

## Full Length Research Paper

# A study of the anatomical features of testa and other associated structures in selected banana ecotypes

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Low and erratic germination in wild banana seed is caused by dormancy due to physical, anatomical or physiological reasons. Imbibition activates germination process and the rate of water uptake during imbibition is influenced by seed molecular composition and internal and external morphological structures. The present study aimed at examining the effect of seed anatomy and seed moisture content on water uptake by wild banana seeds. Matured fresh and dry, intact or scarified seeds of three wild banana ecotypes (*MUSA ACUMINATA* Colla) viz. Krau White, Serdang Red and Serdang Yellow were imbibed for up to 96 h. Percentage increase in seed mass was recorded at different intervals. Internal morphological structures of fresh and dry seeds were viewed using scanning electron microscopy (SEM). Seed mass increased rapidly within first hour and the rate of increase was always higher in dry seed than the fresh seed in all the ecotypes. The SEM observation revealed that the rapid increase of seed mass is likely due to the shrinking of the operculum and the surrounding tissues in the hilum area which resulted in the formation and widening of a water channel in dry seed. This study indicates that operculum does not impede water uptake in mature banana seed during imbibition, suggesting that seed dormancy in these three ecotypes is not due to physical dormancy but may be physiological in nature.

**Key words:** *Musa acuminata*, imbibition, seed coat, scarification, physical dormancy.

## INTRODUCTION

Bananas (*Musa* spp., Musaceae) are an important food crop in the subtropics and tropics and are routinely cultivated for ornamental purposes (Chin, 1996; Wong et al., 2001). Edible bananas (triploid) are believed to have originated from hybridization between two wild banana (diploid) species, *Musa acuminata* Colla and *Musa balbisiana* Colla (Hakkinen and Hong, 2007). *M. acuminata* has larger contribution to the genome of the triploid cultivated species. The diploid wild banana produces fertile and viable seeds. On the other hand, the triploid cultivated bananas are parthenocarpic, which makes them sterile and unable to produce viable seeds. The widespread threat to *Musa* cultivation by fungal

diseases in recent years has heightened interest in *Musa* breeding programme. The improvement of banana through breeding work is hindered due to low seed set as well as by slow and non-uniform germination (Graven et al., 1996).

Malaysia is considered as the centre of genetic diversity of banana (Horry and Jay, 1988; Daniells et al., 2001) and the country has an enormous genetic pool to be exploited and utilized as several subspecies of *M. acuminata* Colla (that is, *truncata*, *microcarpa* and *malaccensis*) have been identified in this country (Wong et al., 2001).

Banana seeds have been classified as orthodox (Roberts, 1973; Ellis et al., 1985; INIBAP, 1997), and mature seeds are reputed to be highly dormant (Purseglove, 1975; Chin, 1996). Hard seed coat or physical dormancy is associated with a barrier to water

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**Table 1.** Seed moisture content (%) of freshly harvested and air dried seeds of three wild banana ecotypes.

Seed type	Ecotype		
	Krau White	Serdang Red	Serdang Yellow
Freshly harvested	58 ± 3	38 ± 5	47 ± 5
Air dried	24 ± 3	15 ± 2	21 ± 3

± indicates standard error of the means; seed moisture content (%): g H<sub>2</sub>O/100 g dry seed mass.

imbibition in some seeds which subsequently leads to a reduced germination or complete germination failure (Baskin et al., 2000). Banana seed consists mainly of endosperm surrounded by multilayer outer integument (testa) and a thin inner integument (tegmen). The embryo is very small and is situated under the operculum or seed lid (Graven et al., 1996). The cells surrounding the embryo (for example, endosperm and testa) are reported to be responsible for blockage of water uptake during germination in some seeds (Finch-Savage and Leubner-Metzger, 2006). The primary site of water entry into seeds is different among species (Hu et al., 2009). Often a specialized water-restriction zone within the water-impermeable layer is responsible for regulating water entry into the seed. Graven et al. (1996) implicated the operculum as a block to water entry into the embryo of wild banana seed. In *Vigna* spp., the hilum is reported to be the initial site of water entry into seeds (Hu et al., 2009). Lens is associated with the point of water entry in soybean (Kikuchi et al., 2006) and sesbania seeds (Hu et al., 2009).

