

# Embryo and Seed Germination of Pisang Klutuk Wulung (*Musa balbisiana* Colla) After Storage

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**Abstract.** Pisang Klutuk Wulung is one of *Musa balbisiana* accessions which has potential for genetic improvement of cultivated banana so that its conservation is essential. This research aimed to study the post-storage germination of Indonesian seeds of Pisang Klutuk Wulung. Storage methods were carried out using a factorial completely randomized design with three factors: packing methods using vacuum and non-vacuum plastic bags; storage temperature at 25°C, 4°C, and -20°C; and storage duration by 7, 14, 30, and 50 days. The germinations were done *in vitro* and *ex vitro*. The results showed that seeds stored at 25°C in non-vacuum plastic bags were infested by molds, contrasting to the non-vacuum treatment. The sterilization method using 25% sodium hypochlorite, Tween 20, and 80% alcohol resulted in less contamination than 96% alcohol. *In vitro* germination from the vacuum treatment had a higher germination rate than non-vacuum treatment. However, *ex vitro* germination was not affected by the storage method. Similar patterns were seen in *in vitro* and *ex vitro* germination as storage in 4°C resulted in better seed germination after 30 and 50 days. In contrast, at -20°C, no embryo germinated in all storage duration treatments. Pisang Klutuk Wulung seeds could not be stored in the long term as they rapidly lost their viability. Our finding showed that airtight condition by vacuum treatment and low temperature at 4°C were able to maintain seed viability for longer period of storage. Thus, this finding was useful to improve *Musa* breeding programs and as an essential step for the long-term conservation of *Musa* genetic resources.

**Key words:** germination, Pisang Klutuk Wulung, seeds, storage, wild banana

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## INTRODUCTION

Pisang Klutuk Wulung is one of *Musa balbisiana* Colla accessions that contains B genome. It is widely spread in Indonesia, especially on Java Island. Indigenous people utilize the leaves for wrapping food (Rahmadhia & Juwitningtyas, 2020) as its structure is denser and contains more waxy layers than other bananas. This accession is known for its resistance to biotic stress, e.g., banana bunchy top disease (Hapsari & Masrum, 2012). The conservation of its existence is necessary as a potential wild diploid parent for the genetic improvement of cultivated bananas.

One of the techniques for germplasm preservation is seed storage. This method can be beneficial in the future for advanced *ex-situ* conservation, phenotyping, and breeding (Kallow

et al., 2020). The wild banana seed is classified as an orthodox seed (Vineesh et al., 2015). It can survive in a low water content state and maintain its viability and germination rate after extensive storage in suitable dry and cold conditions (de Vitis et al., 2020; Vineesh et al., 2015; Walters, 2015). Keeping seeds in a dry state is to avoid the deterioration of seed physiology (Filho et al., 2016).

The success of seed storage is measured by seed longevity, which usually is evaluated by the ability to germinate and seedling germination after storage (Basso et al., 2018; de Vitis et al., 2020). Seed longevity in every species may vary, depending on seed characteristics, for example, orthodox and recalcitrant, and suitable

environmental conditions. Seeds of *Pennisetum glaucum* stored in airtight packaging resulted in a higher germination rate (80%) than seed kept in cement paper (<20%) and wood containers (20%) (Hedimbi et al., 2012). The germination rate of *Musa indandamanensis* seed was 90% after 45 days of storage and fell to 20% if stored in extensive duration to 210 days (Bohra et al., 2020).

Information about seed storage in the genus *Musa* is limited as only ten species have been studied, *M. acuminata* (Indrayanti et al., 2019; Vineesh et al., 2015; Wilson & Tenkouano, 2019), *M. balbisiana* (Singh et al., 2021), *M. indandamanensis*, *M. velutina* (Nagano et al., 2008, 2009), *M. boman*, *M. ingens*, *M. lolodensis*, *M. peekelii*, *M. schizocarpa*, *M. maclayi* (Kallow et al., 2020). The percentage of seeds germination is usually lower than embryo germination, so the latter method is commonly used for banana seed germination (Kallow et al., 2020). However, in this experiment, we also observed the effect of storage on seed germination as it could save a considerable amount of time in the media and embryo preparation. This research aimed to study the post-storage germination in *Musa balbisiana* accession Klutuk Wulung from Indonesia. This study was expected to provide new information to maintain the seed in good physiological and morphological condition with proper seed storage treatment to improve *Musa* breeding programs and as an essential step for the long-term conservation of *Musa* genetic resources.

