DESICCATION AND THE STORABILITY OF DESICCATION-SENSITIVE SEEDS OF A DIPTEROCARP SPECIES, HOPEA ODORATA

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ABSTRACT

Some species, particularly trees, may not be able to be preserved due to sensitive seeds to desiccation. In this paper, the influence of seed moisture content and storage conditions on the germination of Hopea odorata seeds, a tropical forest species was explored. The first part of the study aimed to identify the effect of desiccation on the germination of these seeds. Seeds were dried down to several moisture content levels in silica gel: 40.1%, 39.8%, 34.9%, 32.0%, 21.7%, and 16.1%. The initial moisture content of fresh H. odorata seeds were 45.6% with 100% germination. Reduction of moisture content level to 40.1%, 39.8%, and 34.9% did not affect germination percentage, where germination percentages were maintained at 100%. However, the desiccation of H. odorata seeds from 32.0% to 21.7% moisture content reduced germination from 96 to 50%. No germination (0%) was detected once the seeds were further desiccated to 16.1%. From this observation, the estimated lowest safe moisture content (LSMC) for H. odorata was 32.0% and was desiccation sensitive as expected for recalcitrant seeds. In the second experiment, winged and de-winged seeds were stored under different temperatures and durations; with the presence of vermiculite. Results obtained revealed that the most effective way to store H. odorata seeds is at 20°C for 5 weeks with the wings removed; where 98% of the seeds germinated.

Keywords: *Recalcitrant, desiccation-sensitive, storage, germination, dipterocarp*

INTRODUCTION

Hopea odorata is one of the species in the family of Dipterocarpaceae; a family famously known for its important timber tree species. The tree of this species itself is medium-sized, but it can grow up to 45 m tall, normally found in lowland dipterocarp forests and also in seasonally dry tropical rainforests. Its wood is strong and durable, making it suitable for construction, furniture, and making boats. In a wider application, this species is also used for reforestation in Southeast Asia. In Malaysia particularly, this species is planted extensively as a roadside tree where the trees can easily be identified by the attractive conical-shaped crown. However, this species is considered vulnerable in the wild in Malaysia (Yong *et al*., 2021).

Hopea odorata is propagated by seeds and as the majority of dipterocarps produce recalcitrant seeds, which they are susceptible to desiccation (Berjak & Pammenter, 2008); freeze to death when exposed to sub-zero temperatures due to the formation of ice crystals in the cells (Berjak & Pammenter, 2013); and storage of these seeds for long-term seed banking is almost impossible and limited (Walters, 1998; Elliot 2013). In nature, the mature seeds are shed with high moisture content, high metabolic activity, and poor storage potential, which may constrain the *ex-situ* conservation effort for this species. Moisture content is one of the most important factors in preserving seed viability in storage because it regulates the metabolism of seeds (Harrington, 1972; Shaban 2013). Thus, to store these seeds properly, it is necessary to determine the lowest moisture level that these seeds can tolerate while remaining viable. According to Roberts (1973), a small change in a seed's moisture content affects germination and storage life. Several studies showed that partial drying to just above a critical moisture level has allowed for the storage of recalcitrant seeds (Oliveira (1992); Maluf (2003); Lan (2012); Leao-Araujo, E.F. (2022).

Hopea odorata produces fruit periodically and in Peninsular Malaysia, trees produce mature fruit from May to June. This results in the need to strategize the storage method to assure their seed viability before planting season. On the other hand, seeds of *H. odorata* are categorized as recalcitrant and since they are sensitive to drying and low temperatures, storing *H. odorata* seeds is not always easy in practice. Loss of viability always occurs when this type of seed is stored at a moisture content of below 20 to 30% (Pritchard, 2004). As a result, it is important to develop an appropriate seed storage method that would allow a high percentage of viability and survival for this species.

This paper highlights the findings on the desiccation sensitivity of *Hopea odorata* seeds collected in Peninsular Malaysia and their storage potential under moist conditions with the wings removed, at different storage temperatures.