Banana seeds show non-uniform and slow germination mainly because of dormancy. Baskin and Baskin (2004) opined that dormancy may be related to physical, morphological and physiological factors or combination of these factors. Baskin and Baskin (1998) reported that some members in the family Musaceae have seeds with physical dormancy. This is probably due to the hard and rigid nature of seed coat found in banana seed (Bhat, 1994; Baskin et al., 2000). Dormancy is a dynamic state and is influenced by the response of seed to the change in environment. Banana seeds are not inherently dormant as they germinate as soon as they are extracted from the ripe fruits (Vuylsteke et al., 1990) but become dormant after drying due to secondary dormancy (Chin, 1996). This dormancy could be associated with impeded water uptake due to physical barrier imposed by seed coat and other associated structures (Graven et al., 1996). Thus, the amount of water taken-up by seed during soaking could be used as an indication of the degree of physical dormancy (Baskin and Baskin, 2004). However, reports on the water uptake pattern of banana seed in relation to the testa or other associated structures surrounding the hilum region is highly lacking. Therefore, the objectives of this study were to determine the water uptake pattern of fresh and dry seed with or without scarification and to

relate this with the anatomical and morphological features of testa and other associated structures in selected banana ecotypes.

## MATERIALS AND METHODS

Three ecotypes of wild banana *M. acuminata* subspecies *malaccensis* viz., Krau White, Serdang Red and Serdang Yellow were selected for this study. These ecotypes are generally abundant in the low lying rainforest areas (<100 m in altitude, 3.2°N) of Malaysia. Serdang Red and Serdang Yellow ecotypes were collected from University Putra Malaysia (UPM) area while the Krau White ecotype was collected from Temerloh, Pahang, about 200 km in the east from UPM. Five matured bunches were harvested for each ecotype (when 2 to 3 fruits of a bunch started to turn yellow) during March 2010. The seeds were extracted from fruits within 7 days after bunches were brought to the laboratory and a portion of the extracted seeds were air dried at room temperature (23°C) for approximately 7 days to get dry seeds. Immediately after extraction or air drying the moisture content of the seeds of three wild banana ecotypes were determined on fresh weight basis by drying 25 seeds per replicate in an oven at 105°C for 24 h. The seed moisture contents of the fresh and dry seeds are shown in Table 1.

## Measurements

### Fruit and seed morphological characters

Fruit length and width, number of seeds/fruit and 100 seed weight were recorded based on ten randomly collected fruits per replicate. Seed length, embryo length, testa thickness and hilum gap width were measured from 20 seeds randomly selected per replicate from each ecotype with a stereomicroscope (Leica EZ4D, Leica AG, Heerbrugg, Switzerland) connected to digital imaging systems. Measurements of the seed parameters were made at the base of operculum closest to the embryo.

### Scanning electron microscopy of seeds

Fresh and dry seeds were hand-cut transversely using scalpel to expose the embryo and surrounding tissues. Due to hardness of the testa, the dry seeds were imbibed between moist filter papers for 3 h prior to cutting to prevent seed breakage. The seed samples were not fixed in FAA and thus, no dehydration and drying were performed to avoid collapsing of tissues surrounding the operculum area. The fresh and dry seeds were mounted directly onto aluminum stubs and coated with a thin layer of gold in the Polaron Sputter Coater. The prepared specimens were then viewed at 15 kV to observe detail cell structures of the testa, operculum and surrounding cells using a Scanning Electron Microscope (model

**Table 2.** Fruit and seed morphological characteristics of three wild banana (*Musa acuminata*) ecotypes.

Morphological character	Ecotype		
	Krau White	Serdang Red	Serdang Yellow
Fruit length (cm)	9.28 ± 0.27	9.21 ± 0.26	7.68 ± 0.43
Fruit width (mm)	7.62 ± 0.21	7.08 ± 0.13	5.36 ± 0.11
No. seed fruit <sup>-1</sup>	55.20 ± 5.65	107.3 ± 3.80	28.6 ± 2.99
100-seed weight (g)	4.64 ± 0.02	3.68 ± 0.02	4.01 ± 0.05
Seed length (mm)	6.42 ± 0.17	2.16 ± 0.03	3.05 ± 0.19
Embryo length (mm)	1.10 ± 0.04	0.72 ± 0.16	0.92 ± 0.03
Testa thickness (mm)	0.24 ± 0.01	0.36 ± 0.02	0.18 ± 0.01
Hilum gap width (mm)	0.88 ± 0.05	0.70 ± 0.02	0.78 ± 0.05

± indicates standard error of the means.