## METHODS

The study was done from December 2020 to January 2021 in the Laboratory of Plant Tissue Culture and Plant Physiology, Science Technopark Area BRIN, Cibinong. Pisang Klutuk Wulung seeds were extracted from fruits harvested in Sampora village, Cibinong, Bogor. The harvested fruits were then placed in the ripening chamber for two weeks until they were ripe and soft enough to extract the seeds. The seeds were removed by squishing and brushing fruits under running water until the fruit flesh was utterly detached. Seeds were then air-dried for three days at 20°C in the laboratory.

### Storage of banana seeds

This study was carried out using a factorial completely randomized design with three factors: packing methods (vacuum and non-vacuum); storage temperatures (25°C, 4°C, and -20°C);

storage durations (7, 14, and 50 days for *in vitro* germination and 7, 14, and 30 days for *ex vitro* germination). Both *in vitro* and *ex vitro* germination consisted of 18 treatments examined using 100 dried seeds. Therefore, the total number of seed used in this study were 3600 seeds. This study used two types of plastic, i.e., purpose-built plastic bags for vacuum treatments and plastic clip seal bags for non-vacuum treatments.

### Sterilization and germination of banana seeds after storage

Seed sterilization was done in aseptic condition using laminar airflow. After the storage period, seeds from each treatment were soaked separately in 25% sodium hypochlorite (v/v) with three drops of Tween 20 for 15 minutes, followed by sterile distilled water for 2 minutes, then 80% alcohol for 10 minutes. Seeds used for *in vitro* germination were then hydroprimed by soaking them in 100 mL Erlenmeyer containing 50 mL sterile distilled water for 24 hours at room temperature (Prawestri et al., 2021). Meanwhile, after the sterilization, seeds for *ex vitro* germination were directly sown without being hydroprimed.

Second sterilizations were undertaken after priming. Seeds stored for seven days were only sterilized using 96% alcohol for 3 minutes without rinsing. Nevertheless, seeds stored for 14 and 50 days were re-sterilized using the same technique as the first sterilization. Sterile seeds were then grown *in vitro* through embryo culture and *ex vitro* by seed germination. It has been known that the embryo rescue technique resulted in a higher germination rate.

### *In vitro* germination

*In vitro* germination was done aseptically under Laminar Air Flow. Sixty embryos were extracted from randomly selected sterile seeds by cutting the seed longitudinally using a scalpel and then planting on three petri dishes containing a solid germination medium. Each petri dish consisted of 20 embryos. The medium used in this experiment was solid modified MS (Murashige & Skoog, 1962) containing MS macro and micro salts, MS vitamin, 100 mg/L of myo-inositol, 30 g/L of sugar, and supplemented with 1 mg/L biotin, 1 mg/L proline, and 0.5 mg/L benzyl-adenine (BA) and 3 g/L gelrite as the gelling agent (Prawestri et al., 2021). The medium pH was adjusted to 5.7-5.8 with a few drops of either 1 N NaOH or 1 N HCl before adding the gelling agent. The medium was autoclaved at 121°C for 20 minutes.

After planting on the germination medium, the embryo cultures were transferred to the culture room and placed on a shelf in dark condition at  $\pm 25^{\circ}\text{C}$ . Germinating embryos were observed and recorded for four weeks after culture. Each treatment consisted of 20 embryos in each three replication.

### ***Ex vitro* germination**

The seed germination medium was rock wool moistened with sterile distilled water and placed on a plastic tub. Planting holes were made by perforating the rock wool using tweezers at a 1 x 1 cm distance. As many as 20 sterile seeds from each treatment were randomly selected, planted in the hole, and replicated five times. The seeds were then incubated in a germination chamber at  $30^{\circ}\text{C}$ . Germinating seeds were observed and recorded for four weeks after planting. Each treatment consisted of 20 seeds in each five replication.

### **Data analysis**

Saphiro-Wilk test was used to assess the normality of the residual from a linear regression model. Data with normal residuals were analyzed using Analysis of Variance (ANOVA). Otherwise, data were proceeded by using descriptive analysis.

