Parameter*	*Panicle elongation*		*Anthesis*
	Seven days	14 days	21 days
Panicle length (cm)	3.18 ± 0.89	4.61 ± 0.71	13.46 ± 2.81
Panicle width (cm)	1.17 ± 0.76	2.59 ± 0.83	2.15 ± 0.55
Flower diameter (mm)	0.89 ± 0.18	1.52 ± 0.34	2.66 ± 0,87
Number of flowers	30.25 ± 3.36	45.00 ± 4.33	28.83 ± 5.50

Note : * Age calculations are carried out since the appearance of panicle shoots

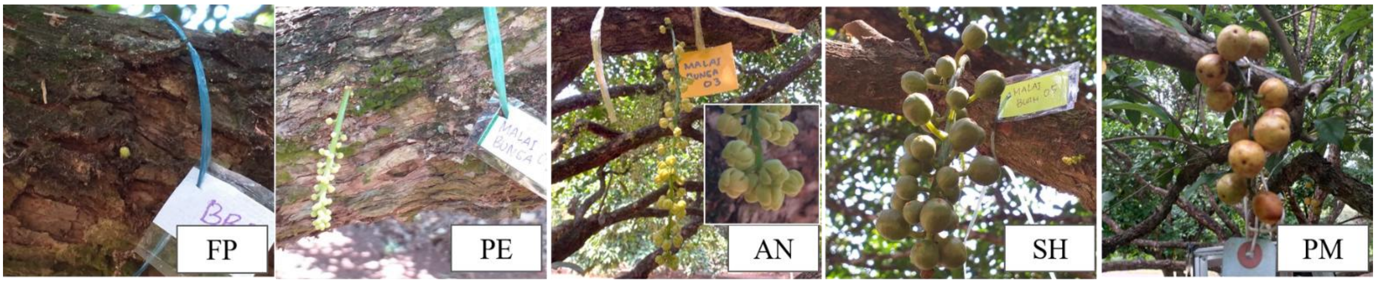

Figure 1. Phenological development stages of rambai flower: flower primordia (FB), panicle elongation (PE), anthesis (AN), Seed hardening (SH), Physiological maturity (PM)

Figure 2. Flower panicle shoots morphology: panicle shoots (a), panicle shoot development into one panicle (b), panicle shoot development into two panicles (c), and panicle shoot development into three panicles (d)

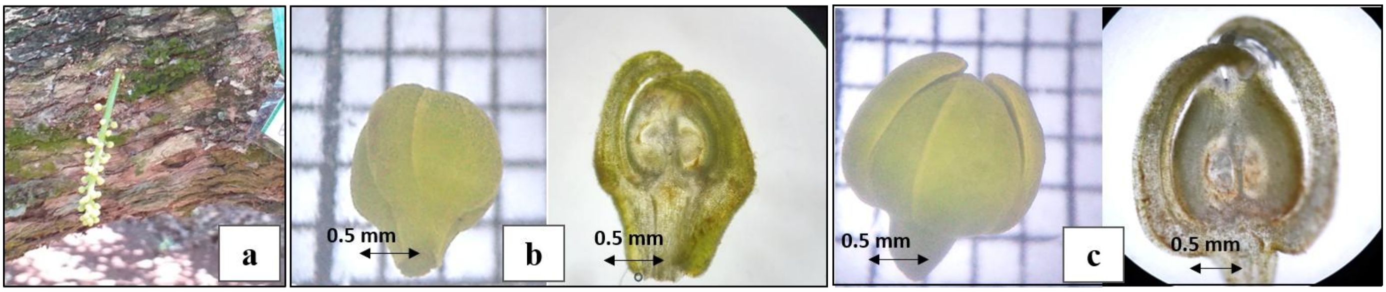

Figure 3. Flower buds (a), flower buds aged seven days (b), flower buds aged 14 days (c)

