# Fruit Ripeness Effects on Characteristics, Germination and Desiccation Tolerance of *Syzygium myrtifolium* Walp. Seeds

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Received: 7 April 2021; Revised: 19 May 2021; Accepted: 28 May 2021; Published: 6 June 2021

## ABSTRACT

To plant *Syzygium myrtifolium* Walp. on harsh urban sites, planting materials of seed origin having better developed tap roots are preferred compared to those propagated from stem cuttings. However, fully ripe fruits at 8 weeks after fruit set are heavily lost to birds. In the current study, the unpalatable semi-ripe fruits at 7 weeks were found suitable for propagation. The seeds from the semi-ripe fruits showed mean germination rate of 93% and developed normal seedlings within 1 week, although continued growth with increases in size and fresh weight (FW) in the fruits and seeds was significant towards completion of fruit ripening. Thus, harvesting of fruits within the short period between these two fruit developmental phases is critical in the seed procurement process of this plant species. At the semi-ripe stage, pericarp exhibiting mixture of red and black colour can be a practical guide to indicate the suitable time for harvesting the fruits. Nonetheless, subsequent desiccation trial aimed to determine the storability of the seeds at postharvest found that the seeds obtained from both the semi-ripe and fully ripe fruits had drastic loss of germinability at seed moisture content of approximately 0.3 g H<sub>2</sub>O/g FW. Storage of the seeds above this relatively high moisture level for future planting was detrimental because the seeds age rapidly when they remain metabolically active with high respiration rates. Microbial infestation will also be an added problem during seed storage. Therefore, the seeds need to be sown fresh.

Keywords: Predation; seed size; seedling; storage; recalcitrant.

# **INTRODUCTION**

*Syzygium myrtifolium* Walp. which belongs to the family Myrtaceae is commonly known as kelat paya, ubah laut, Chinese red wood, wild cinnamon or Australian brush cherry. With its attractive reddish young foliage, it gains popularity as a hedge plant to beautify tropical urban areas and recreational parks. As it has also shown rapid establishment and high tolerance to the harsh urban environment, this plant species has become one of the primary choices for urban planting nowadays. A practical means for securing large quantities of planting materials is vital for the success of such planting plans.

Propagation by seeds produces robust seedlings with balanced architecture and well-developed tap roots and is preferred over planting materials that originate from stem cuttings for urban sites which can vary greatly in soil and microclimatic conditions (Ahmad Nazarudin et al., 2014). However, a high percentage of the fully ripe fruits of *S. myrtifolium* on the tree is lost to avian predation. The juicy spherical fruits of less than 8 mm in diameter are easily swallowed whole by birds (Haron et al., 1995).

In this study, the germinability of the seeds obtained from the unpalatable semi-ripe fruits was compared with that from fully ripe fruits to assess whether early harvesting of the fruits is a viable alternative for procurement of *S. myrtifolium* seeds. Upon successful fruit set, the red colour unripe fruits turn semi-ripe when the pericarp changes colour to be in a mixture of red and black after about 7 weeks. Then, the semi-ripe fruits become fully ripe, being black in colour, within the following 7 to 10 days. The

fully ripe fruits are shed from the tree in the next few days. The short transition period from semi- to full ripeness in *S. myrtifolium* fruits provides clues that the fruits may be physiologically matured at the semi-ripe stage and the seeds within will be germinable (Villa et al., 2019). Selected morphological and physiological parameters of the fruits and seeds were measured in the current study as basic information related to seed germinability.

When there is an abundance of fruits, storage procedures that retain seed viability enable easy management and continuous supply of planting materials. The desiccation tolerance of the seeds determines the optimum storage options for preserving the planting stock. Desiccation sensitive recalcitrant seeds generally die at relatively high moisture content (MC) of 0.2 to 0.3 g  $H_2O/g$  fresh weight (FW) (Boucher et al., 2010; Chen et al., 2011; Hill et al., 2012; Xia et al., 2012; Delahaie et al., 2013; Joët et al., 2013; Sahu et al., 2017; Barbedo, 2018). These seeds cannot be stored at MC that retains their viability with no impairment as they remain metabolically active and are killed within short periods by heat produced with the relatively high respiration rates. Seeds having such high MC are also prone to microbial infestations. Seeds that are sensitive to drying must, therefore, be sown fresh and are difficult to handle.