## **RESULTS AND DISCUSSION**

### **Storage methods**

This experiment used purpose-built plastic bags for the standard vacuum treatment and plastic clip seal bags for the non-vacuum treatment. The seed showed a different response between treatments. Both treatments showed contrast that was noticed after storage. The seeds of non-vacuum treatment were covered with molds after seven days of storage at room temperature ( $25^{\circ}\text{C}$ ). The longer storage duration considerably increased the mold population. Meanwhile, after 50 days of storage, molds in all vacuum treatments and low-temperature non-vacuum treatment were almost unnoticed.

The results showed that the seeds in non-vacuum treatment were more able to respire. Magan et al. (2004) stated that the respiration product then condensates on the plastic clip as it is trapped. This circumstance appeared to promote fungal growth. Factors influencing fungal emergence in seed storages were temperature, oxygen level, interactions with other organisms, chemical preservatives, and storage time.

Data in Table 1 shows that packaging affected seed viability. Seeds stored in vacuumed bags

could maintain germination ability compared to those stored in non-vacuumed bags. Airtight conditions prevent the exchange of gases and moisture from outside so that the seeds remain of good quality. Similar findings stated that seeds stored in vacuum bags possessed less deterioration than those stored without being vacuumed because of fewer seeds respiration as there are less moisture and humidity (Meena et al., 2017). A similar result was shown in Tubbs et al. (2016), that weekly opening of hermetic storage bags stimulated the fungal growth due to an increase in maize grains' moisture content. Grains with 15 – 20% moisture content were high enough to support fungal growth. Aboagye et al. (2017) also added that if grains are dried to 14% moisture or less, minimal or no fungal growth is observed. Therefore, the seeds must be properly dried before storage.

### **Sterilization and *In vitro* germination**

Embryos from vacuum treatment had a higher germination rate than non-vacuum treatment (Table 1). The seeds stored at  $4^{\circ}\text{C}$  resulted in the highest germination rate, while seeds stored in a very cold environment ( $-20^{\circ}\text{C}$ ) resulted in no germinating embryo in both packing treatments. This result was similar to Indrayanti et al. (2019), who reported the best short-term storage of wild banana *M. acuminata* var. *microcarpa* seeds were in  $1$  to  $5^{\circ}\text{C}$ , and 68-84% of the embryos were successfully germinated after 30 days of culture. On the other hand, embryos from seeds stored at  $-20$  to  $-25^{\circ}\text{C}$  for 30 and 60 days showed no response to germination.

After seven days of storage, the *in vitro* germination rate of Pisang Klutuk Wulung dropped from 70% in fresh seed to  $42 \pm 25.65\%$  and  $32 \pm 38.83\%$  in non-vacuum and vacuum treatment, respectively, after being stored at  $4^{\circ}\text{C}$ . However, there were no embryos germinated after being stored at a temperature of  $25^{\circ}\text{C}$ . Those embryos were contaminated by fungi and bacteria even though no fungi were seen in vacuum plastic bags. It seemed that sterilization using only 96% alcohol after priming was ineffective in stored seeds even though it was effective when used in fresh banana seeds (data not shown).

Therefore, the same sterilization method used before priming was performed 14 days after storage (DAS). This sterilization method resulted in the germination rate of embryos from non-vacuum treatment stored at  $25^{\circ}\text{C}$ , which we can increase up to  $42 \pm 7.63\%$ . It also increased germination in a vacuum and  $4^{\circ}\text{C}$  treatment to

57±10.40%, in contrast to the germination rate of non-vacuum storage that fell to 16±23.43%. Although the contamination was lessened, the germination is low due to the presence of unresponsive embryos. However, the sterilization

Pisang Klutuk Wulung also appeared to show intermediate seed storage behaviour with short seed longevity.

Usually, seed viability is reduced after storage for long periods, which is indicated by a decline in

**Table 1.** Germination rate of the embryo of Pisang Klutuk Wulung after storage

Storage duration (days)	Storage temperature (°C)	Final germination (%)			
		Vacuum		Non-vacuum	
		Mean	SD	Mean	SD
7	25	0	0	0	0
7	4	32	38.83	42	25.65
7	-20	0	0	0	0
14	25	42	7.63	0	0
14	4	57	10.4	16	23.43
14	-20	0	0	0	0
50	25	0	0	0	0
50	4	2	2.88	0	0
50	-20	0	0	0	0

method was ineffective in reducing contamination of the stored seeds for 50 days in non-vacuum treatment.

