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GERMINATION OF *Canarium odontophyllum SEEDS AS AFFECTED BY DESICCATION*

(PERCAMBAHAN BIJI BENIH *Canarium odontophyllum* SETELAH PENGERINGAN)

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ABSTRACT

Canarium odontophyllum is a rare tropical fruit that is primarily propagated by seeds. However, there is little information regarding its seed handling and germination. As low seed moisture content (MC), generally that below 10%, is the key determinant to successful seed storage while avoiding microbial damage, the objective of this study was, hence, set to investigate *C. odontophyllum*'s seed germination as affected by desiccation using drying beads (DBs) at room temperature or convection oven at 40℃ to varying seed MCs, from its initial MC of above 20% down to that below 10%. It was found that the seeds tolerated fast dehydration within 24h at room temperature using DBs. They retained 90% germination despite seed MC was reduced to below 10%. Desiccation tolerance with DBs was, however, found only with seeds extracted from fresh fruits. As the fruits started rotting with wrinkled appearance and fungi on the pericarp from four days after harvest onwards, the seeds extracted from them died at MC of below 15%. A slower seed drying method using convection oven at higher temperature of 40℃, on the other hand, took 72h to bring the seed MC down to <10%, as compared to only 24h when DBs were used for the same purpose. However, this 40℃ oven desiccation down to the same low MC of <10% was lethal. Storage of the seeds of this rare fruit species for future planting is, hence, presumed to be impossible, and seeds are best sown fresh.

Keywords: Rare fruit; drying bead; convection oven

1. Introduction

Canarium odontophyllum Miq., or locally known as Dabai, Sibu Olive etc., belongs to the family Burseraceae. It is listed as a rare fruit species in Malaysia. In the recent years, it has started gaining popularity for consumption among Malaysians following its publicity as a specialty fruit with high antioxidant contents by the Agriculture Department of Sarawak (Azrina et al., 2009).

Canarium odontophyllum is primarily propagated by seeds. Vegetative propagation methods of this fruit tree such as bud grafting are barely successful (Chai et al., 2010). Limited documents on the seed germination of this fruit species, on the other hand, indicated the inconsistent and low germination rates (Salma & Khadijah, 2008). As the fruit availability is seasonal, generally available from the months of October to January, good knowledge on the handling of its seeds as planting materials will be useful for the

cultivation of this fruit species and subsequent production of the fruits to meet the market needs.

Information on seed desiccation tolerance is the key to successful seed handling to retain its viability as planting stock. It is also crucial to indicate storage potential, and subsequent germplasm conservation of the plant species. Desiccation tolerant or orthodox seeds caneasily be stored well after postharvest dehydration to moisture content (MC) of 5-7% (Chin & Krishnapillay, 1989; Berjak & Pammenter, 1994; Hong et al., 1996; Tommasi et al., 2006; Berjak & Pammenter, 2013). Recalcitrant seeds, on the other hand, cannot be stored with no impairment as they usually died at MC of 20-25% (Chin & Krishnapillay, 1989; Berjak & Pammenter, 1994; Hong et al., 1996; Berjak & Pammenter, 2013; Umarani et al., 2015).

The effect of dehyration rate on seed survival has frequently been reported for seeds that are sensitive to drying (Liang & Sun, 2002; Berjak & Pammenter, 2013). In some past studies on such seeds, seed viability was generally lost when dried slowly but survival could be retained with fast drying procedures to the same low MC level, or to an even lower MC state (Pammenter & Berjak, 2000; Berjak & Pammenter, 2004; 2013). Rapid drying may help to retain seed germination by preserving membranes and nuclei as seed tissues spend less time at the partially dried state, while under slow drying, seed tissues spend a longer period of time at the intermediate water content, at which deteriorates membrane structure and accumulates damaging metabolites, which, in return, lead to viability loss (Liang & Sun, 2002). Survival ata lower MC has been reported with rapidly dried recalcitrant *Ekebergia capensis* seeds from Meliaceae, when compared to slow drying that completely caused viability loss (Pammenteret al., 1998). Similarly, fast drying also allowed the excised embryonic axes of recalcitrant *Trichilia dregeana* and *Avicennia marina* to retain much higher survival rate despite dehydration to a much lower MC (Varghese et al., 2011; Ntuli et al., 2014).

