Effect of Storage Condition and Duration on Germination Of Agar *(Aquilaria malaccensis Lamk.)* Seed

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Abstract

A nursery trial was conducted at National Forest Seed Centre, Seed Orchard Division, Bangladesh Forest Research Institute, Chittagong to evaluate the effect of storage condition and duration on germination of Agar seed. Agar seed were stored at five different storage condition viz. open air (control), sand, chalk powder, normal refrigerator (0~40C) and saw dust for different storage durations viz. 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33 and 36 days. Storage condition, duration and their interaction were found significant on germination of Agar seed. Refrigerator (0~40C) showed the highest germination (82%) at 3 days duration. It also prolonged the seed viability (12%) up to 33 days. Such technique of maintaining viability of Agar seed may be useful for raising seedlings and plantations at large scale.

সারসংক্ষেপ

বাংলাদেশ বন গবেষণা ইনস্টিটিউটের অধীনস্থ বীজ বাগান বিভাগের ন্যাশনাল ফরেস্ট সীড সেন্টারে আগর বীজের অংকুরোদগমের উপর বিভিন্ন সংরক্ষণ পদ্ধতি ও উহার সময়কালের প্রভাব মূল্যায়নের জন্য বর্তমান পরীক্ষণটি পরিচালনা করা হয়েছে। ৫টি সংরক্ষণ মাধ্যম যথা : খোলা অবস্থায় (কন্ট্রোল), বালি, চক পাউডার, রেফ্রিজারেটর (০-৪° সে.) ও কাঠের গুড়া এবং বিভিন্ন সময়কাল যথা: ৩, ৬, ৯, ১২, ১৫, ১৮, ২১, ২৪, ২৭, ৩০, ৩৩ ও ৩৬ দিন সংরক্ষণ করে আগর বীজের অংকুরোদগমের হার এবং জীবনীশক্তি নির্ণয় করা হয়েছে। এদের মধ্যে রেজিফ্রেজারেটরে (০-৪° সে.) ৩ দিন পর্যন্ত সংরক্ষণ করা বীজে সর্বোচ্চ ৮২% অংকুরোদগম এবং ৩৩দিন পর্যন্ত ১২% জীবনীশক্তি পাওয়া গিয়েছে । এই পরীক্ষার ফলাফল বৃহৎ পরিসরে চারা উত্তোলন ও বাগান সৃজনের ক্ষেত্রে সহায়ক ভূমিকা রাখবে।

Keywords: *Aquilaria malaccensis*, germination, seed, storage condition.

Introduction

Agar(Aquilaria malaccensis Lamk.) occurs predominantly in the Indo-Burma hotspot of biodiversity (Whitmore 1973). It is also found in Nepal, Bhutan, North-Eastern India (Assam, Meghalay, Nagaland, Manipur and Tripura), Myanmar, Thailand, Laos, Vietnam, Cambodia, Indonesia, Malaysia, South-Eastern China, Brunei Darussalam, The Philippines, Papua New Guinea and islands of East India (Baksha et al. 2009, Burkhill 1966). In Bangladesh, it is found naturally in the forests of Sylhet, Chittagong and Chittagong Hill Tracts of Bangladesh (Rahman and Basak 1980). Population of Agar has markedly decreased in natural forests of Bangladesh due to unsustainable harvesting of natural trees for agar wood trade. Hence, it is enlisted in 'The World

List of Threatened Trees' since late 2000s (Chakrabarty et al., 1994). The high value of the wood has resulted in indiscriminate felling of natural populations in some cases. Thus, the knowledge of its regeneration ecology is extensively desired for developing protocols for raising large-scale plantations of Agar(Donovan et al. 2004).