MATERIALS AND METHODS

SEED COLLECTION

Matured *Hopea odorata* seeds were collected from trees in Selangor, Malaysia in May 2022; as soon as three-quarters of the wings became brown. Seeds were brought back to the Seed Technology Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Selangor where the studies were carried out.

SEED MOISTURE CONTENT

A total of fifteen seeds were used in this test, for all experiments involved; with five seeds in every three replicates. The seeds were cut into halves for uniform drying in the oven at 103±2°C for 17 hours according to ISTA (1993). The weight of the seeds was determined before drying (fresh weight) and after drying (dry weight). The percentage of moisture content was calculated using the following formula:

$$\text{Moisture content (\%)} = (FW - DW) / (FW) \times 100$$

SEED GERMINATION

A germination test was carried out using six replicates of ten seeds, where the de-winged seeds were placed on moist paper in a glass petri dish and placed at a temperature of 28±2°C. Germination was observed on an alternate day and was counted as germinated when the radicals had emerged (≥ 1 mm). The germinated seeds were removed from the petri dish. Ungerminated seeds were assessed whether they were rotten or infected by fungus.

SEED DESICCATION

To perform this experiment, the wings were removed and de-winged seeds with an initial moisture content of 45.6% were placed in polythene bags and mixed with silica gel until they reached the targeted moisture content of 35%, 30% 25%, 20%, 10%, and 5%. Control samples were kept in similar bags filled with 100 g vermiculite. All bags were kept at room temperature of 25±3°C. The silica gel was changed when its initial blur color hand changed. When the seeds reached the desired moisture content, they were removed from the bags and subjected to a germination test and moisture content determination, where the actual moisture content was calculated. Seeds germinated under the conditions mentioned earlier.

SEED STORAGE

In this investigation, winged and de-winged *H. odorata* seeds at their initial moisture content were kept in loosely folded polythene bags in the presence of vermiculite. Each bag consists of seventy-five seeds for both moisture content (fifteen seeds) and germination test (sixty seeds). Seed samples were stored at three different temperatures; 12°C, 20°C and at room temperature (26±2°C; 60% RH) for 2,3,4 and 5 weeks. After each storage time, seed moisture content and germination were evaluated. For both winged and de-winged seed sample bags, a 100 g vermiculite was added as a storage medium.

RESULTS AND DISCUSSIONS

SEED DESICCATION

The initial moisture content was determined before conducting the experiments. *Hopea odorata* seeds showed high moisture content which is 45.6%; and is a characteristic of recalcitrant seeds. This type of seed does not go through maturation before being shed from the tree.

Figure 1. The actual moisture content of dried and controlled *H. odorata* seeds at six target moisture contents (35%, 30% 25%, 20%, 10%, and 5%). Bars represent the standard error of the mean

