Effect of Storage Time and Temperature on *Gyrinops walla* Gaertn. Seed Germination

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ABSTRACT

Certain members of Aquilaria, Gyrinops, Gonystylus and Aetoxylon genera of Thymelaeaceae family produce a highly valuable resin called agarwood inside the stem, branches and roots. Agarwood is extensively used for manufacturing perfume, incense sticks, traditional medicine and cultural and religious activities in many countries, especially in Asian and Middle East regions. Gyrinops walla is the only agarwood producing species naturally growing in Sri Lanka which can commonly be found in the low country wet zone. Although agarwood producing ability of G. walla was scientifically discovered in 2012, the information available about this species is limited when compared to its closely related species of Aquilaria origin. Due to the high demand of agarwood, G. walla is important to the economy of Sri Lanka and therefore plantation establishment using this species is expected by many sectors. Since large quantities of seedlings are required for plantation establishment, it is essential to discover the successful nursery practices. Therefore the present study was aimed at identifying the germination pattern and potential of storage of G. walla seeds at selected temperature levels. Fresh and healthy seeds of G. walla were collected from the trees growing in homegardens of Mathugama Divisional Secretariat Division. Three temperature levels were selected to store the seeds before sowing in the nursery beds, which resulted 18 different combinations of treatments and 50 seeds were used for each treatment. The selected temperatures for seed storage were room temperature, 8^0 C and -10^0 C and the storage time varied from 1 to 6 weeks. Stored seeds were sown at weekly intervals and direct sowing was done without storing as the control. Germination of the seeds were determined at weekly interval for 16 weeks by counting the seedlings. Percentage of germination was calculated and General Linear Model was used to determine the effect of storage temperature, storage period and their interaction on seed germination. In accordance with the results, most of G. walla seeds started germination within 1 to 2 weeks after sowing. Control showed the highest germination percentage (73.3%). The second highest germination percentage (26.7%) showed by the seeds stored at 8^0 C for 2 and 4 weeks. Seeds stored for 6 weeks at 8^0 C showed the third highest germination (16.7%). Further analysis

revealed significant differences in storage temperatures and time. The results confirmed that G. walla seeds are sensitive to the desiccation and therefore cannot be stored for a long period.

KEYWORDS: Agarwood, *Gyrinops walla*, Nursery practices**,** Seed germination, Thymelaeaceae

Introduction

Agarwood is a highly valuable, aromatic resin produced in the stems, branches and roots of *Aquilaria, Gyrinops, Aetoxylon* and *Gonystylus* genera (Blanchette, 2003; Compton & Zich, 2002) of Thymelaeaceae family, distributed in South and Southeast Asia. Among those four genera, species of *Aquilaria* and *Gyrinops* are the most common in production of agarwood resins which are used to produce aroma by burning, for manufacturing of perfumes, incense sticks, Chinese medicine and carvings in Asia, Europe and Middle East (Chaudhari, 1993; Persoon & van Beek, 2008).

Production of agarwood is not consistent and extensive harvesting of *Aquilaria* and *Gyrinops* species threatened the reproduction cycle in the natural environment. This situation drew the attention of the Convention of International Trade in Endangered Species of Wild Fauna and Flora (CITES) to control the agarwood trade by restricting the export quota of goods produced from those species (CITES, 1994; 2004). Demand for agarwood related production is well over the potential supply and therefore plantation establishment is becoming popular in the Asian region especially using *Aquilaria* species.

Gyrinops walla is the only agarwood producing species naturally growing in Sri Lanka, which can be found in lower elevations of the wet and intermediate zones. It grows up to 15 m in height with a straight, slender trunk with a small rounded crown. *G. walla* bears bisexual flowers in the inflorescence with small, slender, pubescent and yellowish flowers. Its fruit is about 1.8 cm long ovate shaped capsule and reddish brown in colour. It bears two tadpole-like seeds in two valves (Dassanayake & Fosberg, 1981).

The ability of producing agarwood in *G. walla* was scientifically discovered for the first time by Subasinghe et al., (2012) and Subasinghe & Hettiarachchi (2013). These findings confirmed that the agarwood produced in *G. walla* due to natural causes are chemically similar to that of *Aquilaria* species. Parallel to these findings *G. walla* became popular with the smuggling efforts due to the newly established high demand in many countries for naturally formed agarwood resins. As a solution to rapid resource decline and to fetch the high demand, both the government and private sectors of Sri Lanka is ready to establish *G. walla* plantations at small and medium scale. However, information available is limited for *G. walla* plantation establishment, especially on nursery establishment and plantation management.