Generally seedling is regenerated after storing of seed over variable period. 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. Seed storage is also important for conserving the genetic resources which are ravaged by deforestation as well as by catastrophes such as forest fire, draught and floods. However, storage potential of tree seeds is highly species-specific and large variation has been encountered across the tree species (Berjek and Pammenter, 2002). Based on the inherent storage potential, seeds are grouped into two main categories viz. recalcitrant and orthodox (Berjek and 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 to 30% (Farrant et al. 1988, Pritchard 2004). As Agar seed is recalcitrant, it is imperative to find out a suitable storage method which can prolong its viability to raise seedlings in large scale at nursery. However, there is a scanty literature regarding the storage method of Agar seed. Therefore, the present study was undertaken to evaluate the effect of storage condition and duration on germination and viability of Agar seed.

Materials and Methods

The experiment was conducted at National Forest Seed Centre, Seed Orchard Division, Bangladesh Forest Research Institute, Chittagong during July to August 2014. Mature fruits of Agar were collected from the plus trees in the month of July. Seeds were extracted from depulping the fruits manually.

Fresh seed germination and viability test was conducted with four replications of 100 seeds each. Seeds were sown into moist sand bed and germination was calculated following standard method (ISTA 2006). To test the influence of storage conditions and durations a total of 3,250 seeds were taken and divided equally into five seed lots. Each lot has subjected to a specific storage condition as follows: open air/control, sand, chalk powder, refrigerator and saw dust. Seeds in all conditions were stored for different durations viz. 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36 and 39 days. From stored seed, ten seeds with five replications were taken out after every three days and germination was tested by sowing in moist sand bed. Seeds were sown 0.5 cm depth and 4.0 cm distance between seeds in sand beds and then pressed lightly into the sand. Proper shade was provided until germination starts. Routine watering and weeding activities were carried out. Seeds were considered germinated when the cotyledons protruded from the sand surface. Germinated seeds were remarked with small sticks to differentiate them from newly germinated seeds. Germination was observed on alternate days until completion. The factorial experiment was followed as complete randomized block design with two factors - storage condition and duration. A variation (ANOVA) in germination potential under different conditions was analyzed using statistical package MSTAT.

Results and Discussion

Analysis of variance(ANOVA) for germination potential of Agar seed under different conditions and durations was done and found highly significant within storage conditions , durations and their interaction(Table 1). This indicated that viability of Agar seed is highly dependent on both storage conditions and storage durations which was in accordance with Manjkhola et al(2005) and Panwar et al. (2015).

Germination of Agar was epigeous. Both fresh and stored seeds started germination within 6 to 12 days. Similar results were obsevered for A. crassna where seeds germinated within 9 to 15 days (Soehartono and Newton 2001, Shankar 2012) and for Gyrinops walla Garten. within 7 to 14 days(Alwis et al. 2016). Gemination was completed within 26 days which is similar to Beniwal (1989) and Adelina et al. (2004).