Within the highly diverse *Syzygium* Gaertn. represented by possibly 1,200 species that are distributed in the African and Asian tropics, seeds of some species identified as timbers, edible rare fruits and spices, for example *S. aromaticum*, *S. aqueum*, *S. malaccense*, *S. cumini*, *S. samarangense*, *S. jambos* and *S. polyanthum*, have been known to lose germinability rapidly with postharvest desiccation as typically demonstrated by the recalcitrant seeds (Coronel, 1992; van Lingen, 1992; Haron et al., 1995; Sardjono, 1999; Verheij and Snijders, 1999; Nair et al., 2020; WCSP, 2021). However, fruits and seeds of many other species in this genus are unknown or insufficiently studied, including those of *S. myrtifolium*. Hence, the current study also aimed to determine the germinability of *S. myrtifolium* seeds following ambient air drying as an easy and practical seed desiccation technique to determine the storability of the seeds.

#### **MATERIALS AND METHODS**

## Fruit collection

Semi-ripe fruits which had pericarp in a mixture of red and black colour at 7 weeks after fruit set, and black coloured fully ripe fruits at 8 weeks were collected from a single tree in a recreational park in Shah Alam, Selangor, Malaysia (3°5'51.9"N, 101°26'41.1"E) in June 2019. The area has tropical weather with mean daily temperature of 27°C and annual precipitation of over 2,500 mm.

Branch terminals bearing panicles with fruits were cut using a fruit cutter attached to an aluminium rod, which was extendable to 4 m. For each fruit ripeness, 490(+50) fruits were collected for this study. The fruits were juicy drupes.

After fruit collection, the panicles were trimmed for storage in labelled plastic bags. The plastic bags were tied loosely and brought to Laboratory of Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Puncak Alam, which is 15 km from the site.

#### **Experimental laboratory**

The laboratory was 24-hour air-conditioned with mean temperature of  $25 \pm 2^{\circ}C$  and relative humidity of  $55 \pm 5\%$ .

## Measurement of fruit and seed

Upon receipt of the fruits, fruits were detached from the panicles and FW of single fruits was determined using an analytical balance. For each fruit ripeness, 50 fruits were randomly selected for this purpose. Then, the longitudinal and transversal diameters of each fruit were measured using a Vernier caliper. The longitudinal diameter of the fruit was measured from peduncle scar to the base of the fruit. Its transversal

diameter was measured perpendicular to the longitudinal diameter. The fruits were placed in numbered cells in plastic boxes accordingly for determination of the seed attributes in the next step.

After recording the fruit measurements, the seeds were extracted from the fruits. All fruits were single seeded. Each seed was cleaned separately and thoroughly thrice with distilled water, pat dried with paper towel and placed in its respective numbered cell again. The seeds were air dried for 1 h in the laboratory to remove the remaining surface water. Then, the seed FW, longitudinal and transversal diameters were measured. The longitudinal diameter of the seed was measured from the hilum, which is separated from the fruit peduncle by the pericarp, to the base of the seed.

#### **Determination of seed MC**

For each fruit ripeness, another 40 randomly selected seeds in four replicates, each with 10 seeds, were used for determination of MC by the gravimetric method according to ISTA (2019). In this procedure, the seeds in moisture dishes were dried at 103°C in a convection oven for 17 h. Seed MC in g H<sub>2</sub>O/g FW was calculated as below.