The recent finding classified some banana seeds into intermediate seeds rather than orthodox seeds. The reasons are that they show partial sensitivity to drying and cold storage in particular and can maintain seed viability to a good level of around 70% after short- or long-term storage at 5°C (de Vitis et al., 2020; Kallow et al., 2020; Vineesh et al., 2015). Although banana seeds have been classified as orthodox (Vineesh et al., 2015), some of them are classified as intermediate species that show partial sensitivity to drying and cold storage in particular (Kallow et al., 2020) and maintain good levels (i.e., 70%) of seed viability after short- or long-term storage at 5°C (de Vitis et al., 2020). Seed viability loss in dry storage is one of the characteristic features of intermediate seed storage behaviour (Bohra et al., 2020). Seed longevity could be a characteristic to classify seeds' storage behaviour. Seed longevity measures how long seeds can be stored and remain viable under certain conditions. Seed longevity in storage varies significantly among species. Orthodox seeds can be stored at -18°C for an extended period (i.e. up to 5 years).

In contrast, recalcitrant seeds are vulnerable and should be stored in moist conditions at warm temperatures ( $\geq 10^\circ\text{C}$ ) for under 1 year. Meanwhile, intermediate seeds are freeze sensitive short-lived, and most seeds die within 1-5 years at 18°C but retain viability at 5°C (de Vitis et al., 2020). Hence, in this study, we found that

germination ability (de Vitis et al., 2020). This study showed that the seed longevity of Pisang Klutuk Wulung was relatively short. The embryos could germinate only 2% in airtight conditions (vacuum treatment), and no embryo grown in non-airtight conditions (non-vacuum treatment) after the seeds were stored for 50 days. Earlier reports in other wild bananas such as *M. acuminata* Calcutta 4 (Wilson & Tenkouano, 2019) and *M. indandamanensis* (Bohra et al., 2020) also showed a similar result in decreased germination after four weeks and 210 days of the storage period, respectively.

There was no evidence of embryos germinating after seeds were kept at -20°C. Freezing injury during storage could be the primary reason for germination failure. This injury is related to the seed moisture content as available water in the seed will be frozen, and the embryo could be dead by ice crystallization. However, orthodox seeds seem to have different upper limits of moisture content to maintain their longevity if stored at -20°C. For example, cereal safe seed moisture is around 12.5% to 13.5%, and oily seed species can be kept in lower moisture (Hong et al., 1996).

Furthermore, some *M. balbisiana* seeds used in this study were lack of endosperm, and some other contained no endosperm. Those characters were different compared to *M. ornata*, *M. acuminata*, and *M. balbisiana* accession from India with powdery endosperm thoroughly filling the seed (Dayarani et al., 2014; Kallow et al., 2020; Singh et al., 2021; Vineesh et al., 2015). In some other cases, the endosperm was absent. It might lead to

a rapid decline in seed viability, mainly due to embryo susceptibility to low temperature.

Double sterilization *in vitro* germination appeared to be less effective since embryos were contaminated, mainly in non-vacuum treatment. It was previously stated that the application of sodium hypochlorite and 70% ethanol as a common method for seed surface sterilization still showed high contamination *in vitro* germination of cotton seeds (Barampuram et al., 2014). Other sterilization agents or methods might be needed to sterilize seeds covered with molds.

In general, embryos can germinate in the first week of culture after seeds are stored and mostly stopped at the third week (Figure 1). However, embryos from seed stored at 4°C for 14 days in both packing treatments can still germinate in the fourth week. On the other hand, the germination of embryos stored for 50 days remained constant in observation.

The storage period appeared to be affecting the ability of embryo germination. A shorter storage period resulted in faster initial germination for both packing treatments by more than 15% in 7 DAS and below 10% in 14 DAS and 50 DAS. Despite the higher germination rate, we could see a slight increase of germination per week in 7 DAS. In contrast, on the following storage duration, the germination climbed rapidly on the second week, specifically in the vacuum treatment, and it gradually increased until the fourth week. A more extended storage period leads to a constant low germination ability at 2%.

