

Research Article

Seeds morphology, anatomy, and quality of *Musa balbisiana* and *Musa acuminata* after storageMertya Anugrah ^{1,2}, Abdul Qadir ^{3,*}, Eny Widajati ³, and Sukartini ⁴

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ABSTRACT

The fluctuations in seed food reserves can trigger a cascade of physiological and biochemical events that ultimately manifest as discernible changes in seed morphology and anatomy. The purpose of this research was to evaluate seeds morphology, anatomy, and quality of Musa balbisiana "Klutuk NTT" and Musa acuminata "Sumatrana" accessions and their storability. Morphological evaluation included seed size and the weight of 100 grains. Anatomical evaluations were carried out for seed structure. The seed quality of the Klutuk NTT banana accessions was observed after being stored for 5, 25, and 41 months, while the Sumatrana banana accessions were observed after being stored for 5, 23, and 41 months. The Klutuk NTT seeds are sized 4 mm to 5.8 mm, while Sumatrana seeds are sized 4 mm to 5 mm. The Klutuk NTT and Sumatrana seeds had a seed coat, endosperm, embryo, micropyle, inner and outer integuments, chalazal, and operculum. The embryonic structure of both Klutuk NTT and Sumatrana seeds exhibited an inverted T-shape. After storage, the two accessions showed significant differences in moisture content, germination rate, vigor index, and seedling growth performance. The Klutuk NTT accessions demonstrated a significant increase in moisture content, germination rate, vigor index, and seedling emergence uniformity, suggesting improved seed performance after storage. In contrast, the Sumatrana accessions showed a significant decline in all measured parameters, except for moisture content. These results suggest that the Klutuk NTT accession may possess a physiological seed dormancy mechanism.

Keywords: Banana; germination; true seed; viability; vigor

INTRODUCTION

Bananas are one of the leading fruit commodities in tropical and subtropical regions. The availability of 'mother plants' used as the source of explants is one of the most important limitations for developing in vitro culture in new banana cultivars. Ardhani et al. (2024) stated that conventional propagation can only produce a small number of seeds and a high chance of transmitting pathogens from the parent tree. To increase production in large quantities of uniform and healthy seedlings, the induction of multiplication has been carried out on *Musa acuminata* cv. Mas Kirana (Annisa et al., 2021).

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Häkkinen & Hong (2007) stated that the cultivated bananas known today are mostly the result of hybridization between two wild bananas, namely *Musa balbisiana*, which has the BB genome, and *Musa acuminata*, which has the AA genome. The combination of the two genomes produces several groups of diploid, triploid, and tetraploid bananas.

Some superior characters are often found in wild bananas. *Musa balbisiana* is known to be resistant to Banana Xanthomonas Wilt disease (Goodman et al., 2021). The advantages of the properties possessed by wild bananas can be used for various genetic improvements for breeding activities of high-yielding varieties of bananas (Chang et al., 2019; Hastuti et al., 2019; Vu et al., 2023). According to Suryani & Owbel (2019), exploration activities are one of the efforts to obtain superior germplasm characters. Conservation of plant genetic resources, including the wild relatives of crops, plays an important and well-recognized role in addressing some of the key challenges faced by humanity and the planet, including ending hunger and biodiversity loss (Kallow et al., 2021). The identification and characterization of several genotypes will help determine the distinguishing characteristics between these genotypes.

Anatomical characterization of seeds from different types of wild banana accessions is the first step for conservation, breeding, and development of seed viability testing. Banana seeds can contain intact embryos or no embryos at all. The absence of embryos results in a low percentage of seeds growing (below 33%). MusaNet (2016) stated that there needs to be more in-depth research related to technology and knowledge related to seed biology, seed conservation, and test methods. Germination testing using the full light intensity method was performed by Kallow et al. (2020) and Vu et al. (2023) to study germination in banana seeds. Based on the nature of the seeds during storage, banana seeds are classified as orthodox seeds (Kurniajati et al., 2022). Orthodox seeds are capable of being stored at low moisture content and temperature.

The preservation of banana seeds is crucial for germplasm conservation, breeding programs, and understanding the genetic diversity of this economically important crop (Espitia-Camacho et al., 2021). Although bananas are typically propagated through vegetative means, storing seeds presents an alternative strategy to conserve genetic resources, especially for wild species and unique cultivars exhibiting valuable characteristics, and it is essential to consider the inherent challenges associated with banana seed storage, including dormancy, recalcitrance, and viability (Gebeyehu, 2020).