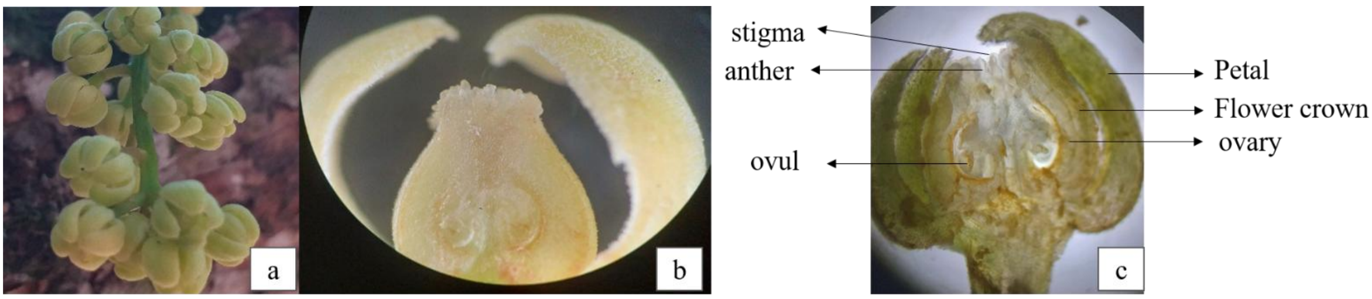

Figure 4. Rambai flower in anthesis phase (a), the cross-section of flower (b), flower parts (d)

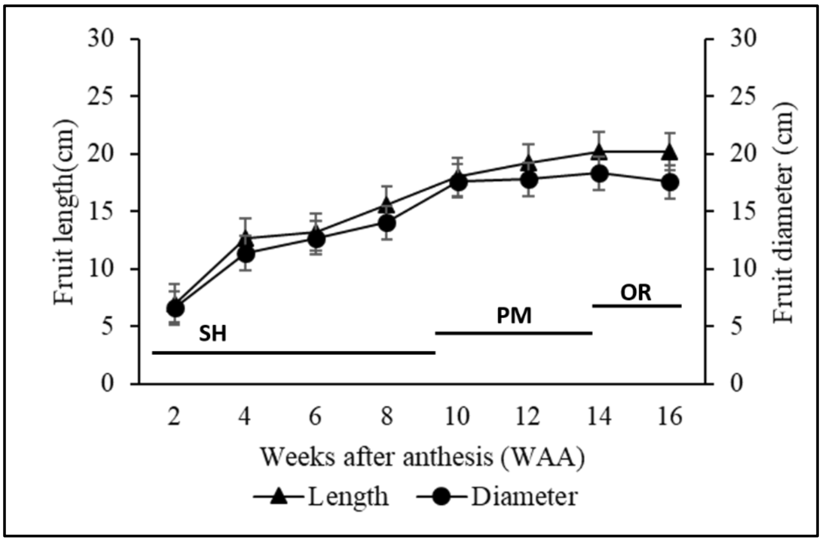

Figure 5. Rambai fruits length and width at various ages

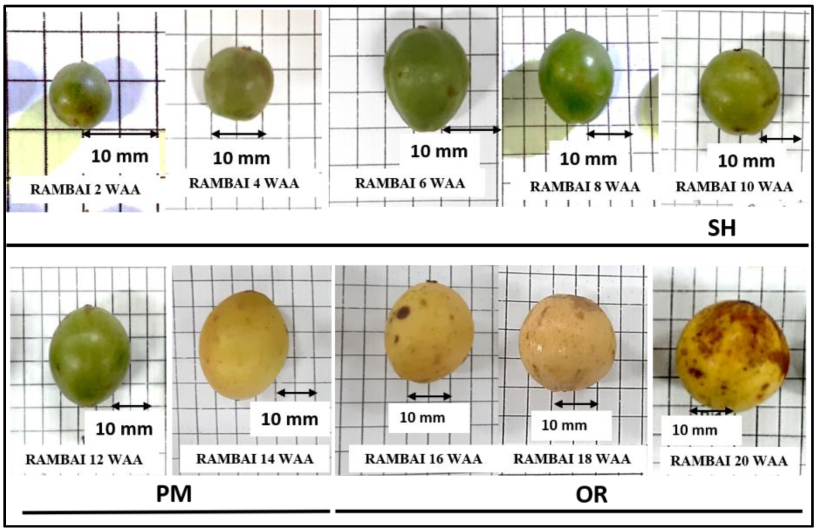

Figure 6. Rambai fruit morphology at various ages
Note: SH: Seed hardening, PM: Physiology ripe, OR: Overripe