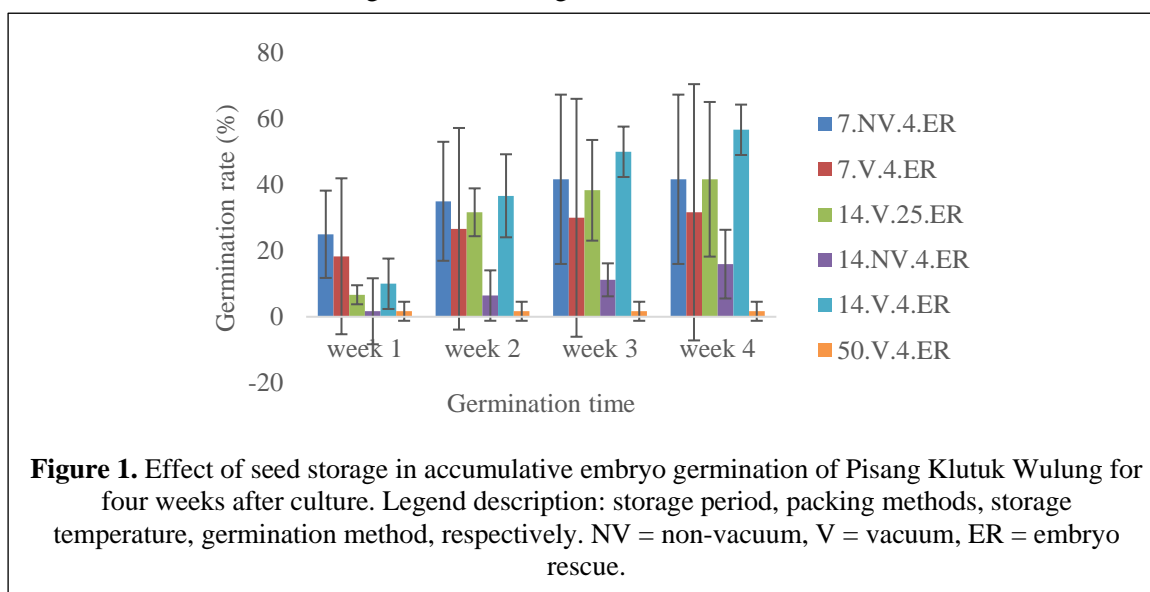
The results showed that Pisang Klutuk Wulung

started to germinate one week after culture. Meanwhile, Indrayanti et al. (2019) reported that most of the embryos from *M. acuminata* var. *microcarpa* seeds stored for 4-10, 30, and 60 days started to germinate in two, three, and five weeks after culture with 6-12, 11, and 11% percentage of germination, respectively.

### Ex vitro germination

In general, seed germination was lower than embryo germination in fresh seed and after storage treatment. However, similar after-treatment patterns were seen as storage in 4°C resulted in better seed germination, whereas at -20°C, there was no embryo germination (Table 2.). Furthermore, seeds stored at 25°C experienced deterioration, in line with a more extended storage period. In contrast with the results of embryo germination, packing treatment appeared to be insignificant on the seed germination rate.

The germination rate of fresh seed was 16% after 30 days, considered low compared to embryo germination. After being stored for seven days, the germination rate of vacuumed seeds stored at 25°C was higher than those at 4°C in contrast to the non-vacuumed seeds. However, the germination of seeds after being stored 14 days at 25°C decreased sharply, compared to germination of seeds kept at 4 °C that were constant. While the non-vacuum treatment that kept the seed at a higher temperature resulted in a slight germination increase by 1%, the opposite occurred in the lower temperature treatment, where the germination fell about 2%.



seeds have a faster germination time than *M. acuminata* var. *microcarpa* seeds through embryo culture. The embryos of Pisang Klutuk Wulung

Seeds stored at 25°C for 30 days on both packing treatments failed to germinate. Meanwhile, around 3% of seeds stored at 4°C were

able to grow. This result indicated that packing treatment for seeds of Pisang Klutuk Wulung in longer storage duration was not as crucial as storage temperature. Whitehouse et al. (2020) mentioned that seed dormancy, germination conditions, and low seed viability caused no or low germination in the seed lot. In addition, some factors, including phytohormones, climate, nutrients, light, and water, affect the germination rate in plenty of plant species (Bareke, 2018). However, in *Musa* species, water is not affecting seed dormancy as the dry seeds could quickly imbibe water. Other forms of dormancy may exist due to physical, anatomical, and physiological reasons that can cause low and erratic germination of banana seeds (Puteh et al., 2011).