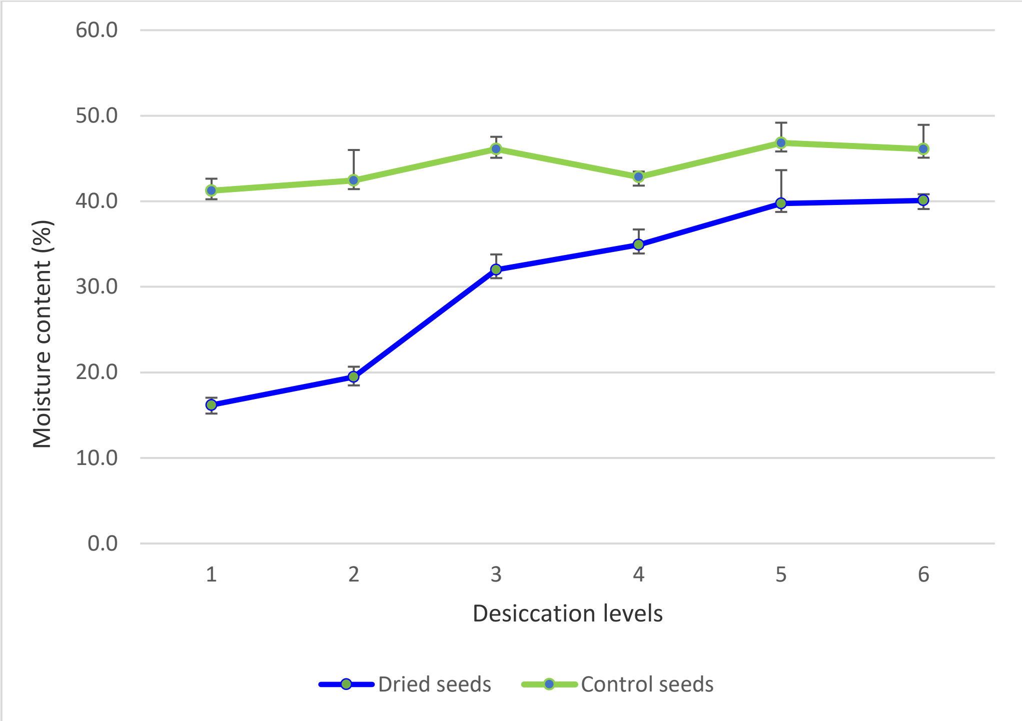
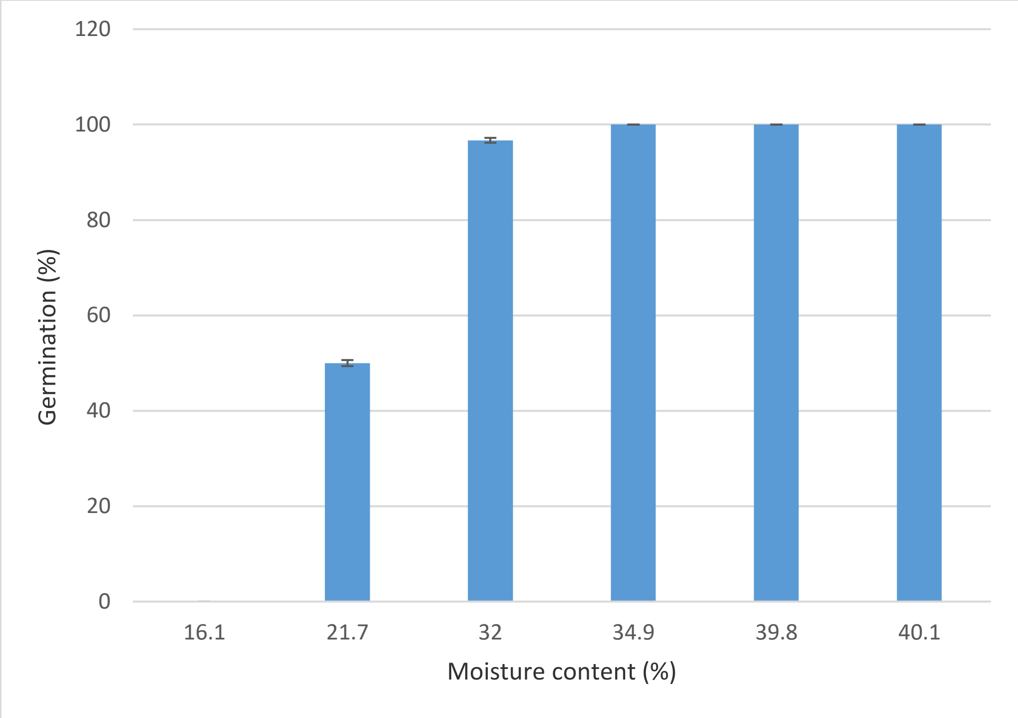


