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Factors affecting seed germination and establishment of critically endangered *Aquilaria malaccensis* (Thymelaeaceae)

Tasso Tabin[1] and Karuna Shrivastava[2]

[1]*Krishi Vigyan Kendra, Department of Agriculture, Upper Siang, Geku, Arunachal Pradesh, India*
[2]*Department of Forestry, North Eastern Regional Institute of Science and Technology (NERIST), Deemed University, Nirjuli, Arunachal Pradesh, India*

ABSTRACT

The recalcitrant seeds of Aquilaria malaccensis Lamk. possess short viability period. Nursery trials were conducted to examine the effect of storage periods (four treatments) and light intensity (three treatments) on Aquilaria seed germination and growth. The maximum germination was obtained with fresh sown seeds (92.0 %) followed by 5 days (39.6 %) and 10 days (4.0 %) stored seeds. No germination took place in 15 days stored seeds at all. The light intensities/shade tolerance capacity of Aquilaria seeds was assessed during germination by using polynets single and double layers as compare to control. The maximum germination (78.0%) obtained under double layer polynet shade (DLPS) as compare to single layer polynet shade (SLPS) and control (75.0 % & 63.8 % respectively). The significant correlation ($p < 0.05$) was observed between seed moisture content and seeds germination percentage. The growth parameters like plant height, leaf area, plumule and radical length were also higher under DLPS. Direct light as well as storage of seeds even for few days reduce seed germination and seedling growth considerably; hence serve as main stressing factors for regeneration of A. malaccensis plant in field. These findings may be useful for raising seedlings at large scale for Aquilaria plantation programme in the state of Arunachal Pradesh, India.

Key words: *Aquilaria malaccensis*; Germination; Recalcitrant Seeds; Shade Tolerance; Storage period

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INTRODUCTION

Due to continuous metabolism in recalcitrant seeds [1], viability is lost if the moisture contents drop below a certain critical level before germination takes place. Studies on seed biology and physiological ecology help to understand the processes such as germination, establishment, succession, and regeneration that occur in plant communities. The changes in light quality and temperature fluctuations are main factors that influence seed germination [2]. Generally, late successional, shade tolerant species have larger seeds with high water content, short or no dormancy with short life period [3]; however, some shade tolerators may have smaller seeds [4] than light demanders. Germination in some small-seeded species, although not possible in the dark, but can occur under conditions of low red/far-red ratios (R/FR) as in the forest understory trees [5]. Tolerance is a term that foresters and ecologists use to indicate a tree's capacity to develop and grow in the shade of, and in competition with, other trees. It is the degree to which a species can share resources on a site and still be successful, most often reflects as shade tolerance. Shade tolerance is the relative capacity of tree species to compete for survival under shaded conditions. This tree trait or functional adaptation although varies between species to species becomes evident in forest succession.

Aquilaria malaccensis Lamk., is a mid canopy tree and propagated readily through seeds. The natural regeneration rate is reported to be quite high [7] however, the seed productions and seedling dispersion in the forest is limited. Seeds are produced once in a year during the monsoon season i.e. during June to August. Its seeds have a short shelf life at normal temperature. In contrast, low reproductive potential of *Aquilaria* seeds have been reported due to variable, slow and short germination rate. An efficient plant regeneration system was established via organogenesis

from shoot developed from seedlings of *A. malaccensis* in artificial culture medium [8, 9]. The high germination percentage in case of fresh seed and low for stored seeds has been reported under nursery condition [10]. At present, very less is known about autecology of this species. A comprehensive research on these aspects may be helpful since germination capacity is also influence by abiotic and other reproductive factors like light availability, soil type and seedling density [8].

Aquilaria malaccensis is an economically important but critically endangered tree species of northeast India [11]. It has been listed in the 'Word List of Threatened Trees' since late 2000s [12]. The people's understanding about valuable and precious agar wood has lead to its heavy extraction from natural forest in recent years. This has put the natural existence of the species under tremendous pressure. Presently, the species has become the focus of increasing conservation concern and is included in the prioritized list for national recovery programme in India [13]. In addition to utilization pressure, *Aquilaria* tree faces limitations from important ecological factors namely, light availability, seed viability period and insect attack both under nursery and field conditions. Being a tremendously important tree species, information is required on the growth and survival of the species in response to different environmental factors in order to manage it sustainably.