Canarium odontophyllum seeds were found to have initial MC of approximately 23% based on some preliminary work (Masarip et al. 2016). This MC of the fresh seed is rather close to that of the orthodox seeds (Chin & Krishnapillay, 1989; Hong et al., 1996; Umarani et al., 2015) but information available so far has not indicated its possible storability. Despite its relatively low initial seed MC, it has large embryo measuring 13-23 mm (Masarip 2016), which is a typical feature of recalcitrant seeds (Hong et al., 1996; Farnsworth, 2000; Liu et al., 2014). It is, hence, important to conduct further study to understand its planting value, especial that for storage for future planting. The objective of the current research work on *C. odontophyllum* was, therefore, aimed to determine its seed germination as affected by fruit freshness and seed desiccation rate, i.e. fast drying using drying beads (DBs) at room temperature and slower seed desiccation using convection oven at higher temperature of 40°C, from the seed initial MC of above 20% to varying lower seed MCs. The fruits are known to have only a few days' shelf life. The rotting fruits are not suitable for consumption but hoped to still be of value for planting purposes.

2. Materials and Methods

2.1. *Fruit collection*

Ripe fruits with purplish-black pericarp (Lau & Brooke, 2013) were collected from a private land in Kuching-Serian Road of Padawan District, Serian (1 ° 22′41.10″N, 110 ° 22′52.5″E). The location was characterized with tropical climate having above 3,000 mm annual rainfall distributed rather evenly throughout the years. Average daily day and night temperatures were 24°C and 33°C, respectively. At least 480 fruits with pedicels attached

were harvested from a single tree as test materials. Removal of pedicels causes rapid fruit deterioration under ambient temperature (Sim & Lau, 2011). Harvested fruits were brought to laboratory, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Samarahan Campus, Sarawak, on the following day.

2.2. *Seed extraction*

On the next day after fruit arrival, 160 fruits were picked randomly and their pedicels were removed. The other 160 fruits were left at room temperature in laboratory for four days priorto seed extraction. Pericarp wrinkled slightly and less than 10% of the pericarp surface had fungal infestation by this point of time (6^{th} day after harvest). The remaining 160 fruits were left further for seven days (9^{th} day after harvest) in the same laboratory prior to seed extraction. The pericarp showed wrinkled appearance, though the fruits still had moist tissues, and fungal infestation on the pericarp was obvious at this further point of time after harvest.

For seed extraction, the fresh fruits and those left for four and seven days in the laboratory as mentioned above were respectively soaked with tap water in a large plastic basins for three days to soften the pericarp and mesocarp. After the soaking procedure, seeds with endocarp attached were extracted carefully from the fruits using a knife.

The seed with endocarp attached was termed as seed and used in this study as test material.Its true seed within endocarp was not employed in this study as it has papery like testa which cannot be separated from the endocarp without damaging the testa, cotyledon and embryonic axis within it. The seeds extracted from fruits were then cleaned with running tap water, pat dried and air dried for 1h before experimentation.

2.3. *Seed desiccation*

Seeds were dehydrated using DBs at room temperature and at 40°C in a convection oven with the fan set to 3 (middle speed), respectively. DBs are modified ceramic materials that are capable of absorbing and holding water molecules very tightly and rapidly in their microscopic pores. They can absorb moisture fast up to 25% of their initial weight even at room temperature, and are rather consistent in absorbing moisture in air-tight containers, and are hence, useful for fast desiccation of seeds. Seed desiccation in the convection oven, on the other hand, is slower, as compared to desiccation using DBs, and can vary in bringing down the seed MC, depending on the ambient relative humidity. Seed drying using DBs or convection oven was designed to achieve MCs of 15% to <20%, 10% to <15% and <10%, and compared with non-desiccated control seeds with MC 20% to <25% on fresh weight basis.Actual seed MC following DBs and convection oven desiccation was determined according to the method described by ISTA (2005). Seed MC determination was carried out with oven drying at 103±2°C for 16±1h in this procedure.