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#### Water uptake/imbibition

The rate of water uptake (imbibition) was monitored for freshly harvested and air dried seeds of each ecotype. Fresh and air dried seeds were imbibed either by intact or scarified (testa cut near the embryo tip) in three replications of 25 seeds per replication for each type. Seeds were imbibed in distilled water at ambient temperature (23°C) on moistened double layered filter papers in 90 mm Petri dishes. The seeds were removed from Petri dishes at 1, 2, 4, 6, 8, 10, 24, 48, 72 and 96 h, they were blotted dry and weighed individually following the procedure by Baskin et al. (2006). Percentage increase in seed mass was determined on a dry weight basis using the following formula:

$$\text{Seed mass increase (\%)} = [(W_i - W_d) / W_d] \times 100, \quad (1)$$

Where,  $W_i$  and  $W_d$  are the mass of imbibed and dry seeds, respectively.

#### Data analysis

Seed mass data were subjected to analysis of variance (ANOVA) using a three factor completely randomized design. The data were arcsine transformed prior to the analysis. Differences in mean values were tested using Tukey's test ( $P < 0.05$ ).

## RESULTS

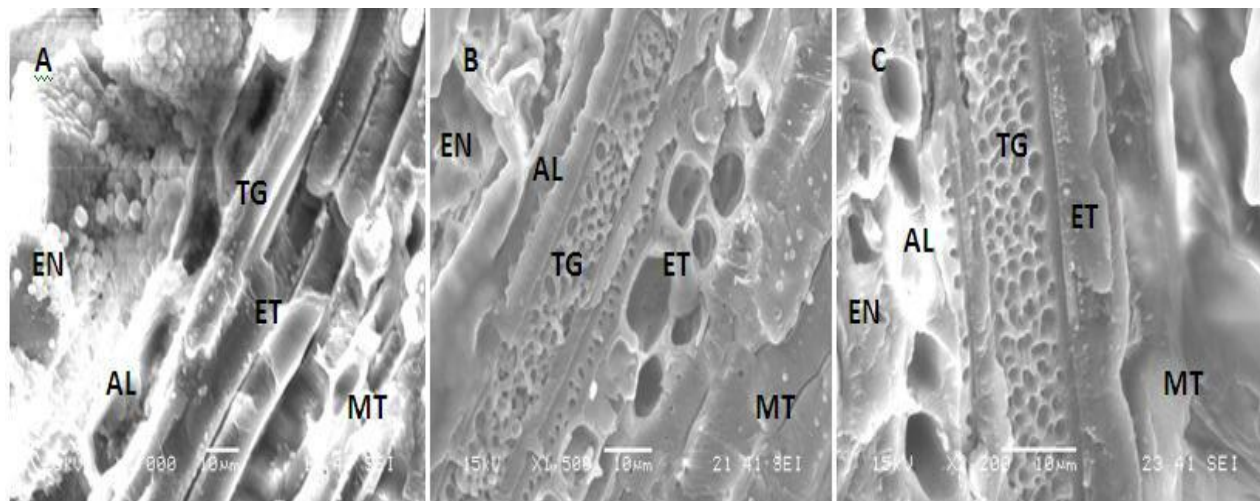
### Fruit and seed morphological characteristics

Fruit length and width of Krau White were  $9.28 \pm 0.27$  and  $7.62 \pm 0.21$  cm, respectively while those for Serdang Yellow were  $7.68 \pm 0.43$  and  $5.36 \pm 0.11$  cm, respectively. The fruit length and width of Serdang Red were very similar to those of Krau White (Table 2). Thus, it appeared that Serdang Yellow ecotype produced smaller fruits than those of Krau White and Serdang Red. Serdang Red produced the highest number of seeds