Regardless of the result, this finding differed from that stated. The seeds of *M. balbisiana* were not germinated after being sown for more than five months at a constant temperature. Our seeds could germinate at a constant 30°C temperature despite a low germination rate. However, controlling temperatures between 27-35°C for 6 to 12 hours and 12-18 °C for 12 to 18 hours appeared to maximize germination up to 99%, higher than in greenhouse conditions that temperatures are altering between 34°C and 19 °C by 83% (Stotzky & Cox, 1962). On the other hand, (Ahmed et al., 2006) reported no seed germination in the greenhouse.

Seed germination mostly started two weeks after being sown (Figure 2). However, a small number of seeds germinated in the first week, and others were not grown until the third week. Similar to embryo germination, the numbers were constant in the fourth week.

Germination occurred at the first week where the seed was stored after seven days at 25°C from vacuum treatment and at 4°C from non-vacuum treatment. However, germination from vacuum treatment increased rapidly to 17% then remained constant until the end of observation. On the contrary, germination from non-vacuum treatment increased gradually until the third week. Other seeds from various treatments were germinated in the second week except for seeds stored for 14 days at 25°C, which germinated in the third week and became stagnant the week after. *Musa* seeds generally need a longer time to grow (Kallow et al., 2020).

This study showed that *in vitro* germination through embryo culture was more effective in enhancing germination rate than *ex vitro* germination. Wilson & Tenkouano

(2019) reported that *in vitro* germination resulted in consistently higher germination than *ex vitro* germination by sowing the seeds. Banana seeds have a hard texture with a thick cuticle layer on the inner side of the seed integument that leads to germination failure due to lack of water supply or exchange of gases (Arun et al., 2013).

Our samples could not be stored in the long term, and this case seems related to the unique character of seeds on each accession in the same species. Seeds characteristic between *Musa* wild species are mainly the same, consisting of the hard seed coat, powdery white endosperm, and mushroom-like embryo. However, it is hard to obtain mature seeds in the exploration, so sometimes, there are empty and non-complete seeds that only consist of either endosperm or embryo (Kallow et al., 2020; Singh et al., 2021). This case becomes a challenge for the seed storage of wild bananas.

This research studied more factors that can influence the success rate of Pisang Klutuk Wulung seeds germination after storage, such as packing methods, storage durations, storage temperatures, and germination methods. We found that packing methods using airtight plastic and germination methods, whether sown seeds in germination chamber or sown embryos in aseptic laminar airflow, affected the germination. This information could be used for developing seed storage strategies, especially banana seeds, in Indonesia.

## CONCLUSION

Various treatments of seed storage influence the viability of Pisang Klutuk Wulung seeds. *In vitro* germination from the vacuum treatment had a higher germination rate than non-vacuum treatment. *In vitro* germination was affected by the storage method, while *ex vitro* germination was not affected. Vacuum treatment affects *in vitro* germination at a higher rate than non-vacuum treatment. Similar patterns were seen in *in vitro* and *ex vitro* germination as storage at 4°C resulted in better seed germination after 30 and 50 days.

In contrast, at -20°C, no embryo germinated in all storage duration treatments. Our finding showed that the seed longevity of Indonesian Pisang Klutuk Wulung was short as the germination rate dropped sharply after storage. Embryo contamination affects germination, so that other seed sterilization methods might be needed after storage.