Mostly trees produce seeds at long intervals, ranging from a few months to many years. Assured supply of seeds during the lean period can be achieved only from the seed stock held in storage (Umarani et al., 2015). Even if fruiting is regular and abundant every year, it may be more cost-efficient to collect surplus seed to cover the needs of few consecutive years. Hence, efficient storage of seeds is necessary to ensure continuous and cost-effective supply of seedlings, which is a prerequisite for the success of any afforestation programme. Further, seed storage is also important for conserving the genetic resources which are ravaged by deforestation as well as by catastrophes such as forest fire, drought and floods. However, storage potential of tree seeds is highly species-specific and large variation has been encountered across the tree species (Berjak & Pammenter, 2002)

Based on the inherent storage potential, seeds are grouped into two main categories, viz. recalcitrant and orthodox (Berjak & Pammenter, 2002). Recalcitrant (desiccation-sensitive) seeds are metabolically active when shed from the mother plant and possess relatively high moisture content. Even under ambient temperature and low relative humidity, their post-harvest life is very short which also depends on the species. Since sensitive to desiccation, these seeds lose viability when their moisture content falls below 20-30% (Farrant et al., 1988; Pritchard, 2004). Orthodox (desiccation-tolerant) seeds are not metabolically active when shed from the mother plant and possess relatively low moisture content (<15%). They can be dried to very low moisture content and can retain viability for a long period of time under ambient temperatures and low relative humidity (Berjak & Pammenter, 2002). However, such information is not available for *G. walla* and therefore the present study was aimed to identify the germination pattern and the potential of storage of *G. walla* seeds at different temperatures.

Materials and Methods

Seed Collection and Storage

Ripened fresh seeds of *G. walla* were collected during June-July of 2015 from the trees growing in three homegardens of Mathugama Divisional Secretariat Division ($6^0$31'25"N, $80^0$6'40"E) of low country wet zone. Altogether18 different treatment combinations were used and 50 seeds were separately used for each treatment. Until the treatments were started, each 50 seed lot was separately stored in cotton bags so that one bag could be used for each sowing.

Prior to the storage, the diameter and length were measured for 10 randomly selected seeds from each 50 seed lot using a vernier caliper. The weight was measured using an electrical balance. Direct sowing was done for 50 seeds as the control without storing. The remaining seed lots were stored in three different experimental temperatures, viz. room temperature ($28^o \pm 2^o$ C), at 8^o C and at -10^o C. Storage time varied from one to six weeks, at one week interval. After sowing in coarse sand beds, seed germination was counted weekly for 16 weeks.

Preparation of Germination Beds and Seed Sowing

This experiment was conducted at the agarwood nursery managed by a private company (Sadaharitha Plantation Ltd) at Ingiriya Divisional Secretariat ($6^0$43'55''N, $80^0$10'35''E) of low country wet zone of Sri Lanka. Seeds were sown in the sand beds of 1×10 m individually keeping the tail-like structure above the medium. Watering was done two times a day after sowing and 50% shade was provided.

Germination Percentage

Germination was recorded weekly for 16 weeks. Seeds with a protruding radicle of about 2 cm were considered as germinated. Percentage of germination was calculated by using equation 1.

$$\text{Germination } \% = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100 \qquad (1)$$

Statistical Analysis

GLM ANOVA was used to determine the effect of storage temperature, storage period and their interaction on seed germination. Germination % of direct sowing at the start of the trial was used as the control which was used to compare the germination at different storage periods and storage temperatures.

Results

The average seed length and diameter without the tail-like structure were 10.54 (±0.13) mm and 6.77 (±0.04) respectively. Average length of the tail-like structure was 13.33 (±0.36) mm. The average weight of a seed is 0.19 (±0.01) g.

Statistical analysis conducted with GLM ANOVA showed significant differences in different storage conditions, storage time periods and their interactions (Table 1).

Table 1. Effect of storage temperature, storage periods and their interaction on seed germination in *G. walla*

Source	F-value	*p*-value
Storage temperature (ST)	112.15	0.000
Storage duration (SD)	23.20	0.000
ST X SD	23.85	0.000

The highest germination (73.3%) was produced by the control, viz. direct sowing without storage (Figure 1) which was followed by 26.7% germination of each of sowing after 2 and 4 weeks of storage at 8^0 C. Seeds stored for 3 weeks had the least germination for all three temperatures. Among the selected temperatures, it was found that 8^0 C had significantly higher germination percentage than the other storage temperatures and seeds stored at -10^0 C had the least significant seed germination percentage for *G. walla* (Figure 1).