( $107.3 \pm 3.80$  seeds/fruit) while Serdang Yellow produced the lowest number of seeds ( $28.6 \pm 2.99$  seeds/fruit). The seed of Krau White fruit is half of that of Serdang Red, but is twice of that of the Serdang Yellow fruit (Table 2). On the other hand, the Krau White ecotype produced the biggest seed ( $4.64 \pm 0.02$  g/100 seed), while the smallest seeds were found in Serdang Red ( $3.68 \pm 0.02$  g/100 seed). Krau White had the highest seed length ( $6.42 \pm 0.17$  mm) and embryo lengths ( $1.10 \pm 0.04$  mm), while those were shortest in Serdang Red ( $2.16 \pm 0.03$  mm and  $0.72 \pm 0.16$  mm, respectively). The testa thickness differed among the ecotypes having the thickest ( $0.36 \pm 0.02$ ) in Serdang Red and the thinnest ( $0.18 \pm 0.01$ ) in Serdang Yellow. On the other hand, the width of hilum gap was the highest in Krau White ( $0.88 \pm 0.05$  mm) and lowest ( $0.70 \pm 0.02$  mm) in Serdang Red ecotype (Table 2).

### Seed coat anatomy

The testa thickness varied among the ecotypes with the highest values ( $0.36 \pm 0.02$  mm) in Serdang Red and lowest ( $0.18 \pm 0.01$  mm) in Serdang Yellow (Table 2). The scanned electron microscopy (SEM) seed coat of different ecotypes revealed that the tegmen was composed of a layer of cells with spongy-like structure in Krau White and Serdang Yellow ecotypes but as a single compressed cell layer in Serdang Red ecotype (Figure 1A, B and C). The mesotesta and endotesta were easily distinguishable with slight differences in cell structures among the ecotypes. The endotesta was consisted of 3 to 4 distinct cell layers in Krau White ecotype, while two layers of sponge-like structures were found in Serdang Red. In contrast, the layers appeared to be collapsing and tightly compressed in Serdang Yellow ecotype. Several distinct mesotesta layers were observed in Krau



**Figure 1.** SEM photomicrographs showing transverse sections of seed coat anatomy of Krau White (A), Serdang Red (B) and Serdang Yellow (C) ecotypes. AL indicates alurone layer, EN indicates endosperm, ET indicates endotesta, MT indicates mesotesta and TG indicates tegmen.

White ecotype, while the mesotesta layers in Serdang Red and Serdang Yellow ecotypes consisted of sclerotic cells with tightly compressed pits (Figure 1A, B and C).

#### Micromorphology of the operculum and surrounding cells

The SEM photomicrographs showed that water channels along the operculum and testa exist in both fresh and air-dry seeds (Figure 2). The width of the water channels appeared to be narrower in fresh seeds (38 to 58% seed moisture, Figure 2 A, C and E) compared with the air-dried seeds (15 to 24% seed moisture, Figure 2 B, D and F). Drying of seeds to <25% moisture content resulted in shrinking of the operculum tissues and the surrounding cells resulting in widening the width of the water channel between testa and operculum (Figure 2B, D and F).