With commencement of fruit soaking prior to seed extraction, DBs were regenerated by heating them at 230°C for at least 2h in the convection oven to release the water molecules trapped in their microscopic pores. Then, these beads were poured into an air-tight glass jar to cool to room temperature for at least one day.

To determine the suitable amount of DBs to bring down the seed MC to a desired level, information on water holding capacity (WHC) of DBs, initial MC of *C. odontophyllum* seeds and weight of the seeds to be dried were needed. WHC of regenerated DBs was determinedby placing 100 g DBs on a raised or standing stainless mesh tray in an air-tight container with 100 ml tap water beneath the tray. The DBs were then left for 1h under air-tight condition to absorb the water molecules within the container up to their saturation level. WHC of DBs was calculated as:

$WHC\ (\%) = (H_1 - H_0)\ x100 / H_0$
where,
H_0 = Initial weight of regenerated BDs (g)
H_1 = Weight of BDs after absorbing water molecules to saturated level (g)

With reference to the WHC of DBs, the amount of DBs in relation to weight of seeds to be dried was determined based on a reference table of initial seed MC-desired seed MC provided by the manufacturer of DBs. Seed desiccation using DBs was carried out at room temperature for 24h in air-tight plastic containers to bring seed MC to 15% to <20%, 10% to <15% and <10%, respectively, as explained above, while the control were non-desiccated seeds.

On the other hand, desiccation using convection oven at 40°C to achieve MC of 15% to <20%, 10% to <15% and <10%, respectively, was achieved simultaneously by drying the seeds laid out as monolayers for 1, 2 and 3 days in the oven. These drying periods were determined by preliminary studies on oven desiccation of *C. odontophyllum* seeds. The desiccation treatments using DBs and convection oven above were applied to seeds extracted from fresh fruits, fruits left for four and seven days at room temperature in the laboratory, respectively, as described above.

2.4. *Data collection and statistical analysis*

Actual MC and germinability of seeds extracted from fresh fruits, fruits stored for four and seven days as above, respectively, were recorded after drying treatment using DBs or convection oven. A total of 10 seeds were picked up randomly from each drying treatment for determination of actual seed MC on wet weight basis according to ISTA (2005) as described above. The other 10 seeds were sown in moistened sand sized 0.2 - 2 mm as germination test. Seed germination count was carried out daily for three weeks.

The germination test was terminated after three weeks as all the non-germinated seeds were found rotting (dead). Data were subjected to descriptive analysis.

3. Results and Discussion

Seeds extracted from fresh fruits and fruits left for four and seven days in laboratory did not differ much in their initial MC. Their MC ranged from 24.01% to 24.96% and the average MC was 24.5%.

Seed desiccation using different amount of DBs to achieve MC of 15% to <20%, 10% to <15% and <10% within 24h, respectively, was rather reliable; average seed MCs achieved were 16.37±1.25, 12.66±0.70 and 9.01±0.67 as planned (Table 1). On the other hand, slower seed desiccation method using convection oven at 40°C for 1, 2 and 3 days resulted in slightly lower seed MCs of 15.00±0.54, 9.91±1.96 and 7.56±0.98, respectively, as compared to the planned seed MC ranges (Table 1).

Table 1: Actual seed MC (%) as affected by drying using DBs and convection oven

Desiccation method	Planned seed MC range (%)	Mean actual seed MC±SE (%)
DBs	<10	9.01±0.67
	10 - <15	12.66±0.70
	15 - <20	16.37±1.25
	20 - <25 (Initial MC)	24.4±0.87
Convection oven	<10	7.56±0.98
	10 - <15	9.91±1.96
	15 - <20	15.00±0.54
	20 - <25 (Initial MC)	24.6±0.41

In seed desiccation using DBs, fast dehydration caused visible fine cracks on the endocarp of approximately 3 mm in thickness (Figure 1). As high as 90% of the seeds dried to MC of 15 - <20% (mean MC of 16.37%) using DBs had fine cracks on the endocarp. Desiccation using greater quantity of DBs to reduce seed MC to 10 - <15% (mean MC of 12.66%) and
<10% (mean MC of 9.01%), respectively, resulted in similar fine cracks on the endocarp of all the seeds. The ability of DBs to withdraw moisture rapidly from the endocarp and seed (embryo) could have cracked the endocarp. There was, however, no such visible cracks on theendocarp of the seeds subjected to desiccation using convection oven at 40°C for even up to three days to reduce seed MC to <10% (mean MC of 7.56%).