Seed MC (g H<sub>2</sub>0/g FW) = 
$$\frac{(W_1 - W_2)}{(W_1 - W_3)}$$

where,

 $W_1$  = weight of seeds in moisture dishes before oven drying (g)  $W_2$  = weight of seeds in moisture dishes after oven drying (g)  $W_3$  = weight of moisture dishes (g)

## **Determination of seed germinability**

Seed germinability as affected by fruit ripeness was tested on paper towel moistened with distilled water in 90 mm glass Petri dishes (ISTA, 2019). For each fruit ripeness, 40 seeds in four replicates, each with 10 seeds, were randomly selected for the germination test. The seeds were germinated on a germination rack in the air-conditioned laboratory away from direct sunlight. The light availability for the germinating seeds was approximately 10  $\mu$ mol/m<sup>2</sup>/s during daytime. Distilled water was added to the paper towel as needed to always keep it moist.

The seed was noted to have germinated with visible radicle protrusion. Seed germination count was recorded daily. Ungerminated seeds in this study were found rotting within 2 weeks and the rotting seeds were discarded immediately. Germination rate (%) and mean time to germination (MTG) were calculated using the formulae below.

Germination rate (%) = 
$$\left(\frac{\sum_{i=1}^{k} n_i}{N}\right) x 100$$
  
 $MTG = \frac{\sum_{i=1}^{k} n_i t_i}{\sum_{i=1}^{k} n_i}$ 

where,

 $n_i$  = number of germinated seeds on the i<sup>th</sup> day (not the accumulated number)  $t_i$  = number of days from commencement of germination test N = total number of seeds

k = last day of seed germination

Germinated seeds were withdrawn after daily germination count and kept on moistened paper towel in separate labelled Petri dishes. They were observed for initial growth of roots and shoots to determine their fitness as planting materials. Fruit Ripeness Effects on Characteristics, Germination and Desiccation Tolerance of Syzygium myrtifolium Walp. Seeds

## **Desiccation trial**

The study on the desiccation tolerance of the seeds was based on a factorial experiment arranged in a completely randomized design. For each fruit ripeness, 360 seeds were placed as monolayers in plastic boxes and air dried on the germination rack in the laboratory at  $55 \pm 5\%$  relative humidity, as described above, for up to 5 days. Daily, 30 randomly picked seeds, in three replicates, were determined for MC as described above. At the same time, another 30 seeds, also in three replicates, were drawn randomly for germination test on moistened paper towel in glass Petri dishes as above. Seed germination was also carried out similarly in the laboratory. Daily germination count was recorded for a period of 2 weeks. Germination rate and MTG were also calculated. The germinated seeds were also observed for initial growth of their roots and shoots after radicle protrusion.

#### Statistical analysis

Statistical analysis was performed using SPSS version 25. The FW and diameter measurements of fruits and seeds, seed MC, germination rate and MTG as affected by fruit ripeness were subjected to descriptive analysis and independent sample T-test at 5% probability. In the seed desiccation trial, analysis of variance (ANOVA) was carried out. The effects of fruit ripeness and desiccation period (seed MC) on seed germination rate and MTG were determined. Percentage of germination was transformed to arc-sine value before ANOVA. Treatment means were compared using least significant difference (LSD) test at 5% probability.

#### **RESULTS AND DISCUSSION**

#### Morphometrics and physiological attributes of fruits and seeds

By size, the semi-ripe fruits with mean longitudinal and transversal diameters of 4.18 mm and 5.08 mm, respectively, were 1 to 1.5 mm smaller than the fully ripe fruits (Figure 1a). Physiologically, the semi-ripe fruits differed markedly from the fully ripe fruits in FW where there was 1.7-fold increase in fruit FW to be  $0.1667 \pm 0.0385$  g at full ripeness (Figure 1b). Box and whisker plots (n=50) showed that there was a relatively wide range of fruit size (longitudinal and transversal diameters) and FW within each fruit developmental stage as studied. The coefficients of variation (CV) for diameter measurements were 8 to 10% whilst those for FW were 21 to 23%.



Figure 1. Quartiles (box plot) and range (whiskers) for (a) longitudinal and transversal diameters, and (b) fresh weight (FW) of fruits according to ripeness; blank and grey bars represented attributes with semiand fully ripe fruits, respectively; o indicated outlier.