Germination of the most of *G. walla* seeds started within 1 to 2 weeks after sowing (figure 2) and the seed viability declined within a short time period. Seeds stored for 1 week at room temperature and at -10^0 C showed 6.7% germination (figure 2a). Seeds stored for 2 weeks at -10^0 C showed 3.0% germination (Figure 2b) and seeds stored for 5 weeks at 8^0 C showed 10% germination percent (Figure 2e). However, the seeds sown after 3 weeks of storage at all three temperatures did not germinate at all (Figure 2c).

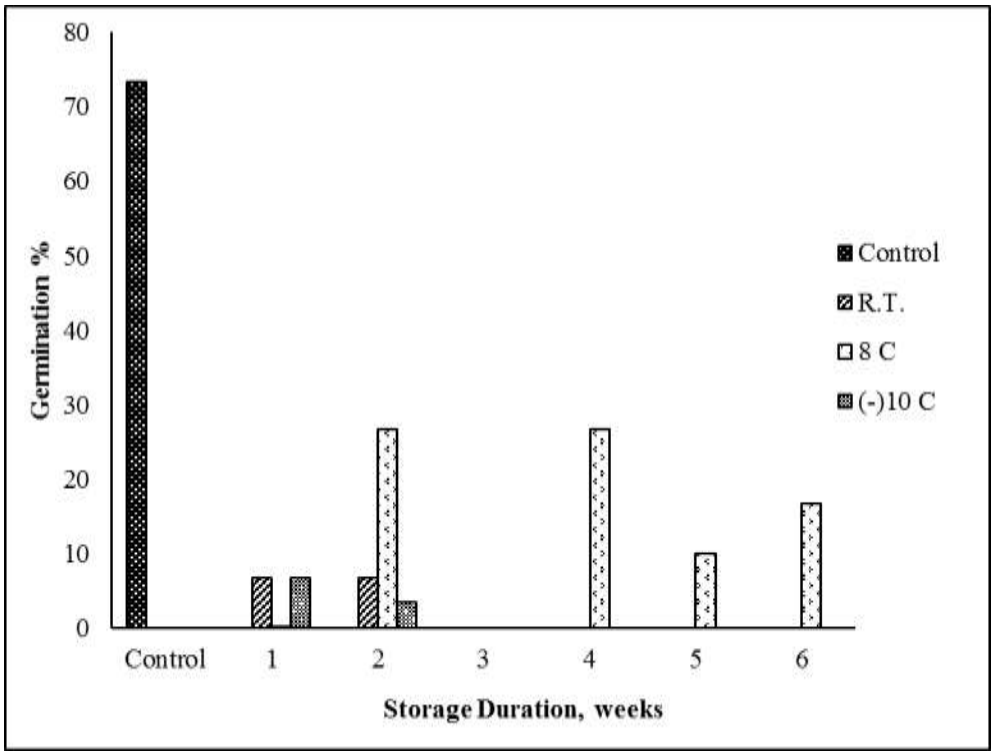

Figure 1. **Effect of storage conditions and storage period on seed germination percentage after 16 weeks of *G. walla* seeds**