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## REFERENCES

- Aboagye, D., Darko, J. O., & Banadda, N. (2017). Comparative study of hermetic and non-hermetic storage on quality of cowpea in Ghana. *Chemical and Biological Technologies in Agriculture*, 4(1), 1–7. <https://doi.org/10.1186/s40538-017-0091-y>
- Ahmed, K. Z., Remy, S., & Swennen, R. (2006). Germination of *Musa balbisiana* seeds and embryo. *XVII International Meeting of the Association for the Cooperation in Investigations of Banana in Tropical America and the Caribbean*, 1–5.
- Arun, K., Uma, S., Saraswathi, M. S., Backiyarani, S., & Durai, P. (2013). Effects of whole seed priming on the in vitro germination of hybrid banana embryos (*Musa* spp.). *Seed Science and Technology*, 41(3), 439–451. <https://doi.org/10.15258/sst.2013.41.3.10>
- Barampuram, S., Allen, G., & Krasnyanski, S. (2014). Effect of various sterilization procedures on the in vitro germination of cotton seeds. *Plant Cell, Tissue and Organ Culture*, 118(1), 179–185. <https://doi.org/10.1007/s11240-014-0472-x>
- Bareke, T. (2018). Biology of seed development and germination physiology. *Advances in Plants & Agriculture Research*, 8(4). <https://doi.org/10.15406/apar.2018.08.00336>
- Basso, D. P., Hoshino-Bezerra, A. A., Sartori, M. M. P., Buitink, J., Leprince, O., & da Silva, E. A. A. (2018). Late seed maturation improves the preservation of seedling emergence during storage in soybean. *Journal of Seed Science*, 40(2), 185–192. <https://doi.org/10.1590/2317-1545v40n2191893>
- Bohra, P., Waman, A. A., & Jerard, B. A. (2020). Seed germination and storage studies in seed fertile *Musa indandamanensis* and its conservation. *South African Journal of Botany*, 128, 161–166. <https://doi.org/10.1016/j.sajb.2019.09.022>
- Dayarani, M., Dhanarajan, M. S., Arun, K., Uma, S., & Narayani, P. (2014). Embryo culture and embryo rescue studies in wild *Musa* spp. (*Musa ornata*). *Journal of Applied Horticulture*, 16(2), 126–130. <https://doi.org/10.37855/jah.2014.v16i02.21>
- de Vitis, M., Hay, F. R., Dickie, J. B., Trivedi, C., Choi, J., & Fiegenger, R. (2020). Seed storage: maintaining seed viability and vigor for restoration use. *Restoration Ecology*, 28(S3), S249–S255. <https://doi.org/10.1111/rec.13174>
- Filho, C. P. H., Goneli, A. L. D., Masetto, T. E., Martins, E. A. S., & Oba, G. C. (2016). The effect of drying temperatures and storage of seeds on the growth of soybean seedlings. *Journal of Seed Science*, 38(4), 287–295. <https://doi.org/10.1590/2317-1545v38n4161866>
- Hapsari, L., & Masrum, A. (2012). Preliminary screening resistance of *Musa* germplasm for banana bunchy top disease in Purwodadi Botanic Garden, Pasuruan, East Java. *Buletin Kebun Raya*, 15(2), 57–70.
- Hedimbi, M., Ananias, N. K., & Kandawa-Schulz, M. (2012). Effects of storage conditions on viability, germination and sugar content of pearl millet (*Pennisetum glaucum*) grains. *Journal of Research in Agriculture*, 1(1), 088–092.
- Hong, T. D., Linington, S., & Ellis, R. H. (1996). Seed storage behaviour: a compendium. In *Handbooks for Genebanks No. 4* (Vol. 26, Issue 1).
- Indrayanti, R., Putri, A. R., Adisyahputra, A., & Sutanto, A. (2019). In vitro germination of wild banana *Musa acuminata* Colla var. *microcarpa* (Becc) after storage periods at different temperatures. *Empowering Science and Mathematics for Global Competitiveness*, 71–80. <https://doi.org/10.1201/9780429461903-12>
- Kallow, S., Longin, K., Sleziak, N. F., Janssens, S. B., Vandeloek, F., Dickie, J., Swennen, R., Paofa, J., Carpentier, S., & Panis, B. (2020a). Challenges for ex situ conservation of wild bananas: seeds collected in Papua New Guinea have variable levels of desiccation tolerance. *Plants*, 9(9), 1–21.
- Kallow, S., Longin, K., Sleziak, N. F., Janssens, S. B., Vandeloek, F., Dickie, J., Swennen, R., Paofa, J., Carpentier, S., & Panis, B. (2020b). Challenges for ex situ conservation of wild bananas: Seeds collected in Papua New Guinea have variable levels of desiccation tolerance. *Plants*, 9(9), 1–21. <https://doi.org/10.3390/plants9091243>
- Magan, N., Sanchis, V., & Aldred, D. (2004). The role of spoilage fungi in seed deterioration.