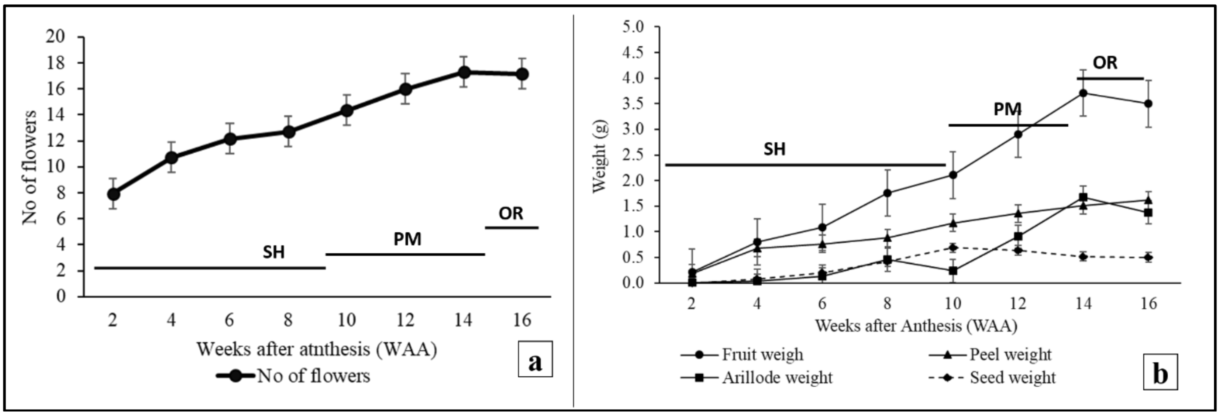

Figure 7. Number of fruit (a) and differences in fruit weight, seed, skin, and arils (b) during fruit development

Note: SH: Seed hardening, PM: Physiology ripe, OR: Overripe

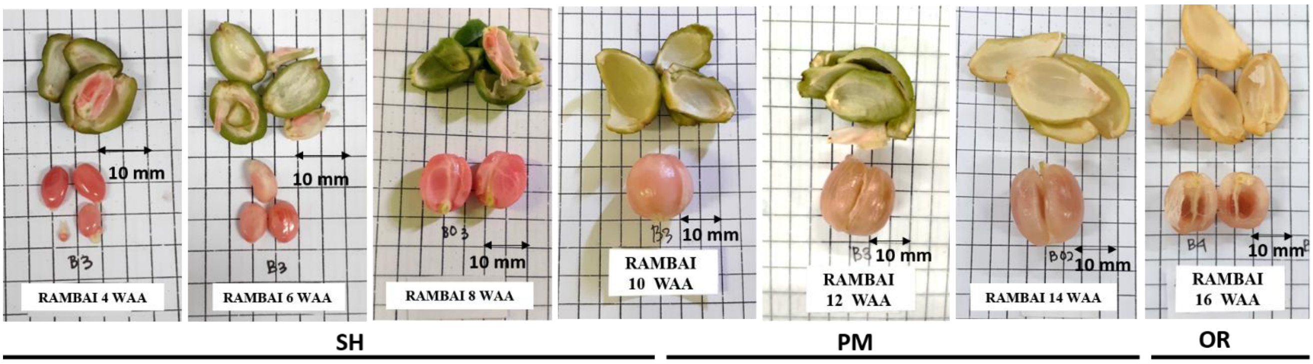

Figure 8. The appearance of rambaiarillodes and peel during the ripening period
Note: SH: Seed hardening, PM: Physiology ripe, OR: Overripe