Figure 1: Fine cracks on endocarp as circled following desiccation using DBs

Germination of *C. odontophyllum* seeds was found affected by both the fruit freshness and desiccation rate. With seeds extracted from fresh fruits, these seeds could retain viability of 90-100% with fast dehydration using DBs, even with desiccation to seed MC of <10%(Figure 2A). In contrast, seed germination was relatively lower at 60% at MC of 15 - <20% following slower desiccation at 40 ° C in the convection oven for the same period of 24h (Figure 2D). Further drying for two and three days in the convection oven to reduce seed MC to 10 - <15% and <10%, respectively, was lethal (Figure 2D). The effects of drying rate on seed viability can be associated with several factors including the nature of the seed coverings like endocarp in the current study (Farrant et al., 1993; Hong et al.,

1996; Berjak & Pammenter, 2008; Hill et al., 2012; Lan et al., 2012).

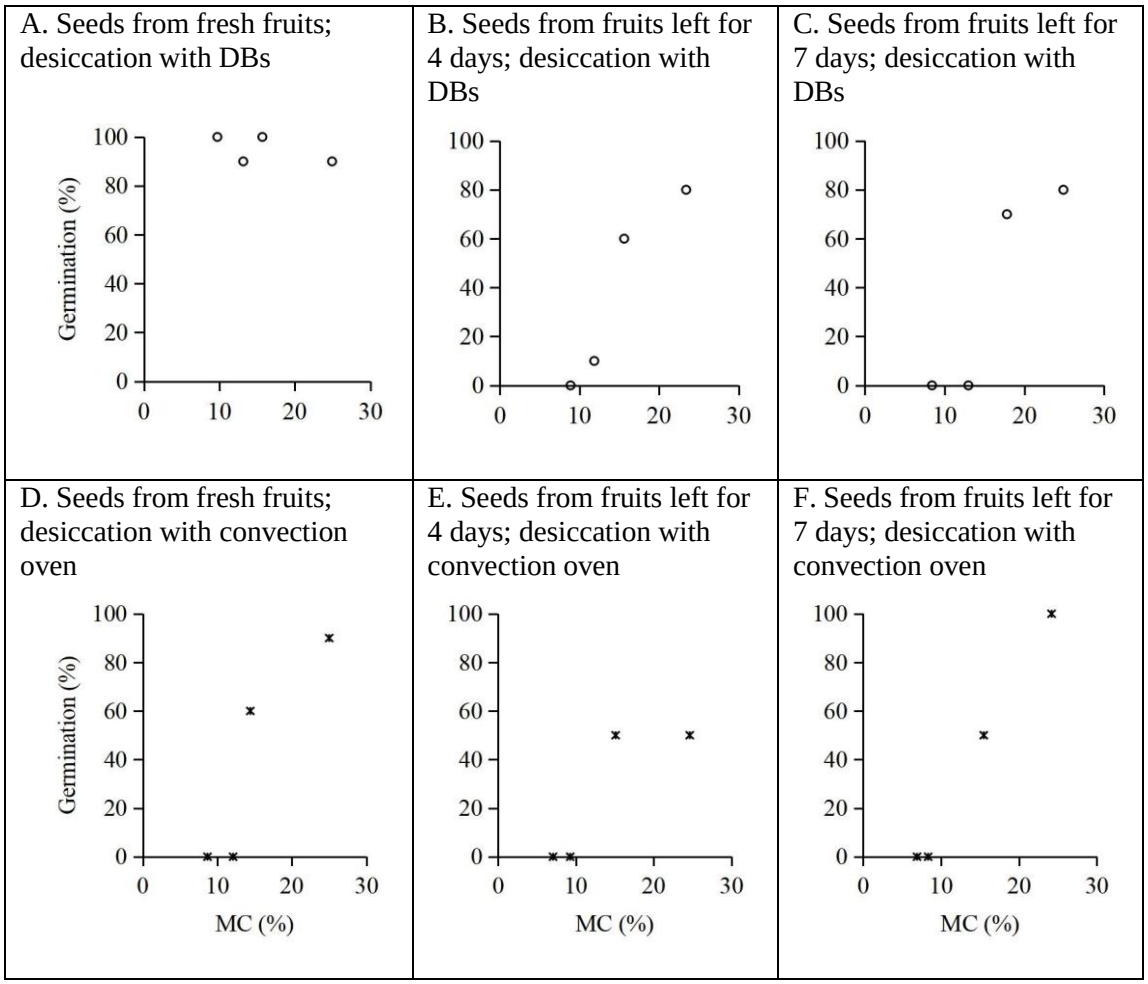

Figure 2: Seed germination as affected by fruit freshness and desiccation

With the seeds extracted from rotting fruits as they were left for four and seven days at room temperature in the laboratory, the non-dehydrated seeds could still retain their value for planting with 50-100% germination as shown in Figures 2B, 2C, 2E and 2F. However, the seeds obtained from the rotting fruits were sensitive to further desiccation. Those desiccated using DBs to MC of 15 - <20%, as in Figures 2B and 2C, showed lower germination of 60- 70% while such fast desiccation with DBs could retain germination of 90-100% with the seeds extracted from fresh fruits, as in Figure 2A. Further desiccation of these seeds extracted from deteriorating fruits to MC of below 15% caused 90-100% mortality, despite rapiddesiccation using DBs (Figures 2B and 2C).

Drying the seeds extracted from the rotting fruits in the convection oven of 40°C, on the other hand, resulted in similar seed germination trend as that carried out on the seeds obtained from fresh fruits, i.e. 50% seed germination at seed MC of 15 - <20%%, while total loss of germination was recorded with lower seed MC of 10 - <15% and <10%, respectively (Figures 2E and 2F).