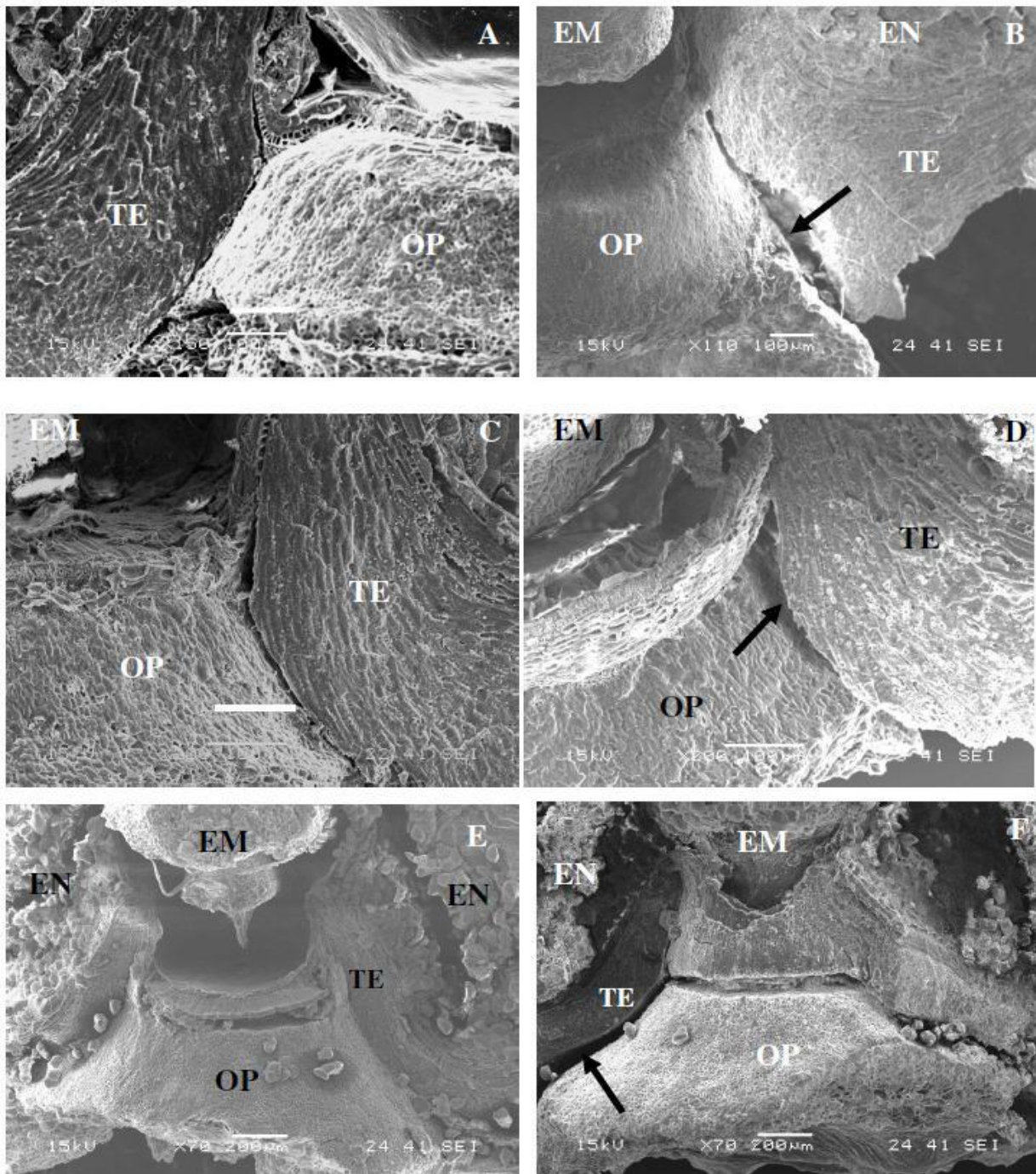
#### Imbibition

Ecotype, seed moisture content, scarification and imbibition interval and their interactions had significant effect on increase of seed mass during imbibition although, interaction of seed moisture content and scarification, seed moisture content and imbibition interval and, seed moisture content, scarification and imbibitions interval did not show any significant effect (Table 3). The analysis of variance indicated that the ecotype has a strong influence in the increase of seed masses during the imbibition period. The non-significant effect of combinations of seed moisture content and scarification or imbibitions interval suggest that scarifying the seed of high moisture has little effect on water uptake during imbibition.

Percent increase in seed mass was higher in both intact and scarified dry seeds than fresh seeds in all three wild banana ecotypes (Figure 3). The dry seeds of Serdang Yellow ecotype showed the most rapid water uptake within less than 10 h with the increase in mass of >100% in scarified (cut seeds) and approximately 82% for control (unscarified) seeds. The fresh seeds, both intact and scarified, showed <40% increase in seed mass throughout the imbibition period. In Krau White and Serdang Red ecotypes, the increase in percent seed mass was <60% for both intact and scarified dry seeds throughout the imbibition period. The Serdang Red ecotypes had the lowest percent increase in seed masses (approximately 10%) throughout the imbibition period for both intact and scarified fresh seeds (Figure 3).