- In *Fungal biotechnology in agricultural, food, and environmental applications* (Issue January 2004, pp. 311–323).
- Meena, K. K., Sorty, A. M., Bitla, U. M., Choudhary, K., Gupta, P., Pareek, A., Singh, D. P., Prabha, R., Sahu, P. K., Gupta, V. K., Singh, H. B., Krishanani, K. K., & Minhas, P. S. (2017). Abiotic stress responses and microbe-mediated mitigation in plants: The omics strategies. *Frontiers in Plant Science*, 8(February), 1–25. <https://doi.org/10.3389/fpls.2017.00172>
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15, 474–497. <https://doi.org/https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
- Nagano, S., Mori, G., & Oda, M. (2008). Effects of temperature and moisture content of the substrate during storage on embryo development and germination in seeds of *Musa velutina* Wendl. & Drude. *Journal of Horticultural Science and Biotechnology*, 83(1), 33–36. <https://doi.org/10.1080/14620316.2008.11512343>
- Nagano, S., Mori, G., & Oda, M. (2009). Seed germinability in *Musa velutina* Wendl. & Drude is markedly lowered by 1 week in dry-storage. *Journal of Horticultural Science and Biotechnology*, 84(3), 325–328. <https://doi.org/10.1080/14620316.2009.11512525>
- Prawestri, A. D., Riastiwi, I., Rahayu, R. S., Handayani, T., Leksonowati, A., & Poerba, Y. S. (2021). Hydropriming improves germination and plant recovery during embryo rescue of wild banana *Musa acuminata* var. *tomentosa*. *Jurnal Biodjati*, 6(1), 11–22. <https://doi.org/10.15575/biodjati.v6i1.10528>
- Puteh, A. B., Aris, E. M., Sinniah, U. R., Rahman, M., Mohamad, R. B., & Abdullah, N. A. P. (2011). Seed anatomy, moisture content and scarification influence on imbibition in wild banana (*Musa acuminata* Colla ) ecotypes. *African Journal of Biotechnology*, 10(65), 14373–14379. <https://doi.org/10.5897/AJB11.1241>
- Rahmadhia, S. N., & Juwitningtyas, T. (2020). Physicochemical Properties of Klutuk banana leaves (*Musa balbisiana* Colla) Susu and Wulung cultivars with its potential as antioxidant. *Journal of Agri-Food Science and Technology*, 1(1), 18. <https://doi.org/10.12928/jafost.v1i1.1942>
- Singh, S., Agrawal, A., Kumar, R., Thangjam, R., & Joseph John, K. (2021). Seed storage behavior of *Musa balbisiana* Colla, a wild progenitor of bananas and plantains - Implications for ex situ germplasm conservation. *Scientia Horticulturae*, 280(January), 109926. <https://doi.org/10.1016/j.scienta.2021.109926>
- Stotzky, G., & Cox, E. A. (1962). Seed germination studies in *Musa*. II. alternating temperature requirement for the germination of *Musa balbisiana*. *American Journal of Botany*, 49(7), 763–770. <https://doi.org/10.1002/j.1537-2197.1962.tb15007.x>
- Tubbs, T., Baributsa, D., & Woloshuk, C. (2016). Impact of opening hermetic storage bags on grain quality, fungal growth and aflatoxin accumulation. *Journal of Stored Products Research*, 69, 276–281. <https://doi.org/10.1016/j.jspr.2016.10.003>
- Vineesh, P. S., Skaria, R., Mukunthakumar, S., Padmesh, P., & Decruse, S. W. (2015). Seed germination and cryostorage of *Musa acuminata* subsp. *burmannica* from Western Ghats. *South African Journal of Botany*, 100, 158–163. <https://doi.org/10.1016/j.sajb.2015.05.024>
- Walters, C. (2015). Orthodoxy, recalcitrance and in-between: describing variation in seed storage characteristics using threshold responses to water loss. *Planta*, 242(2), 397–406. <https://doi.org/10.1007/s00425-015-2312-6>
- Whitehouse, K. J., Hay, F. R., & Lusty, C. (2020). Why seed physiology is important for genebanking. *Plants*, 9(5), 1–16. <https://doi.org/10.3390/plants9050584>
- Wilson, V., & Tenkouano, A. (2019). Effects of storage and priming on seed emergence in soil and embryo culture of *Musa acuminata* Calcutta 4. *Asian Plant Research Journal*, 2(1), 1–9. <https://doi.org/10.9734/aprj/2019/v2i129795>