Based on the above results, retaining viability of *C. odontophyllum* seeds can, hence, be difficult without impairment. Only seeds obtained from fresh fruits, as in Figure 2A, could tolerate low seed MC of below 10% while maintaining above 90% seed germination following rapid desiccation using DBs. Seed desiccation in convection oven of 40°C, on the other hand, was less favourable, even for the seeds extracted from fresh fruits. Desiccation for two days or longer in the convection oven to reduce the seed MC was found detrimental.

As the fruits started rotting after four days, the seeds within them had very much reduced planting values, besides being unsuitable for consumption. Great loss of viability of 90-100% was recorded with the seeds extracted from them following desiccation to <15% MC, irrespective of the desiccation speed of either using DBs or in convection oven of 40°C.

Despite tolerance to low MC of below 10% as achieved using DBs, the seeds, however, could not be stored by the conventional method in non-aseptic hermatic containers as seed drying using DBs resulted in cracks on endocarp. The cracks expose the very thin testa and embryo (cotyledons and embryonic axis) within endocarp to possible microbial infestation. The seeds must, therefore, be sown fresh without desiccation procedure, and are considered difficult to handle and cannot be kept for future planting, while aseptic in vitro storage may bethe other option for storing and conserving this rare fruit germplasm.

4. Conclusion

Canarium odontphyllum seeds are best sown fresh. Seeds extracted from fresh fruits could tolerate fast desiccation to MC of below 10% using DBs while retaining 90% germination but such desiccation method caused fine cracks on the endocarp, which is deemed unsuitable for safe seed storage. Slower seed desiccation to MC of below 10% in the convection oven of 40°C, on the other hand, was lethal. Seeds extracted from fruits with wrinkled appearance and fungi on the pericarp had relatively lower germinability, besides being sensitive to seed desiccation with either DBs or in convection oven.

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