Comparatively few studies on regeneration of *Aquilaria* species using different technologies have been under taken [8, 9] as most seeds become incompatible within very short periods [14]. Keeping in view the degradation of natural population and biological conservation of *Aquilaria* in India, investigations were carried out with the main objective to understand the response of *Aquilaria* seedlings to environmental factors, such as timing of seed sowing after harvest and effect of shades on germination and establishment of *A. malaccensis* seeds in nursery and green house conditions.

MATERIALS AND METHODS

Effect of shelf life on seed germination of A. malaccensis - Germination tests of *A. malaccensis* seeds **(Figure 1)** were conducted with four treatments (0, 5, 10 and 15 day's stored seeds) in the forest nursery of Forestry Department, NERIST during June to August. Fresh and healthy seeds of approximately same sized were collected from standing trees and separated into four lots of fifty seeds each in three replicates. From each category, the seeds were sown in the nursery after measuring their fresh weight. The number of seedlings was counted up to 30 days after sowing.

The germination % was calculated using the following formula:

$$\text{Germination (\%)} = \frac{\text{No. of germinated seeds}}{\text{Total number. of seeds sown}} \text{ X } 100$$

The moisture content of seeds was measured as per gravimetric method [15] to calculate and assess its influence on germination.

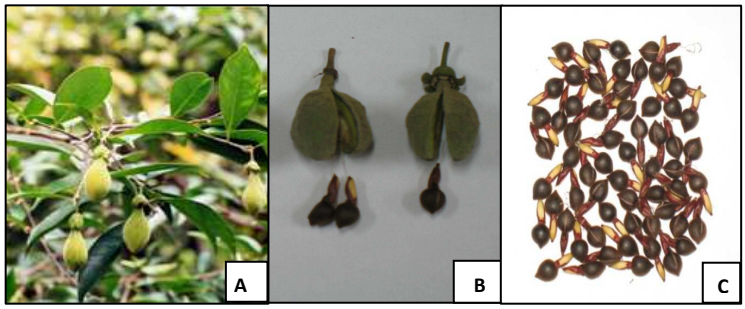
$$\text{Moisture content (\%)} = \frac{(W2-W3)-W1}{W2-W3} \text{ X } 100$$

Where,
W1 = Weight of empty plate (g),
W2 = Weight of fresh seeds + plate (g),
W3 = Dry weight of seeds + plate (g).

Effect of shades on seed germination of A. malaccensis - The effect of shades on seed germination was tested with three treatments i.e. no shade, single layer polynet shade (SLPS) and double layer polynet shades (DLPS) by partitioning the green house with polynets into three compartments. The nets were positioned immediately below the roof of green house in each nursery plot. The experimental design was a randomized complete block (RCB) with three replicates of fifty seeds each. The emergence of seedlings was counted up to 30 days after sowing and germinations percentage was calculated using the formula mentioned above. The plumule and radical were measured after three days of germination whereas, the plant height and leaf area were measured after 30 days of germination using leaf area meter.

Data analysis - The germination and growth parameters of *A. malaccensis* seedlings were analyzed using Microsoft Excel Spreadsheet and STATISTICA software.

Figure 1: A) Fruits on tree; B) Seed from fruit and C) Dissected single fruits of *A. malaccensis*



RESULTS AND DISCUSSION

Effect of seed shelf life on germination of A. malaccensis - The fruits of *Aquilaria* desiccate very fast (Shankar, 2012) and may bear one or two seeds per fruit (**figure 1**). Germination percentage of seeds was found to be directly proportional to seed's storage period. The germination started on 14th day and continued up to 35th day after sowing. The results (**table 1)** show that the highest germination percentage (92.0 %) was recorded with fresh seeds sown immediately after harvest which reduced drastically by 5 days (39.6 %) and 10 days (4.0 %) stored seeds. The 15 days old seeds showed no germination at all. The seeds stored for 15 days became hard, shrunken and turned black in colour. The highest average fresh weight of seeds was recorded as 39.1 g /50 seeds which reduced to 35.4 g after 5 days, 32.8 g after 10 days and 30.0 g after 15 days. Seeds lost turgidity very fast and their original appearance after 15 days. The survival percentage of seedlings was calculated after 30 days and recorded highest (80.0 %) in case of fresh seeds followed by 5 days (33.2 %) and 10 days (2.8 %) stored seeds respectively. The moisture contents of the seed were found significantly correlated ($p < 0.05$) with the germination of seeds (**table 2** and **figure 2)**.