Figure 1 shows the actual moisture content obtained after the seeds were desiccated in silica gel at six different levels. Seed moisture content loss was drastically reduced from 32.0% to 21.75% after 23.17 hours of drying. The controlled seeds' moisture content, on the other hand, was maintained at 41.2% to 46.8%; which is in the range of the seed's initial moisture content (45.6%). Our present findings demonstrate the time needed to reduce the moisture content of *H. odorata* seeds from the initial moisture content of 45.6% to 16.7% was 30.5 hours, which was relatively slow (Table 1). In another study on *Shorea assamica* seeds, also a dipterocarp, the time required to desiccate the seeds was longer, i.e. 52 hours (Nashatul et al., 2022). Similar findings were obtained when seeds of *Baccaurea montleyana*, a tropical fruit species were dried. These seeds took 52 hours to be desiccated from their initial moisture content of 54% to 20% (Ghani et al., 2020).

Table 1. The time required (hour) to reach different moisture content (%) levels during the desiccation of *H. odorata* seeds

Desiccation level	Moisture content (%)	Time required to reach moisture content (h)
1	40.1	3.0
2	39.8	5.0
3	34.9	7.25
4	32.0	16.1
5	21.7	23.17
6	16.7	30.53

Figure 2. Germination of *H. odorata* seeds at different levels of moisture content. Bars represent the standard error of the mean



Desiccation of *H. odorata* seeds from the initial moisture content (45.6%) to 40.1%, 39.8%, and 34.9% of moisture, did not affect the germination percentages; where at these levels 100% of seeds germinated. A slight reduction in germination was observed (96%) at 32% of moisture content. However, at 21.7% of moisture content, the germination percentage dropped drastically; from 96% to 50%. No germination was detected in *H. odorata* seeds when they were dried to 16.1%. From this result, it is shown that seeds of *H. odorata* were desiccation sensitive and the lowest safe moisture content tolerable was 32.0%. These results are comparable with the findings by Nguyen et al. (2021) where the critical moisture content for *H. odorata* seeds collected in Vietnam, was 35%. However, in our findings, *H. odorata* seeds viability loss occurred at a higher moisture content (16.1%) whereas in Nguyen et al. (2021) the seeds lost viability at 8% moisture content. Several research on other Hopea species have also been conducted. Rajeeswari & Kaveriappa (2000) in their paper mentioned seeds of *H. parviflora* and *H. ponga* lose germinability when dried to 26 or 28% MC (wet mass basis), respectively. Meanwhile, Song *et al.* (1983) detected various ultrastructural damages in *H. hainanensis* seeds when the moisture content decreased from 34.9 to 25.6%, where the germination percentage declined from 65 to 20%. In another work, Nashatul *et al.* (2017) found that the critical moisture level for *H. subalata* was between 33 to 34% and this is close to the moisture content level we found with *H. odorata* seeds.

Seed death caused by desiccation occurs at or below critical moisture and as a result of membrane-related physiological damages as suggested by King and Roberts (1980), or some membrane dysfunction during desiccation (Hanson 1984) in Joseph et al. (2011). Leprince et al., (1999) suggested that desiccation sensitivity in recalcitrant seeds may be related to their inability to effectively suppress their metabolism during desiccation causing lipid oxidation, membrane degradation, and as a result, the death of the desiccation-sensitive seeds which is related to the accumulation of the reactive oxygen species (ROS).

SEED STORAGE

Figure 3. Germination of *H. odorata* seeds at different storage temperatures (12°C, 20°C, and RT) for 2, 3, 4 and 5 weeks. Seeds were stored with wings that remained intact and in the presence of vermiculite. Bars represent the standard error of the mean.