The longevity and viability of seeds during storage are intricately linked to the preservation of their food reserves, encompassing carbohydrates, proteins, and lipids, which serve as the primary energy source for germination and seedling establishment (Gebeyehu, 2020). Fluctuations in these reserves, whether due to enzymatic degradation, oxidation, or microbial activity, can trigger a cascade of physiological and biochemical events that ultimately manifest as discernible changes in seed morphology and anatomy (Alemayehu et al., 2023). For instance, the degradation of storage proteins can lead to a reduction in seed size and weight, while the oxidation of lipids can result in the formation of toxic compounds that disrupt cellular membranes and impair embryo development (López-Fernández et al., 2018). The purpose of this study was to evaluate seeds morphology, anatomy, and quality of *Musa balbisiana* “Klutuk NTT” and *Musa acuminata* “Sumatrana” accessions and their storability.

MATERIALS AND METHODS

The research was carried out from December 2020 to July 2021 at the Biotechnology and Genetics Laboratory and the greenhouse of the Sumani Agricultural Technology Research and Technology Study, the Research Center for Tropical Fruit Plants (Balitbu Tropika), West Sumatra, Indonesia. The seeds were obtained from exploration in the wild population of a particular region. *Musa balbisiana* seeds were obtained from East Nusa Tenggara (Nusa Tenggara Timur province), and *Musa acuminata* seeds were obtained from Sumatra.

Seed morphology

Morphological characterization of seeds using materials for observation, namely *Musa balbisiana* accession Klutuk NTT and *Musa acuminata* accession Sumatrana. The seeds had been stored for 4 months in -4 °C freezer. The material was 10 seed grains per accession, with 5 replications. The characterization of seed morphology was carried out by measuring seeds and calculating the weight of 100 grains of seeds. The seed weight was evaluated from 8 replications. The color of the Klutuk accession seed was black, and Sumatrana was greyish brown.

Seed anatomy

A microscope connected to digital devices with 40x magnification, scalpels, and tweezers were used. The material was 10 seed grains per accession, with 5 replications. The seeds were cut longitudinally using scalpels and observed using a Meiji Techno EMT Series (EMT-1-P-MA503) stereo microscope with 15x magnification. The seed diameter was measured with a vernier caliper on the largest part.

Seed quality after storage

Accession of Klutuk NTT seeds had been stored for 5, 25, and 41 months, while the Sumatrana seeds had been stored for 5, 23, and 41 months. Klutuk NTT and Sumatrana seeds were stored using large plastic clips measuring 35 cm by 45 cm, which were stored in the refrigerator (freezer) at -4 °C.

Observation of seed quality included moisture content, germination percentage, vigor index, and uniformity of seedling emergence. Seed moisture content was measured by using the direct method of oven with high temperature (130-133 °C) for 2 hours.

The seeds were germinated in a germination box using a planting medium of charcoal husk and sand (ratio 2:1). The humidity of the planting medium was 90%. A 50 seeds were planted in each storage period with 5 repetitions. Seeds were planted with a spacing of 1 cm x 1 cm and a depth of ± 0.5 cm. Germination was carried out in a screen house with daily temperatures ranging in the morning between 22-24 °C, at a day 30-32 °C and at night 19-21 °C, and light intensity ranging from 3,600-9,100 lux. Media humidity was maintained by covering the germination box using clear plastic. Measurements of the planting medium were carried out every day using a digital thermohygrometer type SDL598844587.

Germination was determined based on the number of seeds that germinated and formed normal sprouts. The normal banana seedling was considered to have primary and secondary roots, hypocotyls, cotyledons, epicotyls, and plumules that grow normally and healthily. Normal sprouts should also be about twice the length of the seeds. The germination rate of seeds is observed based on the formula based on ISTA (2018).

The seed vigor index was calculated from the percentage of normal sprouts on the first count of the germination, the vigor index follows Sadjad (1999). Uniformity of seedling emergence is calculated based on Sadjad (1999). Data were analyzed using t-tests at a level of 5% using Minitab 17.