Figure 1. A) Fruits, B) Seeds and C) Seedlings of Agar

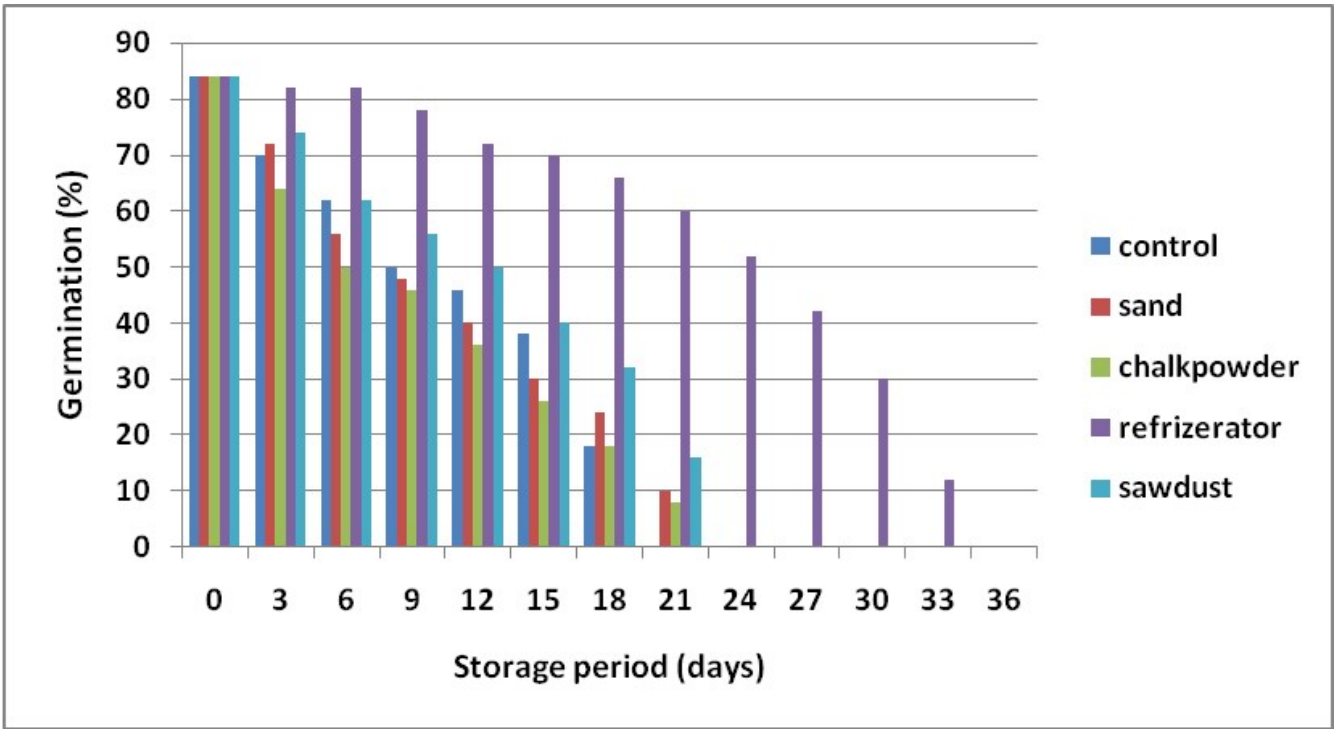
The highest germination (82%) was recorded with both fresh seeds sown immediately after harvest and stored in refrigerator (0~4^{0}C) for 3 days. Tabin and Srivastava (2014) recorded 92% and Adelin et al. (2004) recorded 70-80% germination when direct sowing.

Table 1. Two-way ANOVA for seed germination at different storage conditions in relation with storage periods

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob
Storage condition(C)	4	458.240	114.560	110.7346**	0.0000
Storage duration (D)	10	1559.542	155.954	150.7466**	0.0000
C X D	40	108.240	2.706	2.6156**	0.0000
Error	220	227.600	1.035		
Total	274	2353.622			

**Values are significant at p > 0.01 level

Figure 2. Effect of storage conditions and durations on germination of Agar seeds



A reduction trend on seed germination percent over the period was evident and significant differences in the germination among the storage conditions and durations used in the experiment. The seeds stored for 3 days in refrigerator showed the highest (82%) germination (Fig. 2) followed by the seeds stored in saw-dust (74%), sand (72%), control (70) and chalk powder (64%). After a period of 21 days storage, the seeds of refrigerator found 60 % germination followed by the seeds of saw-dust, sand and chalk powder 16%, 10% and 8% respectively, although the seeds of control had no germination. After the storage of 24, 27 and 30 days, the seeds of refrigerator showed 52%, 42% 30% and 12% of germinability respectively, while others storage conditions showed no germination at all. Present study revealed that storing seeds in cool conditions such as in a refrigerator can prolong viability12% up to 33 days. No more seeds were found with germinability after storage of 33 days.

Analysis of variance (ANOVA) for viability potential under different media and durations was done and F value was found highly significant (Table 1) within the interaction of media and storage durations. LSD values (1.267 at 5% and 1.672 at 1% level) were for grading the combination more precisely. Germination level in earlier interval period of followed all storage media were found all most same and maximum. But, emphasizing the prolonging period of viability in desired level, seeds stored in refrigerator, germination after 15, 18 and 21 days were found statistically same and optimum at 5% & 1% level of significance. Seed viability of Agar other than those conditions in various interval periods were found poor & below desired level (Table 1).

