Moisture Equilibration on Germinability and Seedling Performance of Cacao (*Theobroma cacao*) Seed

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ABSTRACT Cacao (*Theobroma cacao*) produces abundant fruits regularly. Thus, little attention is given to the storing of seeds. The recalcitrant nature of cacao seeds further poses challenges to the growers in ensuring the availability of high-quality seeds when required. In the scope of cacao seeds desiccation-sensitivity, the appropriate hydrated storage could minimize the loss of seed quality. This study aimed to evaluate the responses of moisture equilibration on the germinability and seedling performance of cacao seeds stored at different temperature and relative humidity (RH) conditions. Seeds from ripened cacao pods of PBC 123 clone were extracted, demucilaged and placed in zip-lock polyethylene bags to be stored at (i) 16°C, 40% RH, (ii) 16°C, 80% RH, (iii) air-conditioned room temperature, RT (25±2°C, 55±5% RH), and (iv) control (seeds freshly extracted from pods). Seeds were sampled every 24 hours over for 144 hours and evaluated for moisture content (MC), leachate conductivity (LC), germinability, and seedling performance. Seeds with higher MC had a significantly lower germination rate index (GRI) (r = -0.49). Seeds stored at RT and 16°C, 40% RH showed minimal fluctuations of MC (45 to 52%), a minimal decrease of seedling dry biomass (MASS), with higher GRI. However, seeds stored at RT germinated more and were fungi invaded during storage. Seeds stored at 16°C, 80% RH equilibrated at higher MC (49 to 56%) showed the lowest GRI and decreased of MASS after 96 hours of storage. Regardless of the RH, seeds stored at 16°C showed increase LC over time, which might enhance their deterioration. Despite the deterioration associated with seed aging, the storage condition of 16°C, 40% RH showed some promise in minimizing the loss of cacao seed quality within 144 hours of storage durations.

KEYWORDS: Cacao seeds; Desiccation-sensitive; Hydrated storage; Relative humidity; Temperature
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INTRODUCTION

Seed storage is required to ensure the continuous and cost-effective supply of tree seeds (Umarani *et al*., 2015). In recalcitrant-seeded species, generally directly pass from development to germination due to the lack of a dehydration stage during seed ripening (Pukacka *et al*., 2011). Hence, those seeds contain high moisture content (MC) and active metabolism as soon as they shed from the parent plant. They are vulnerable to drying, which complicates their prolonged conservation (Suma *et al*., 2013). Seed deterioration often results in the accumulation of harmful by-products. This process decreased the ability of the seeds to survive, and usually occurred through their physiological changes over time (Sun, 2002). While the seed MC exerts the greatest influence on their storage longevity; temperature, and relative humidity (RH) are the determinants of the type of deteriorative reactions (Mbofung, 2012). Recalcitrant seeds placed in the relatively high-water content environment would keep the metabolism in seed active, leading to germination. Early germination involved three phases of an increase in seed fresh weight; Phase I, imbibition of water by the seed; Phase II, active metabolic activity but little water uptake; Phase III, radicle protrusion and additional water uptake (Bewley *et al*., 2013). Recalcitrant seeds, particularly tropical species, are often chilling-sensitive and cannot be stored at temperatures below 15°C (Tommasi *et al*., 2006). Cacao (*Theobroma cacao*) trees are propagated using seedlings; thus, the handling of good quality seeds is important for conservation. Despite the falling cacao beans production in recent years, cacao is still the third most important agricultural export after palm oil and rubber for Malaysia. Categorized as recalcitrant, cacao seeds are better stored at their shedding water content (FAO, 2013). To date, reports on the cacao seed reactions in the short-term of low-temperature storage are still scarce. A recent study demonstrated that they could be stored at 16°C and 25°C in sealed polyethylene bags for at least 144 hours, and still produced higher germinability, lower abnormal, with no dead seedlings recorded (Shahruddin *et al*., 2019). A further experiment on the manipulations of storage RH may yield useful information regarding the loss of seed quality. Therefore, the objective of this study was to evaluate the effect of moisture equilibration on the germinability and seedling performance of cacao seeds stored at different temperatures and RH.

METHODOLOGY

Plant Materials

Ripened cacao pods of PBC 123 clone were provided by the Malaysian Cocoa Board, Tawau, Sabah. The harvested pods were transferred soonest possible to the laboratory at Universiti Malaysia Sabah (UMS), Sandakan, Sabah, Malaysia. Upon arrival, seeds were extracted from pods, demucilaged using sawdust, and cleaned using a soft sponge.