RESULTS AND DISCUSSION

Seed morphology

Seed diameter of NTT klutuk accession was 5.8 ± 0.89 mm, and Sumatrana was 6.2 ± 1.02 mm. Seed weight 100 index of NTT klutuk was 6.78 ± 1.29 g and Sumatrana was 2.17 ± 0.46 g (Table 1). *Musa acuminata* seeds had different testa thicknesses on each ecotype. *Musa acuminata* Colla var. *bantamensis* Nasution has a seed diameter of 4.9-5.5 cm (Sulistyaningsih, 2016). *Musa acuminata* var *flava* (Ridl.) has a seed diameter of 2.19 ± 0.15 mm (Trimanto et al., 2022). Seed morphology is presented in Figure 1.

Table 1. Diameter and 100-seed weight of banana seeds of Klutuk NTT and Sumatrana accessions.

Variable	Klutuk NTT	Sumatrana
Diameter (mm)	5.8±0.89	6.2±1.02
Weight of 100 seeds (g)	6.78±1.29	2.17±0.46

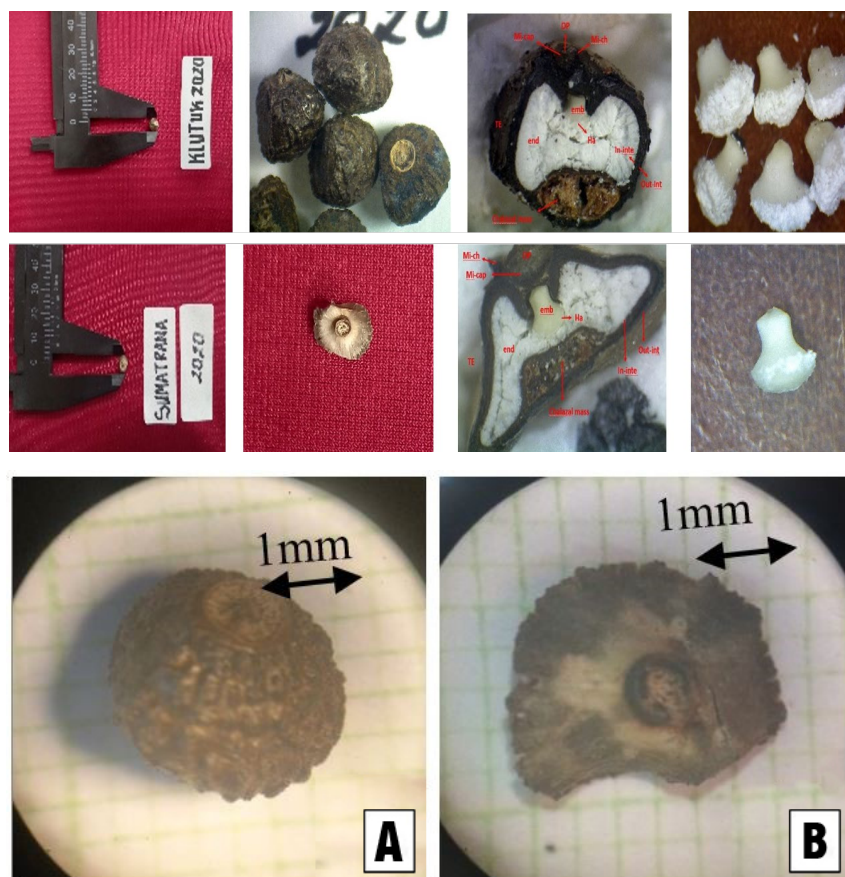


Figure 1. The first four figures in the upper part, from left to right, are the size of Klutuk seeds, seeds appearance, the longitudinal section, and the embryo. The middle four figures from left to right are the size of Sumatrana accession of seed size, appearance, longitudinal section, and embryo. Lower figures are banana seed of Klutuk NTT (A) and Sumatrana (B).

Seed anatomy

A longitudinal section of banana seeds is presented in Figure 2. Klutuk NTT and Sumatrana seeds had different diameters, with irregular, round, and flat shapes. The banana seeds of accession Klutuk NTT had an irregular, round shape, and the color of the seed skin was black with an uneven seed skin texture. The Sumatrana accession seeds had a flattened shape, and the color of the seed skin was gray-black to black with a rough seed skin texture. *Musa acuminata* Colla var. bantamensis Nasution has black seeds when ripe and has an irregular shape (Sulistyaningsih, 2016). Kurniajati and Martanti (2019) stated that wild banana seeds (*Musa acuminata* var. zebrina, malaccensis, banksii) have a thick skin.