On account of the thin pericarp, the seeds within the fruits were simply 1 to 2 mm smaller than the fruits in size (Figure 2a). Despite also spherically shaped, the seed had a less spherical side, making this side slightly flattened. The transversal diameter of seed as shown in Figure 2a was the average value for this flattened side as well as its quarter turn. In terms of variation, the seeds obtained from the semi-ripe fruits had relatively higher CV of 11 to 12% for size, whilst the seeds obtained from the fully ripe fruits had more consistent sizes with CV of 8 to 11%.

Despite having thin pericarp, the FW of seeds was only 41% of fruit FW for both the studied fruit ripeness. This was attributed to the juicy pericarp. Hence, the FW of seeds in the fully ripe fruit was also 1.7-fold higher than that in the semi-ripe fruits (Figure 2b). In conjunction with the less dispersed size, FW of seeds in the fully ripe fruits was more consistent with lower CV (22%) compared to that in the semi-ripe fruits (CV was 29%).



Figure 2. Quartiles (box plot) and range (whiskers) for (a) longitudinal and transversal diameters, and (b) fresh weight (FW) of seeds, and mean ± SE for (c) seed moisture content (MC), (d) germination rate, and (e) mean time to germination (MTG) according to fruit ripeness; blank and grey bars represented attributes with semi- and fully ripe fruits, respectively; o indicated outlier.

In contrast to growth and assimilate accumulation, MC of seeds decreased significantly (P < 0.05) from 0.54 to 0.47 g H<sub>2</sub>O/g FW with the completion of fruit ripening (Figure 2c). Nonetheless, the seeds extracted from semi-ripe fruits did not differ significantly (P > 0.05) from those in the fully developed ripe fruits in germinability. The seeds had mean germination of 93 and 100% with semi-ripe and fully ripe fruits, respectively (Figure 2d). The greater variation for the size and FW of fruits and seeds could have resulted

in a less consistent seed germination rate at the semi-ripe stage. In terms of germination time, the seeds germinated fast within 1 week where those extracted from the semi-ripe fruits had MTG of  $4.22 \pm 0.94$  days, but germination was even faster for the seeds obtained from the fully ripe fruits (MTG was  $2.92 \pm 0.34$  days) (Figure 2e). All the germinated seeds demonstrated normal growth of roots and shoots by 1 week after radicle protrusion.

# **Desiccation tolerance of seeds**

In the desiccation trial, seed MC was affected significantly (P < 0.05) by interaction between fruit ripeness and desiccation period (LSD was 0.0125) (Table 1). For the seeds extracted from the semi-ripe fruits, the MC was reduced linearly (y = 0.5516 - 0.0653x,  $R^2 = 0.98$ ) and significantly (P < 0.05) to 0.2 g H<sub>2</sub>O/g FW from the initial MC of 0.54 g H<sub>2</sub>O/g FW after air drying for 5 days in the laboratory (Figure 3). The rather similar MC reduction rate (y = 0.4794 - 0.0611x,  $R^2 = 0.99$ ) was experienced by the seeds obtained from the fully ripe fruits but MC was reduced to lower level of 0.16 g H<sub>2</sub>O/g FW from the lower initial MC of 0.47 g H<sub>2</sub>O/g FW.

Table 1. F-value and	probability of	of ANOVA	for moisture	content (MC	) and g	erminability	of desiccated
	1 2				, 0	J	

seeds							
Variable	Seed MC	Germination	Mean time to germination				
		rate	(MTG)				
Fruit ripeness	615.022***	120.164***	289.209***				
Desiccation period	1527.836***	74.637***	23.387***				
Fruit ripeness x Desiccation period	3.164*	25.497***	57.248***				

\*, \*\*\* indicated probability < 0.05, < 0.001, respectively.