Storage Conditions

Cacao seeds were packed into (28 x 20 cm) zip-lock polyethylene bags (250 seeds per bag) and stored at; (i) 16°C, 80% RH, (ii) 16°C, 40% RH, (iii) air-conditioned room temperature, RT (25±2°C, 55±5% RH), and (iv) control (seeds freshly extracted from pods). Seed hydration involved the use of a microprocessor-controlled console-style germinator (Seedburo MPG-3000, USA).

Experimental Design and Statistical Analysis

The experiment was arranged in a split-plot randomized complete block design (RCBD) with three (3) replications. Seeds were sampled every 24 hours for 144 hours and were evaluated for moisture content, leachate conductivity, germinability, and seedling performance. Data were subjected to analysis of variance with SAS version 9.4, and the significant means were separated by the least significant difference (LSD) test at $p < 0.05$.

Measurement of Seed Moisture Content (MC)

Ten seeds per replication were drawn and dried at 105°C for 16 hours (ISTA, 1999). MC was determined by the following formula:

$$\% \text{ MC} = \frac{(\text{Fresh weight - dry weight})}{\text{Fresh weight}} \times 100$$

Measurement of Leachate Conductivity (LC)

Five seeds per replication were drawn, weighed, and soaked in 25 ml ultrapure water (Mullet and Wilkinson, 1979). After 24 hours, the leachate was decanted, and LC was measured using the Eutech PC2700 (Conductivity) Meter.

Measurement of Germination Percentage and Germination Rate Index (GRI)

Germination was conducted in trays and maintained at the laboratory (25±2°C). Fifty seeds per replication were kept between moist cloth towels and observed for 14 days. Seeds were considered germinated when 2 mm protrusion of the radicles were visible (ISTA, 1999). GRI reflects the speed of germination (Kader, 2005), and calculated by the following formula:

$$\text{GRI (\% per day)} = G1/1 + G2/2 + \ldots + Gx/x$$

Where; G1 is germination percentage x 100 on the first day after sowing.

Measurement of Seedling Dry Biomass and Evaluation of Normal, Abnormal and Dead Seedlings

Thirty germinated seeds were sown in a mixture of 20% chicken manure and 80% topsoil, in the black polyethylene bags (22 x 10 cm). Seedlings were grown under rain shelter with 70% shade. Seedlings were harvested at the end of the 6th week, were dried in an oven at 80^{o}C for 24 hours, and the weight was recorded (Vanitha *et al*., 2005). The percentage of normal (morphologically complete), abnormal and dead seedlings were recorded at the end of the test period. The abnormalities stunted and tip dry in seedlings were the common deformities observed in this study.

RESULTS AND DISCUSSION

In this study, the moisture content (MC) of cacao seeds was recorded to be in the range of 45 - 56% throughout 144 hours of storage. The seeds might already in their Phase II of water uptake but with different metabolism rates among storage conditions. This concurred by Vertucci (1993), who reported that among the five states of hydration levels in seeds, respiration activity was first observed in the type 3 water level (approximate 26 - 45% MC). Seeds at RT (25±2°C, 55±5% RH) showed no significant differences in MC than the control (seeds freshly extracted from pods) at all storage durations (Figure 1A). The same pattern of MC displayed by seeds at 16^{o}C, 40% RH, except it declined from 49.1 to 44.9% after 96 hours of storage. Seeds at 16^{o}C, 80% RH showed a significant increase of MC from 49.1 to between 52.3 and 56.2%, after 48 to 120 hours of storage. Umarani *et al*. (2015) reported that slow-drying (drying of seed tissues with testa or in closed containers) at the ambient (25 - 28^{o}C) could help to reduce the mechanical damage to the seeds due to the delay time taken to cross the critical seed MC. The constant adjustments of seed MC at RT might further stabilize it to be at the same level as the control. However, the on-going metabolic activity could expose seeds to increase respiration, heating, and fungal invasion (Suma *et al*., 2013). Dresch *et al*. (2014) reported that under laboratory conditions (25˚C), the recalcitrant *Campomanesia* sp. seeds with 31.2% MC exhibited primary root protrusion and allowed for the fungal invasion during storage. In this study, more germinated seeds and fungal invasion was evident during storage at RT than seeds at 16°C. In contrast, regardless of the RH, seeds at 16^{o}C showed minimal fungal invasion with no seeds germinating during storage. Thus, it might eliminate or reduce the fungal invasion as one of the factors contributing to the seed deterioration process.