Klutuk NTT and Sumatrana seeds had seed shells, endosperm, embryos, microphiles, inner and outer integuments, and operculum (Figure 2). Anatomical observations of seeds have been reported in several previous studies on subspecies of *Musa acuminata* (Kallow et al., 2020) and other wild banana species *Musa* (Bohra et al., 2020), and accession of *Musa acuminata* var. *acuminata*. *flava* (Ridl.) (Trimanto et al., 2022). The seed skin of the

accession of Klutuk NTT has a thicker seed skin with an irregular, round shape, and the Sumatrana accession has a thin and flattened skin presented in Figure 1.

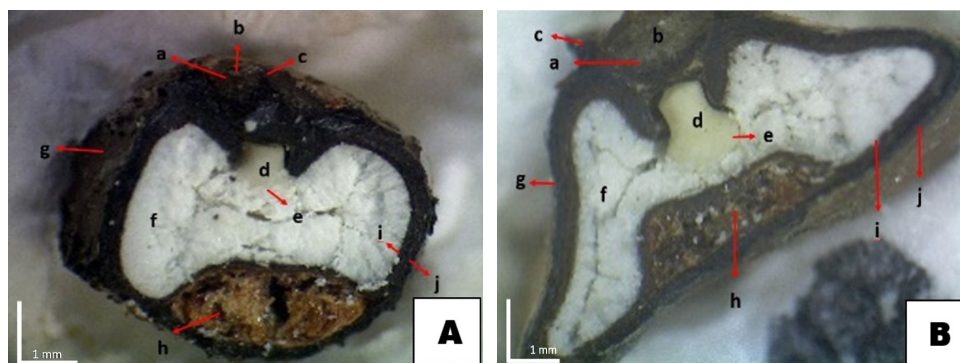


Figure 2. Longitudinal section of banana seeds: (A) *M. balbisiana* Klutuk NTT and (B) *M. accuminata* Sumatrana; a: micropyle cap, b: operculum, c: micropyle duct, d: embryo, e: haustorium, f: endosperm, g: testa, h: chalaza, i: inner integument, j: outer integument.

The embryo was located at the end of the inner microphile and had ivory white, slightly different from the milky white endosperm around it (Figure 2). Banana has a T-shaped embryo, with well-developed haustoria and meristematic stems (Prawestri et al., 2021). Banana seed embryos Klutuk NTT and Sumatrana had inverted T-shaped, with a yellowish-white color. The embryonic part of a banana seed consisted of an embryo and a haustorium (embryonic endosperm). The embryonic form of the accession seed of Klutuk NTT had a larger and longer shape, while the embryo in the Sumatrana accession seed in the haustorium was widened and inverted T-shaped (Figure 3).

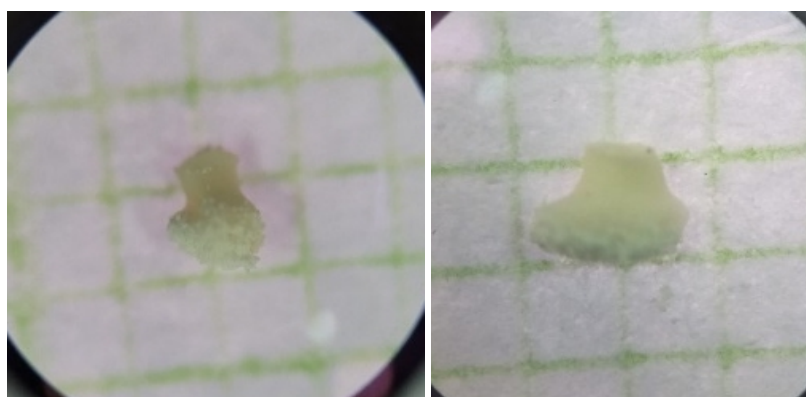


Figure 3. Seed embryo shape of accession banana: (A) Klutuk NTT and (B) Sumatrana.

Seed quality after storage

Moisture content was increased significantly in Klutuk NTT (10.25%-12.16%) after being stored for 41 months, while in Sumatrana it was not significantly different (11.02%-8.92%) (Table 2). According to FAO (2014), genebank standards for storage conditions of accessions are <10% MC and <15% RH. Panis et al. (2020) studied that *Musa* seeds can maintain viability for at least five years on a seed moisture content of less than 10%. Accordingly, Singh et al. (2021) stated that the initial moisture content of storage for the banana seeds of *Musa balbisiana* was in the range of 5-10%. Kaya et al. (2020) studied the percentage thresholds of seed moisture content used during cryopreservation were 16.7% and 16.3% (*M. velutina* and *M. acuminata*), so that seed viability could be maintained.