Therefore, viability period of Agar in desired level could be selected for the period prolonging 21 days in refrigerator condition. In the same condition Agar seed prolonged only 12% germinability up to 33days.

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References

Adelina, N. ; Harum, F.;Schmidt, L. and Joker, D. 2004. Seed Leaflet: *Aquilaria malaccensis* Lamk. Forest & Landscape, Denmark.

Baksha, M. W; Akhter, S.; Basak, A. C. and Rahman, M. S. 2009. *Bangladeshey agar chas o agar kutir silpo* (Agar cultivation and agar cottage industry in Bangladesh). Bangladesh Forest Research Institute, Chittagong. 20pp. (a booklet in Bangla)

Barjak, P. and Pammenter, N. W. 2002. Orthodox and recalcitrant seeds. In. Vozzo J. A. (Ed.) Tropical *Tree Seed Manual* USDA Forest Service, Washington DC.

Beniwal , B. 1989. Silviculture Characteristics of Aquilaria agallocha Roxb. Indian Forester, 5(1): 17–21

Burkhill, I. H. 1966. .A dictionary of the economic products of the Malay Peninsula 1870–1965

Chakrabarty, K.; Kumar, A.and Menon, V. 1994. Trade in Agarwood. Traffic India and WWF-India, New Delhi, 51p.

Donovan, D. G. and Puri, R. K. 2004. Learning from traditional knowledge of non-timber forest products: Penan Benalui and the autecology of Aquilaria in Indonesian Borneo. Ecol. Soc. (Online), 9(3): 3.

De Alwis, H.N.; Subasinghe, S. M. C. U. P. and Hettiarachchi, D. S. 2016. Effect of Storage Time and Temperature of *Gyrinops walla* Garten. Seed Germination. *Journal of Environmental Proffessionals Sri Lanka*, 5(2): 16–24

Farrant, J.M.; Pammenter, N.W. and Berjak, P.; 1988 Recalcitrance – a current system. Seed Science and Technology 16, 155–166

ISTA, 2006. International Rules for Seed Testing. The International Seed Testing Association. Zurich,Switzerland

Manjkhola, S.; Dhar, U. and Rawal, R. S. 2005. Phenology and biology of Arnebiabenthamil: A critical endangered medicinal plant of the Himalaya, *Proceedings of the Indian Natural Science Academy,76: 283–287*

Panwar, G. S. and Srivastava, S. D. 2015. Seed germination and seed storage behavior of *Eremostachys superba:* An endangered medicinal and ornamental herb of India, *Indian Forester, 141(7), 762–765*

Pritchard, H. W. 2004. Classification of seed storage types for ex situ conservation in relation to temperature and moisture. In : Guerrant, E., Havens,K. and Maunder, M.(Eds) Ex situ plant conservation : Supporting Species Survival in the World, Island Press, Washington DC.

Rahman, M. A. and Basak, A. C. 1980. Agar production in agar tree by artificial inoculation and wounding. Bono Biggyan Patrika 9(1&2) 87– 92

Shankar, U. 2012. Effect of seed abortion and seed storage on germination and seedling growth in *Aquilaria malaccensis* Lamk. *CURRENT SCIENCE*, 102(4): 596-604

Soehartono, T. and Newton, A. C. 2001. Reproductive ecology of *Aquilaria* spp. in Indonesia. For. Ecol. Manage., 152: 59–71.

Tabin, T. and Srivastava, K. 2014. Factors affecting seed germination and establishment of critically endangered *Aquilaria malaccensis* (Thymelaeaceae). *Asian Journal of Plant Science and Research, 4(6): 41– 46*

Whitmore, T. C. 1973. Thymelaeaceae. In Tree *Flora of Malaya* (ed. Whitmore, T. W.), Longman Press, Kuala Lumpur, Malaysia, vol. 2, pp. 383–391.