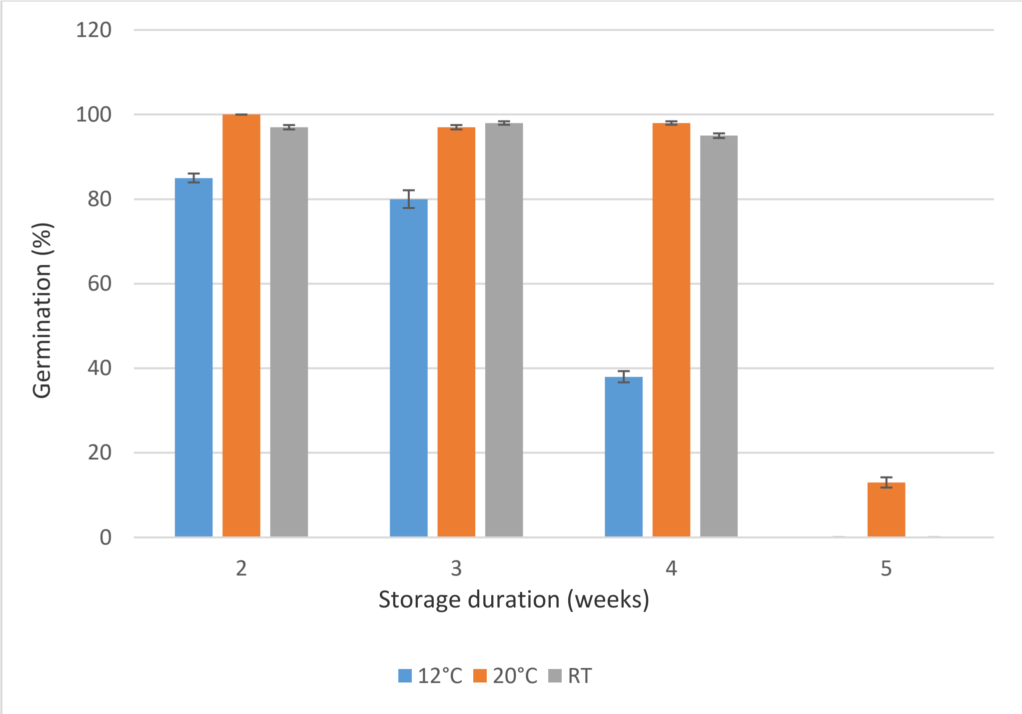
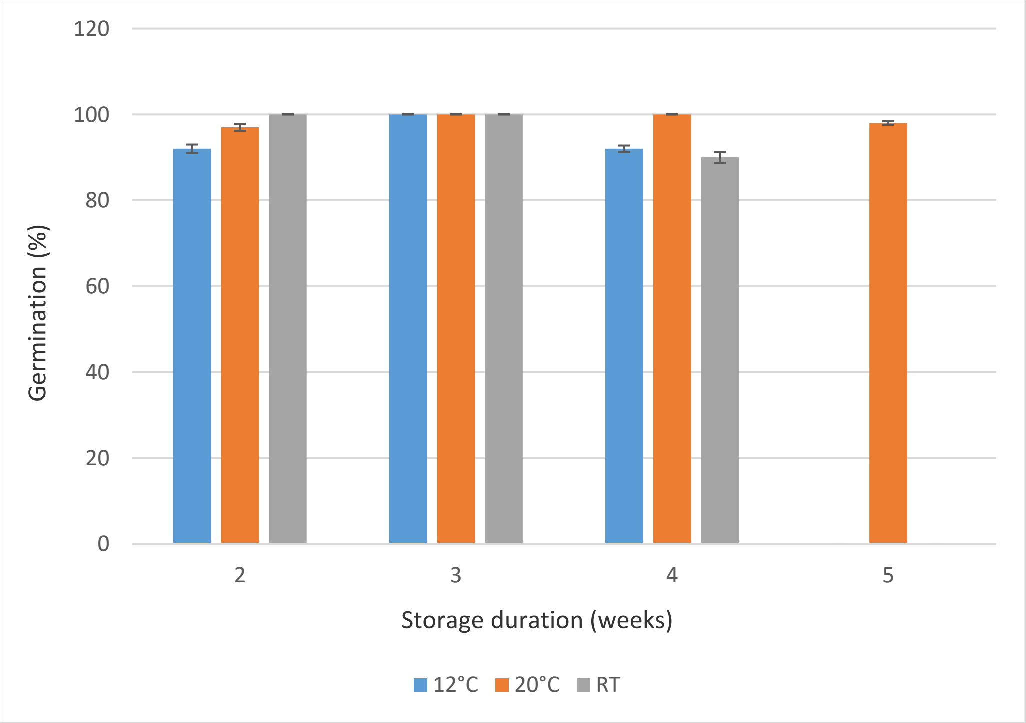