Table 2. Moisture content of Klutuk NTT and Sumatrana accession seeds in several storage periods.

Accession	Storage period (months)	Moisture content (%)
Klutuk NTT	5	10.25c
	25	10.73b
	41	12.16a
Sumatrana	5	11.02a
	23	10.86a
	41	8.92a

However, not all musa seeds retain their viability after drying. According to a recent assessment, seeds obtained from the wild were susceptible to drying (Kallow et al., 2020). Furthermore, the rate of drying and seed maturity have been studied to be related to desiccation sensitivity (Singh et al., 2021). Seed survival and longevity may also be impacted by other factors, such as the process used to extract fruit pulp, post-harvest ripening, drying intensity and rate, and more (Kallow et al., 2022).

The results showed that Klutuk NTT and Sumatrana accessions, which had been stored for 41 months, had significant values on all observed variables. Klutuk NTT accession showed significantly increased values in germination rate, vigor index, and uniformity of seedling emergence after being stored. Oppositely, Sumatrana accession showed significantly decreased values in all observed variables (Table 3). It indicated that the Klutuk NTT accession has a seed dormant mechanism. Dormancy in banana seeds is thought to be due to the thick skin of the seeds, thus making the seeds not subject to imbibition, which makes the seeds difficult to germinate. Burgos-Hernández et al. (2014) stated that seed viability and the presence and degree of dormancy appear to be species characteristics.

Table 3. Germination, vigor index, and uniformity of seedling emergence of Klutuk NTT and Sumatrana accession seeds in several storage periods.

Accession	Storage period (months)	Germination rate (%)	Vigor index (%)	Uniformity of seedling emergence (%)
Klutuk NTT	5	51.6c	18.8b	48.0b
	25	63.6b	24.0b	51.6ab
	41	79.2a	46.8a	63.6a
Sumatrana	5	97.6a	46.8a	76.8a
	23	42.4b	4.8b	42.4b
	41	18.0c	0.0b	4.8c

According to Bohra et al. (2020), *Musa indandamanensis* was classified as an orthodox seed as it has decreased in germination rate after 90 days (53%) and 210 days (21%) of storage. Sumatrana seeds stored for 5 months have a germination capacity of > 80%; this shows that germination above 80% is the minimum threshold for determining quality seeds. P80 is the minimum limit for determining quality seeds of horticultural plant seeds (Ministry of Agriculture, 2019).

Seed moisture does not have a statistically significant linear correlation with any of the tested seed viability parameters (germination, vigor index, or uniformity of seedling emergence) under the conditions of this study (Table 4). A normal banana sprout is presented in Figure 4. The P-values for all treatments are above the conventional significance level of 0.05, suggesting that any observed trends could be due to random variation. Furthermore, the R-squared values are generally low, indicating that seed moisture explains only a small to moderate proportion of the variability in these seed viability traits. This implies that other factors likely play a more dominant role in determining seed viability.

Table 4. Regression results between seed moisture and seed viability.

Treatment	P value	R-square (%)	Equation
Germination	0.372	20.12	$-88+12.3x$
Vigor index	0.150	44.10	$-110.2+12.55x$
Uniformity of seedling emergence	0.623	6.60	$-15+5.9x$

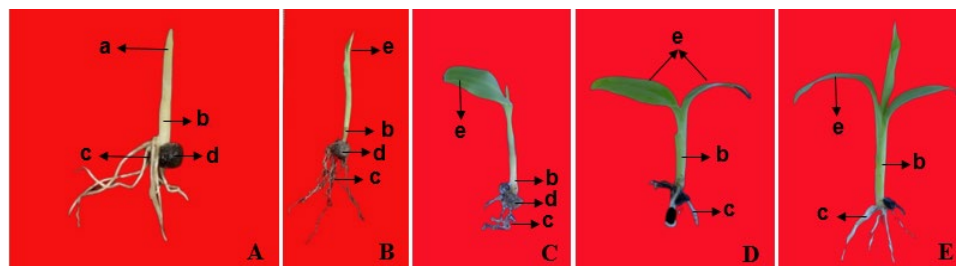


Figure 4. Criteria for normal banana sprouts; Description: (A) Plumule has filled the entire coleoptile, (B) Plumule has emerged through the coleoptile, (C) Normal sprouts have formed one leaf, (D) Normal sprouts have shown a) Two leaves, (E) Normal sprouts have formed three leaves; a) Coleoptile b) Mesocotyl c) Radicle (potential root) d) Seed coat e) Plumule (potential leaf).