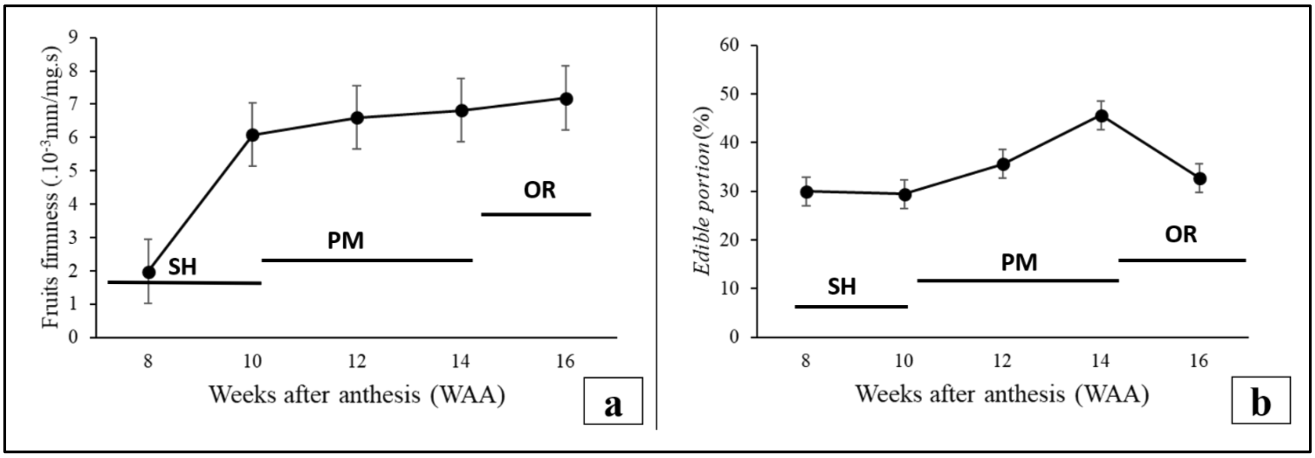

Figure 9. Physical characteristics of fruit: fruit firmness (a), edible portion (b)
Note: SH: Seed hardening, PM: Physiology ripe, OR: Overripe

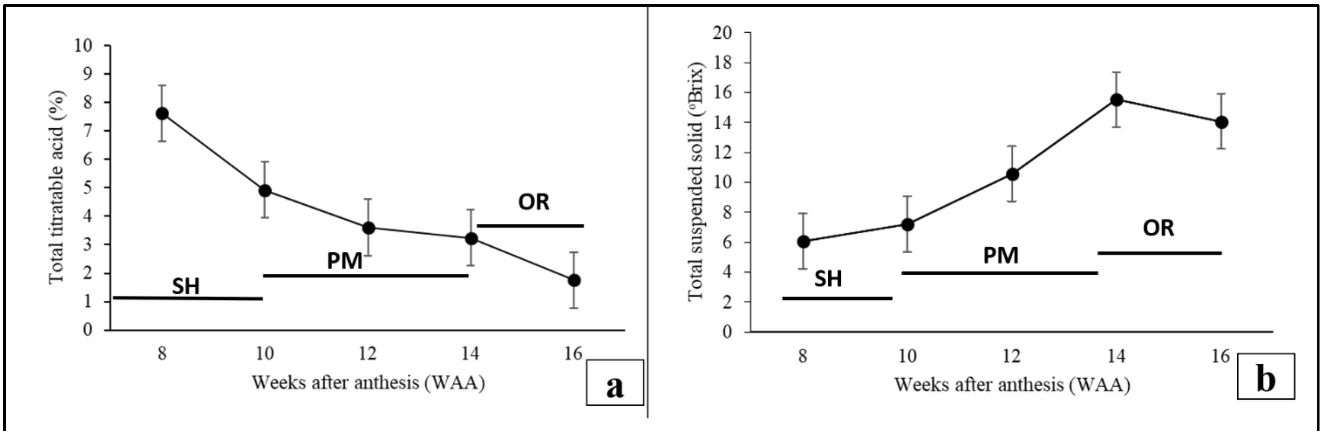

Figure 10. Chemical characteristics of fruit: Total titratable acid (a), Total suspended solid (b)
Note: SH: Seed hardening, PM: Physiology ripe, OR: Overripe