#### DISCUSSION

Natural openings located on the seed coat primarily serve as an avenue of water entry into the seed. The structures responsible for water uptake in some seeds include the lens, micropyle, hilum or extrahilar region (Taylor, 2004; Kikuchi et al., 2006; Koizumi et al., 2008; Hu et al., 2009). In banana seed, the testa is highly silicified (Graven et al., 1996), thus, water uptake is most likely through the hilum area where the operculum is embedded. In this study, water uptake by fresh seeds was lower than the dry seed. The lower water uptake is probably due to partial blockage of water entry by operculum and the adjacent cells surrounding the hilum. When the seed is dried, the operculum and the adjacent cells surrounding the hilum tend to shrink as was evident in the SEM observation. The width of the water channel was narrower in the fresh seeds than the dry seeds. Drying



**Figure 2.** SEM photomicrographs showing transverse sections of seed components (EM; embryo, EN; endosperm, OP; operculum and TE; testa) of wild banana ecotypes. A and B are fresh and dry seeds, respectively, of Serdang Red ecotype. C and D are fresh and dry seeds, respectively, of Serdang Yellow ecotype. E and F are fresh and dry seeds, respectively, of Krau White ecotype. Arrows indicate the water channel formation between TE and OP. Scale bar = 100 µm for A, B, C and D and 200µm for E and F.

may have resulted in the formation and widening of water channel along the operculum and the testa, thus, allowing greater amount of water entry into the seed.

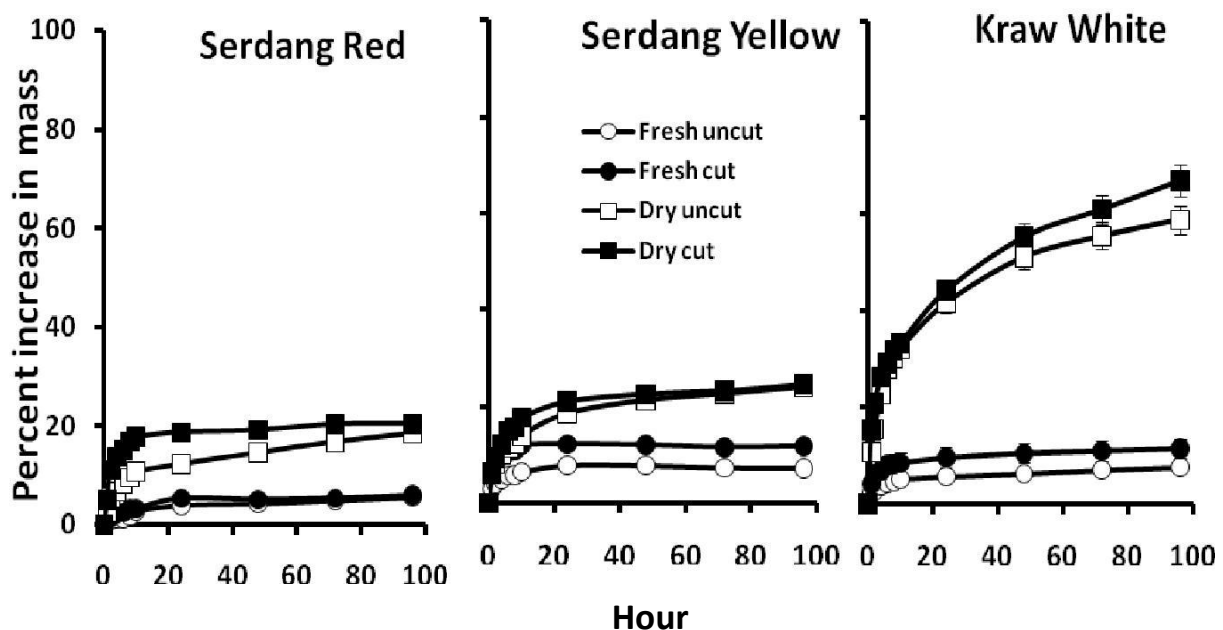
The increase in seed mass was significantly higher in scarified seeds compared with non-scarified (intact)

seeds suggesting that scarifying the seed coat will further facilitate water entry into the seed. It was found that when the air dried seed was scarified, a very rapid water uptake was observed especially in Serdang Yellow ecotype. This is probably due to damage to the thin testa

**Table 3.** Mean squares from the analysis of variance of ecotypes, scarification, seed moisture content and imbibition intervals on increase in seed mass.