The higher leachate conductivity (LC) reflected the poorly structured membranes and is associated with the seed deterioration process (Bewley *et al*., 2013). Seeds at RT exhibited a significant increase in LC from 82.1 to 175.1 µS g^{-1} FW after 48 hours (Figure 1B). The higher storage temperature could ameliorate the membrane damages through the humidification of the dry membrane to a more fluid gel state (Bewley *et al*., 2013). Less leakage might occur for seeds at RT, due to the reduction of LC after 96 hours of storage. In contrast, seeds at 16^{o}C, 40% RH, led to show the significant gradual increase of LC, starting after 24 to 144 hours of storage (from 82.1 to between 128.3 and 197.4 µS g^{-1} FW) (Figure 1B). The water potential (WP) of cacao seed tissues tended to change rapidly under low RH (Liang and Sun, 2000). Thus, the well-organized seed tissues at the lower RH (as compared to the ones at 80% RH) might be disturbed by the uneven and rapid volumetric change and caused more mechanical stresses. Once the seeds exposed to higher water content or vapor from surroundings, it results in a phase transition back to the hydrated bilayer membrane, and severe leakage might occur during this transition phase (Bewley *et al*., 2013).

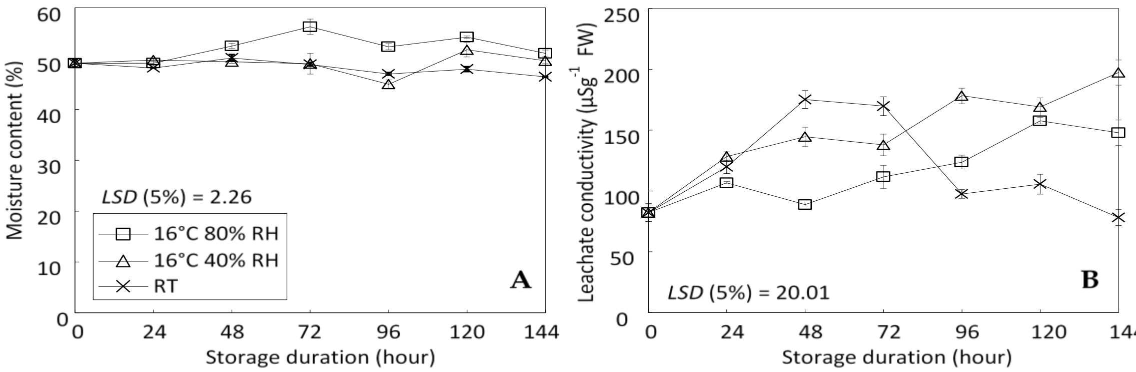

Figure 1. (A) Moisture content and (B) leachate conductivity of cacao seeds stored under different storage conditions. Data are means ± standard error.

All storage conditions recorded no variations in the final germination percentage than the control at all storage durations (Figure 2A). On the other hand, seeds at RT displayed a significant gradual increase of germination rate index (GRI) from 43.4 to between 53.2 and 75.4% per day after 48 to 96 hours of storage (Figure 2B). The favorable condition could increase the efficiency to utilize food stocks, thus, contribute to the higher accumulation of dry matter in the seed (Bahri *et al*., 2018). Seeds at RT displayed a better seedling performance through the production of 100% normal seedlings (Figure 2D), with the seedling dry biomass (MASS) at the same level as the control at all storage durations (Figure 2C). In contrast, seeds at 16°C, 80% RH showed significantly lower GRI from 23.8 to 32.8% per day (Figure 2B). In this study, negative correlations produced between MC and GRI (r = -0.49). In parallel with that, seeds at 16°C, 80% RH showed a significantly higher MC than the control, during the middle of storage durations (Figure 1A). There will be a WP gradient between the seeds and their surrounding due to the increase of cell expansion through the conversion of stored polymeric reserves to more osmotically active building blocks in the seed, e.g., starch to sugars (Bewley *et al*., 2013). The greater solutes concentration lowering the WP in seed tissues and resulted in a driving force for water uptake during Phase II, which explained the increase of seed MC. Reductions of WP in the low storage temperature might cause slow respiration rates in seed, and the extension time required for germination (Bewley *et al*., 2013). As the seeds stayed longer at the intermediate water contents, it might induce the aqueous-based deleterious processes from the disruption of coordinated metabolism to the failure of antioxidant systems (Liang and Sun, 2000). Since repairing the disturbed metabolism in seeds is a time-consuming process, it resulted in the delay in germination, which could be the earliest signs of deterioration exhibited by crop seeds (Suma *et al*., 2013).