A significant combined effect of fruit ripeness and seed MC on germinability of *S. myrtifolium* seeds was evidenced (Table 1). Seeds from the fully ripe fruits were more sensitive to desiccation with germination rate declining drastically to 27% at seed MC of 0.3 g H<sub>2</sub>O/g FW after air drying for 3 days in the laboratory (Figure 4a). For the seeds extracted from the semi-ripe fruits, germination rate was still above 90% at slightly higher MC of 0.31 g H<sub>2</sub>O/g FW after air drying for 4 days (Figure 4a). Nonetheless, the seeds from the semi-ripe fruits showed a significant drop in germination rate (33%) after another day of desiccation, which reduced the seed MC to 0.2 g H<sub>2</sub>O/g FW. The lowest safe MC for the seeds was estimated to be 0.3 g H<sub>2</sub>O/g FW, taking into account the greater variation of seed size and FW at semi-ripe stage. At moisture levels that retained viability, the desiccated seeds had always been capable of developing normal seedlings after radicle protrusion.

Regardless of fruit ripeness, MTG for the desiccated seeds was relatively longer in comparison to that for the non-desiccated control (Figure 4b), although germination of the desiccated seeds had also been noted within a short period of seven days.



Figure 3. Air drying effect on moisture content (MC) of seeds extracted from semi-ripe (x) and fully ripe (•) fruits; whiskers indicated 95% confidence intervals



Figure 4. (a) Germination rate and (b) Mean time to germination (MTG) as affected by moisture content (MC) for seeds extracted from semi-ripe (x) and fully ripe fruits (•); whiskers indicated 95% confidence intervals

### DISCUSSION

Both the fruits and the seeds of *S. myrtifolium* showed continuous mass accumulation implied by significant quantitative increases in size and FW between the semi-ripe phase and full ripeness. The amount of water in the seeds, on the other hand, was presumed to remain similar or only reduced slightly in the time towards full ripeness. The lower seed MC of 0.47 g H<sub>2</sub>O/g FW with fully ripe fruits, in comparison to that with semi-ripe fruits (0.54 g H<sub>2</sub>O/g FW), must be attributed to the mass gain over the ripening period. In other word, the seeds did not experience maturation drying. Despite their small sizes of less than 6 mm, seeds of *S. myrtifolium* contrasted with some other small sized orthodox seeds that demonstrate maximum reserve or biomass accumulation prior to moisture loss during the final phase of seed development, through which a quiescent state is achieved before the orthodox seeds are shed from the trees (Hay and Probert, 1995; Bareke, 2018).

In terms of germinability, *S. myrtifolium* seeds from the semi-ripe fruits needed a very short period of warm and moist incubation before radicle protrusion could take place. This was inferred by the slightly longer mean to germination time (MTG) as demonstrated by the seeds obtained from the semi-ripe fruits

during the germination test, but the seed germination rates from both the semi-ripe and fully ripe fruits were high and comparable, being above 90%. Germination time as an indicator of seed vigour and quality implied that the seeds from the fully developed fruits with more consistent morphological and physiological characteristics would be more favourable for planting but those from the semi-ripe fruits could turn germinable with warm and moist incubation during the germination procedure. Such advancement to be germinable is rather similar to the after-ripening process to overcome morphological dormancy in seed, through which cell development events continue and ultimately allow protrusion of the radicle (Pollock and Roos, 1972). The capability of the seeds to sprout despite detachment from tree prior to full ripeness of fruits has also been described for *Physalis peruviana* (Solanaceae) (Diniz and Novembre, 2019), *Poincianella pluviosa* (Leguminosae) (Silva et al., 2015), some epiphytic plants from the family Bromeliaceae (Correa and Zotz, 2014) and *Phalaenopsis amabilis* hybrids (Orchidaceae) (Schwallier et al., 2011). In the case of *S. myrtifolium*, the germinability of the seeds in the unpalatable semi-ripe fruits, followed by normal seedling development, could be an adaptation that is vital for the regeneration success of this plant species, as the ripe fruits are highly in demand by animals.