Source	df	Increase in seed mass
Ecotype (E)	2	0.0035866***
Seed moisture content (SMC)	1	0.0094861***
Scarification (S)	1	0.0043513***
Imbibition interval (I)	9	0.0003606 ***
E x SMC	2	0.0004971***
E x S	2	0.0032021***
E x I	18	0.0000802***
SMC x S	1	0.0000032 <sup>ns</sup>
SMC x I	9	0.00005519***
S x I	9	0.0000109 <sup>ns</sup>
E x SMC x S	2	0.0000425***
E x S x I	18	0.0000316***
SMC x S x I	9	0.0000136 <sup>ns</sup>
E x SMC x S x I	36	0.0000144***
Error	240	

\*\*\* Significant at  $\alpha < 0.01$  and non significant denoted as ns at  $\alpha = 0.05$ .



**Figure 3.** Increase of seed mass of cut and uncut fresh and air dried seeds of three wild banana ecotypes during imbibition period.

layer caused by cutting which facilitated a rapid increase in water uptake in this particular ecotype. Water uptake by mature banana seed is very rapid within the first 8 h and tends to equilibrate after 10 h with no or little increase in seed mass. Similar pattern of increase in mass was observed in both scarified and intact seeds for all ecotypes. The rapid water uptake in *Phaseolus vulgaris* and *Vigna angularis* was found to be about 10 h

(Kikuchi et al., 2006), but it was up to 17 h in *Moringa oleifera* (Moravec et al., 2008).

The increase in moisture content of intact seeds indicates that mature banana seed imbibes water naturally through the hilum on testa. The water uptake was higher in dry seeds than the fresh seeds because of differences in width of water channel. Thus, the width of water channels between the operculum and testa appears

to play a significant role by regulating the rate of water uptake into the seed. The width of hilum gap where the operculum is embedded appears not to play a major role in regulating water entry into the seed. For instance, the width of the gap in Krau White ecotype (0.88 mm) was wider than the Serdang Yellow ecotype (0.78 mm), but the water uptake was much slower in this ecotype (Figure 2).

The present study shows that mature banana seed are able to imbibe water regardless of initial seed moisture content. The rate of water uptake varies between ecotypes due to the difference in width of water channels. The presence of water channel between testa and operculum appears to play a significant role in water entry into the seed. Seed scarification by cutting the testa seems to contribute to better water uptake. In some seeds, the brachysclereids, osteosclereids and macrosclereids strongly impede water entry into the seed (Li et al., 1999). But those cell layers are not formed in banana seed, thus, the hard mesotesta and endotesta layers are not likely the barriers to water entry into the seed. Physical dormancy is caused due to water impermeability to seed in the chalazal region. For example, SEM observation of *Malva parviflora* showed a wide fissure in the centre of chalazal region for seeds subjected to fluctuating temperatures that contributed to water entry, but control seeds had a small slit-like cleft impermeable to water (Michael et al., 2006). In the present study, dry seeds showed rapid water uptake and thus, chalazal region might have posed no barrier to water entry in these wild banana *Musa* seeds.

A significant interaction was detected between seed moisture content and imbibitions interval. However, the difference in seed moisture content prior to imbibition appears not to be the major reason for the variations in the rate of water uptake among the ecotypes. For instance, the dry seed of Serdang Red ecotype had 15% seed moisture but the increase in percent seed masses never exceeded 60%. On the contrary, the air-dried seed of the Serdang Yellow ecotype had 21% seed moisture but the increase in percent seed mass exceeded 100%. The difference in the rate of water uptake among the ecotypes is most likely due to presence of macromolecular components in the testa. A high amount of lignin has been quantified in *Musa* spp and the high concentrations in the endotesta have been implicated as a barrier to water entry into the seed (Graven et al., 1996).

This study reveals that the seed tissues of three wild banana ecotypes offer no barriers to water uptake and the seeds can readily imbibe water. The water uptake of dry seed is much higher than in fresh seed. The SEM observation proved that dry seeds had wider water channel that contributed to rapid and more water uptake compared with fresh seed. The present study indicates that seed dormancy present in wild banana ecotypes is not related to seed coat imposed barrier and therefore, other forms of dormancy may possibly exist.

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