In contrast, the metabolic activity prior to the germination of seeds at 16°C, 40% RH, seemed to counteract their membrane changes and displayed GRI at the same level as the control (Figure 2B). This is also supported by Liang and Sun (2000), which suggested that the mechanical and metabolic stress in seed tissues during drying could have been minimized when their WP increases and decreases, respectively. Seeds at 16°C, 40% RH generally showed better germination characteristics, and the lower abnormal seedlings than seeds at 80% RH. Nevertheless, seeds at 16°C, 80% RH showed better MASS, after 24 to 96 hours of storage. Bahri *et al*. (2018) found that cacao seeds with higher MC might have higher growth potentials. Seeds at 16°C, 80% RH, which equilibrated at higher MC (49 to 56%), showed a significant increase of MASS from 1.8 to between 1.9 and 2.1 g/plant, after 24 to 72 hours of storage (Figure 3A). However, it might lead to 'starvation' of meristem tissues due to the reduction of the food reserves in seeds through increased respiration prior to germination (Saajah and Maalekuu, 2014). The results suggested that a more severe deterioration process might occur in seeds at 16°C, 80% RH, which showed the higher decreasing pattern of MASS (59% of decreased) than seeds at 40% RH, after 48 hours of storage.

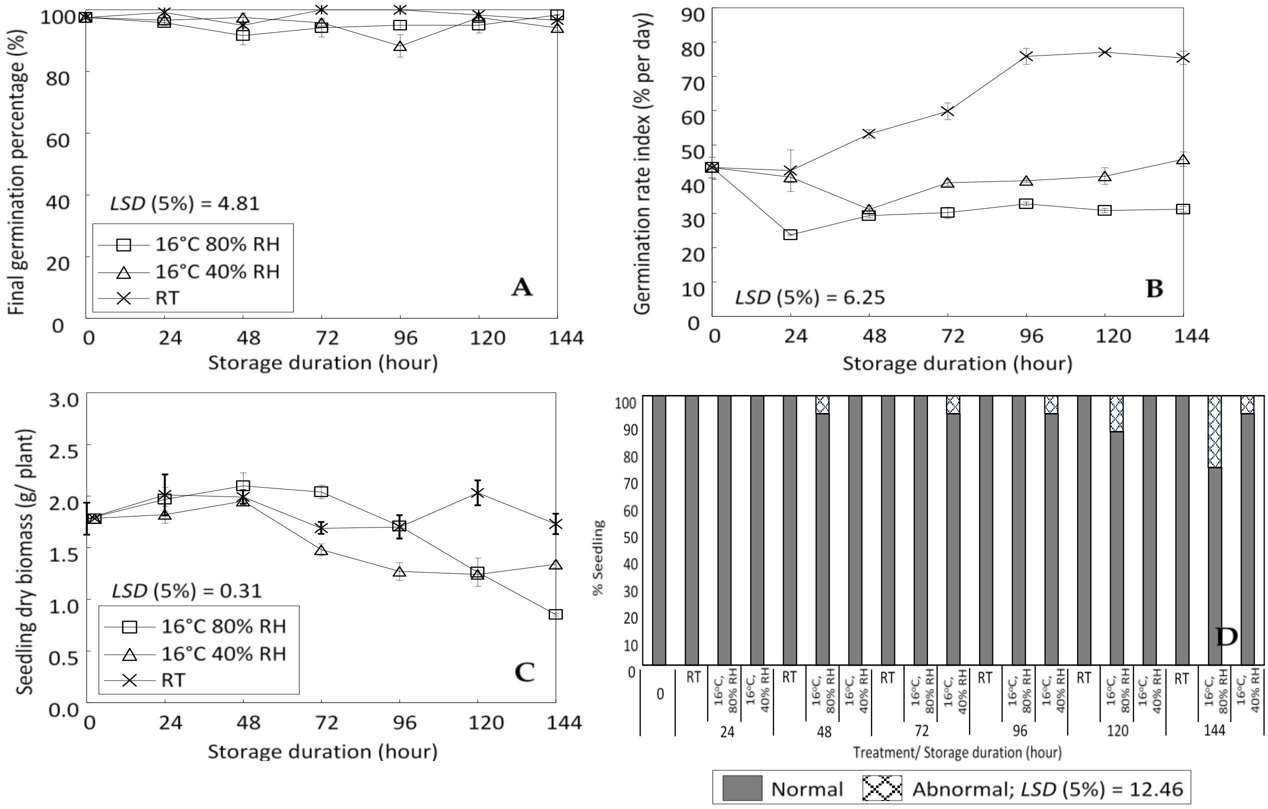

Figure 2. (A) Final germination percentage, (B) germination rate index, (C) seedling dry biomass, and (D) seedling performance of cacao seeds stored under different storage conditions. Data are means ± standard error.

CONCLUSION

Cacao seeds with high moisture content had a relatively low germination rate index. Despite the deterioration associated with seed aging and increased pattern of leachate conductivity, the storage condition of 16°C, 40% RH showed more potential to improve germinability or germination characteristics, with less reduction in seedling performances. Further experiments will be necessary to evaluate biochemical changes, which could be associated with the deterioration of cacao seeds.

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