With postharvest desiccation, the seeds of semi-ripe and fully ripe fruits died at MCs typical for recalcitrant seeds (Roberts, 1973; Mayrinck et al., 2016; León-Lobos and Ellis, 2018). Despite the small seed size of less than 6 mm and 1,000 seed weight of 39.1 g and 69.1 g for the seeds extracted from the semi-ripe and fully ripe fruits, respectively, the seeds were spherically shaped with lower surface area to volume ratio, a characteristic associated with recalcitrance (Chin et al., 1984; Hong and Ellis, 1996; Daws et al., 2005; Lan et al., 2014; Gentallan Jr et al., 2018). In contrast, most small seeds with flattened appearance remain viable with postharvest drying to low MC of 0.05 to 0.07 g  $H_2O/g$  FW. These seeds have metabolism and growth held in an arrested state at low moisture levels. Upon rehydration, the seeds have been found to resume normal growth and development. In other word, seeds that tolerate drying to such low moisture levels are favourable as they can be kept hermatically for long periods for future sowing (Roberts, 1973; Ellis and Roberts, 1980). Other small seeds, for example those from the Arecaceae family, tolerate only a certain degree of drying. They generally exhibit intermediate seed behaviour. Among the documented species in the genus of Syzygium, there are considerable differences in the shape and size of their seeds (WCSP, 2021). The larger sized seeds have diameters in the range of 15 to 35 mm whilst others are small with diameter comparable to that of S. myrtifolium (Shareef et al., 2013; Sujanapal et al., 2013; Mohamed et al., 2014; Karuppusamy and Ravichandran, 2016; Snow et al., 2016). In connection with desiccation tolerance, the seeds of only a limited number of species in this genus have been investigated. Notwithstanding the seed size, the studied *Syzygium* were found to lose seed viability rapidly. While size can be a not so significant indicator for tolerance to desiccation in seeds (Pritchard et al., 2004), the lack of maturation drying in seed during fruit ripening and the relatively high initial seed MC with S. myrtifolium could be the more reliable clues for predicting the viability loss with postharvest drying of its seeds, making storage of the seeds impossible, regardless of fruit ripeness as studied.

The rapid radicle protrusion demonstrated by *S. myrtifolium* seeds obtained from both semi-ripe and fully ripe fruits, as shown by MTG, also indicated the possible need for the seeds to have roots to source for soil water at the soonest possible for survival. Fast germination could be a profound strategy to avoid desiccation induced seed death in nature (Parsons, 2012). The seeds must be capable of exploiting the short period of favourable conditions for germination and establishment. The rapid germination of *S. myrtifolium* seeds could also be attributed to the thin seed coat that imbibes water readily as necessary to avoid loss of viability. With substantial loss of germinability below relatively high MC of 0.3 g H<sub>2</sub>O/g FW, the seeds of this plant species were best sown fresh and not stored with conventional seed storage methods.

#### **CONCLUSIONS**

Semi-ripe fruits of *S. myrtifolium* at 7 weeks after fruit set, as indicated by pericarp in a mixture of red and black colour, contained seeds with mean germination rate of 93%, and germination was achieved within 1 week. The fruits and seeds at semi-ripeness had continued growth as correlated with the size and weight

increases while completing the fruit ripening process in the following week, which enabled 100% seed germination rate as the fully developed fruits attained more consistent morphological and physiological attributes. Thus, early harvesting of the fruits at the semi-ripe stage can be planned to reduce seed losses to predation with negligible differences for propagation. At postharvest, the seeds were sensitive to desiccation and must be planted with minimum delay.

# **AUTHORS CONTRIBUTION**

FYT conceived and designed the work. FYT and NFA performed the analysis. FYT wrote the paper, and checked and approved the submission.

# **CONFLICT OF INTEREST**

The authors declare that they have no competing interests.

# FUNDING

The authors thank Universiti Teknologi MARA for funding the work [500-BPD(BKK.14/5/4)(188427)].

# ACKNOWLEDGEMENTS

The authors are grateful to the Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA for access to facility and other resources during the course of the study.

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