The analysis reveals strong and statistically significant positive correlations between DB (%) and IV (%) ($r = 0.842$, $p = 0.035$), as well as between IV (%) and KST (%) ($r = 0.881$, $p = 0.021$). A strong positive correlation was also observed between DB (%) and KST (%) ($r = 0.783$, $p = 0.066$), though it was not statistically significant at the 0.05 level. Conversely, KA (%) did not exhibit any strong or statistically significant linear relationships with the other variables. The most robust linear relationships are observed between DB and IV, and between IV and KST (Figure 5). This suggests that IV (%) plays a significant role in connecting DB (%) and KST (%). KA (%) did not have any strong or statistically significant linear relationships with the other variables in this dataset.

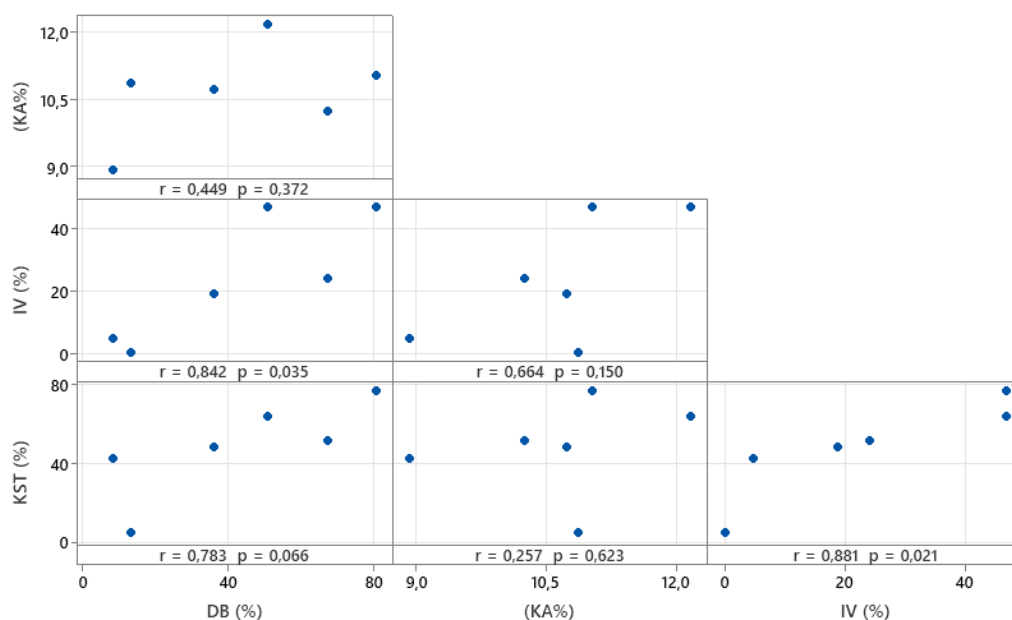


Figure 5. Correlation between seed moisture and seed viability. DB = Germination rate, IV = Vigor index, KA = Moisture content, KST = Uniformity of seedling emergence.

CONCLUSIONS

The seed size of the Klutuk accession was 4-5.8 mm, while Sumatrana was 4-5 mm. Both accessions had a seed coat, endosperm, embryo, microphyll, inner and outer integument, khalazal, and operculum. The embryo was inverted T-shaped, with the haustorium of Klutuk NTT embryo was rounded, while Sumatrana was wider and depressed. Seeds of Klutuk NTT increased moisture content, germination rate, vigor index, and uniformity of seedling emergence after being stored. On the contrary, Sumatrana accession showed decreased values except for moisture content, indicating Klutuk NTT accession had a seed dormant mechanism. There were positive correlations between DB (%) with IV (%), and between IV (%) with KST (%). Conversely, KA (%) showed no significant linear association with any of the other measured variables. These findings suggest that factors beyond seed moisture are predominant in influencing seed viability parameters, and that specific inter-parameter relationships, particularly involving IV (%), are key determinants of overall seed quality.

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