Figure 3 illustrates the germination percentage of *H. odorata* seeds without the removal of wings, in storage for five weeks at different temperatures. Generally, seeds stored at 12°C showed lower germination percentage in which only 85% of seeds germinated after two weeks of storage, and further reduced to 38% after four weeks. Finally, no seeds germinated after the fifth week. In contrast, winged seeds kept at 20°C and at room temperature showed high germination (more than 95%) after two weeks of storage until the fifth fourth week. But when germination evaluation was conducted on the fifth week, only seeds stored at 20°C germinated although the percentage was low (13%). At this time, all seeds stored at 12°C and room temperature were heavily contaminated by fungi.

Figure 4. Germination of *H. odorata* seeds at different storage temperatures (12°C, 20°C and RT) for 2, 3, 4 and 5 weeks. Seeds were stored with the wings removed and in the presence of vermiculite. Bars represent the standard error of the mean.



Keeping *H. odorata* seeds under the same storage condition as above but with the wings removed, demonstrated better results (Figure 4). Seeds stored at all temperatures for two to four weeks showed 90% to 100% germination. But after five weeks in storage, none of the seeds kept at 12°C and under room temperature, germinated; except those seeds at 20°C maintained high germination percentage (92%). This observation was similar to those of winged seeds at 12°C and room temperature but with the removal of wings, higher germination was obtained (98%). Based on the results obtained, longer storage for *H. odorata* seeds is expected to be achievable when the wings are removed and stored in moist conditions.

This study chose vermiculite as a storage medium due to its moisture-retention capacity to prevent seed dehydration. This type of storage is known as moist storage. Other examples of storage media with similar functions are perlite, sawdust, coconut dust, damp charcoal, and moist sand (Wen, 2009). The application of moist storage is appropriate mainly for seeds with recalcitrant traits where storage in a completely dry condition is almost impossible. Dried seeds lost their ability to absorb water and they eventually failed to germinate. Work by Nashatul *et al.* (2013) suggested keeping *Shorea leprosula* seeds, an important dipterocarp species, at 20°C in vermiculite with the wings removed. At this condition, 85% of seeds germinated after six weeks of storage.

Corbineau & Côme (1989) revealed that maintaining *H. odorata* seeds for 2 to 3 months at 20°C in cotton wool imbibed with deionized water gave 100% viability; but decreased to 75% and 0% at 15°C and 10°C. Meanwhile, in another study of seeds of the same species, germination was only 10% after 27 days of storage in refrigerator conditions of 0-4°C (Hoque et al. 2020). In this present study, similar observations were recorded where all seeds did not survive 5 weeks of storage at 12°C. These research findings were in line with a statement that storing *H. odorata* seeds at below 20°C caused a reduction in viability where chilling damage and injury could occur (Bonner, 2008).

CONCLUSION

Seeds of *H. odorata* were found to be desiccation sensitive where desiccation to a moisture content of 16.1% killed all the seeds. The lowest safe moisture content for *H. odorata* was 32.0%. Generally, high germination percentages were obtained (85 to 100%) when the de-winged seeds were stored at all temperatures (12°C, 20°C, and at room temperature) for up to five weeks; as compared to the winged seeds. Our results show that to maintain a high germination percentage in storage, it is suggested that *H. odorata* seeds should be partially dried above 32% of moisture content, and kept at 20°C with the wings removed; in moist storage of vermiculite. Findings from this study show the potential method of storing desiccation-sensitive seeds which can be applied in other laboratories handling tropical forest tree seeds.

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