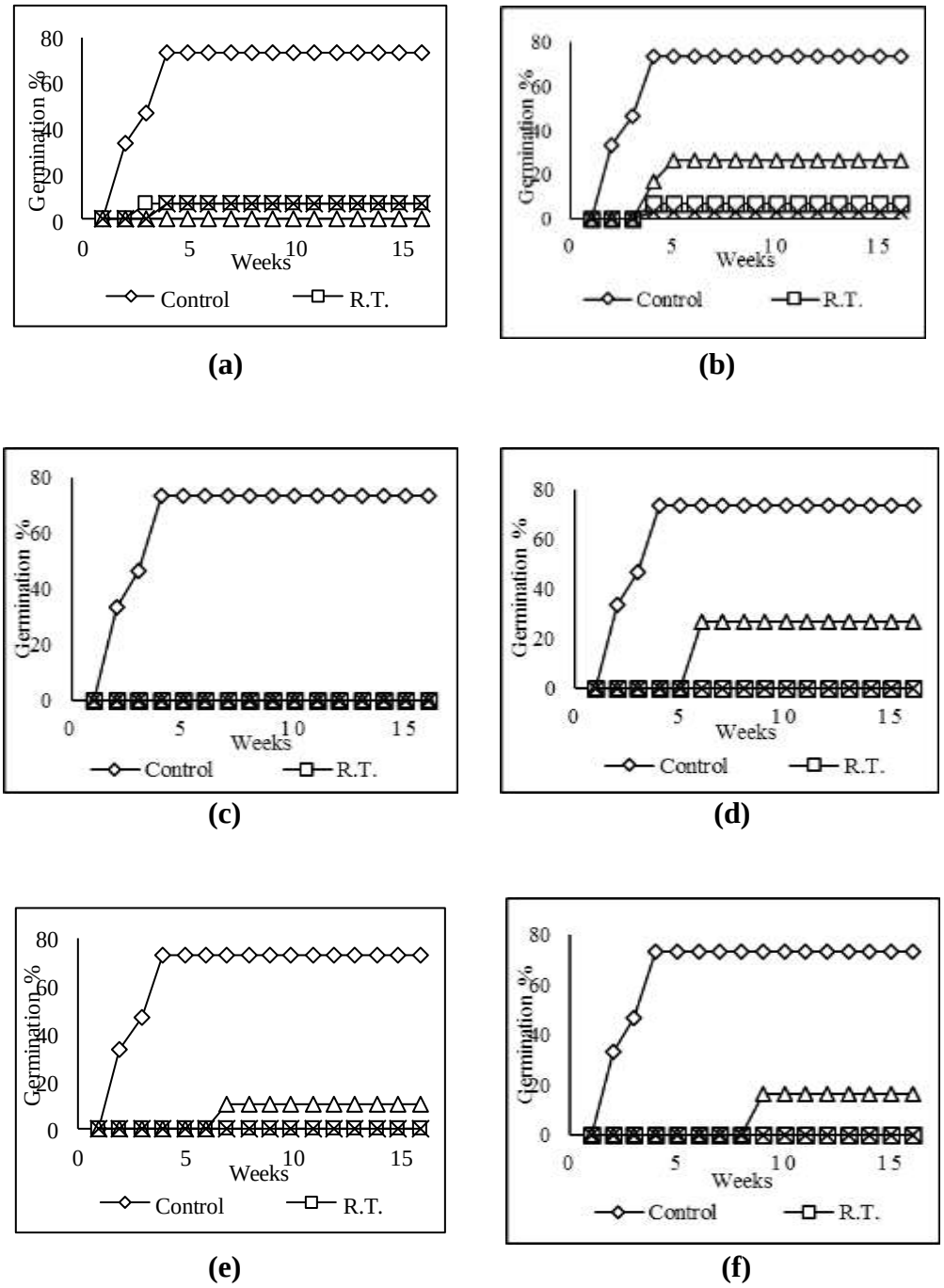

Figure 2. **Effect of storage temperature and storage period on seed germination percentage of *G. walla* seeds (a) 1week; (b) 2 weeks; (c) 3 weeks; (d) 4 weeks; (e) 5 weeks; (f) 6 weeks after storage; (R.T: room temperature)**

Discussion

Present study revealed that most *G. walla* seeds started germination within 1 to 2 weeks after sowing in the nursery beds. Same germination results were recorded for *A. crassna*, where seeds germinated in 9 to 15 days after sowing (Suhartono & Newton, 2001). Experiments conducted with *A. agallocha* in India indicated similar results, where most seeds germinated within three weeks after sowing (Beniwal, 1989). Most fresh seeds of *A. malaccensis* also germinated within three weeks after sowing (Adelina et al., 2004). However, a study conducted on *G. ledermannii* in Indonesia revealed that first signs of germination occurred after 22 days (Gunn et al., 2004).

The highest germination (73.3%) was recorded in this study when *G. walla* seeds were sown directly without any storage period. Suhartono and Newton, (2001) reported that *A. crassna* seeds had the highest germination (up to 92%) when sown directly without storage, while *A. malaccensis* showed 70-80% germination when direct sowing (Adelina et al., 2004).

Present study revealed that the germination ability of *G. walla* seeds rapidly declines after falling from the trees. Similarly, seeds of *Aquilaria* sp. showed the highest germination at room temperature for maximum of 3 days and then germination rate declined. (Aroonrungsikul et al., 2009; Aroonrungsikul & Wongsatain, 2010; Tabin & Shrivastava, 2014) which is similar to certain seeds of other species like *Santalum album* (Gamage et al., 2010). Among the storage temperatures, *G. walla* seeds showed the highest germination percentage (26.7%) at 8^{o} C, which was, however, significantly lower than the control. Luu & Loc (2001) stated that the optimum storage condition for *A. crassna* seeds can be 8^{0} C for two months which gave the germination of 22%.

According to the findings of this study, *G. walla* seeds could be recalcitrant type due to their short life span. Therefore the present study suggest that the *G. walla* seeds should be directly sown within a very short time period of collection to obtain the best germination.

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