Table 1: Effect of seed's shelf life on germination and survival of *A. malaccensis* in Arunachal Pradesh, India

Storage Period (in Days)	Seed Germination Characteristics		
	Average Seed Weight (gm/seeds)	Germination (%)	Survival (%) (after 20 days of germination)
0	39.1±2.31^{a}	92.0±3.82^{a}	80.0±1.76
5	35.4±3.11^{b}	39.6±2.76^{b}	33.2±8.18
10	32.8±3.33^{c}	4.0±0.11^{c}	2.8±6.00
15	30.0±2.41^{d}	0.0	0.0

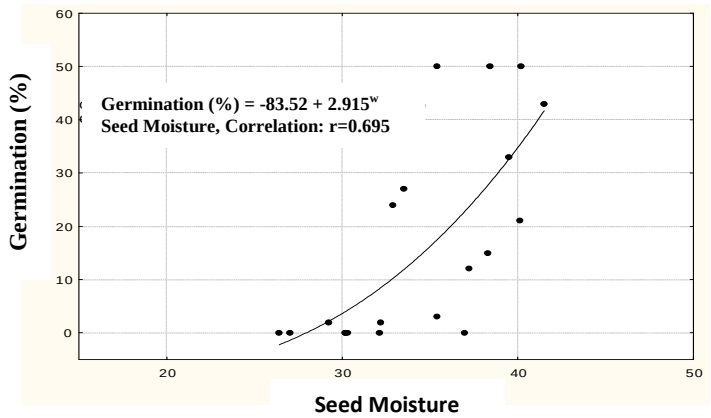
Values with different superscripts across each column are significant different at P<0.05 (n=5)

Table 2: Two-way ANOVA for seed moisture in relation with storage periods of *A. malaccensis*

	df	MS	F	*P*
Stage	1	224.68	**34.184***	0.000002
Storage Period	3	46.51	**7.077***	0.000885
Stage*Storage Period	3	45.01	**6.849***	0.001079
Error	32	6.57		

**Values are significant at P<0.05 level; df-Degree of freedom, F-Frequency*

Fig 2: Correlation between moisture content and germination % of seeds of *A. malaccensis*



The germination trial on seeds using different shelf life and polynet shades under nursery/green house condition have shown high germination percent (<90 %) in case of fresh seeds sown immediately after harvesting which supported earlier findings[10]. The seed germination percentage also declined drastically with increase in storage period from 31% for seeds without storage to 25% for 5 days of storage and only 1% for 14 days shelf life [14]. The recalcitrant seeds of *A. malaccensis* regenerate freely under natural conditions in the forest probably due to availability of necessary moisture and light under the shaded canopy. The fleshy covering of *Aquilaria* fruits also provides moisture to the seeds for their survival **(figure 1**). Saikia and Khan [16] noticed germination of *Aquilaria* seeds up to 45 days with a decreased germination percentage by increase in storage period and 100% viability on Tetrazolium test of freshly collected seeds. The natural regeneration rate was found moderately higher [7] in spite of intensive extraction of agar wood for economic benefits [17], limited seed productions and dispersion in the forest [8]. The annual seed production period is also relatively shorter i.e. from mid June to August which might be a contributing factor for relatively low density of naturally regenerated plants putting tremendous pressures on this forest species. The continued existence of the species may only be substantiated through artificial propagation and serious conservation efforts. The seeds moisture contents decreased considerably with the increase of storage period **(table 2)**. The percent seed germination was inversely proportional to their storage period, as storage reduces moisture contents and in turn viability of seeds. The seed weight of stored seeds after different shelf life revealed the positive relationship between moisture content and seeds physiological process (**figure 2**). Therefore, the probability of seed germination depends on seed's moisture content, physiological processes and in turn embryonic activities. The germination percentage was significantly correlated with seed weight and moisture contents in our findings and supports earlier findings [18, 19]. Generally, seeds are associated with behavioral differences in morphological, physiological, anatomical structure and biochemical composition during storage periods [20]. These factors might have caused storage of seeds difficult for commercial exploitation, cultivation and conservation of this species hence needs some biotechnological intervention for sustainable management.

Effect of shades on seeds germination of A. malaccensis - *A. malaccensis* is a medium sized tree grows naturally underneath canopy of dominant trees in the forest. The experiments were conducted to assess the shade tolerance capacity of *Aquilaria* seeds in relation to germination percentage by creating three artificial shadings i.e.SLPS and DLPS and no layer as control (**figure 3**). The seeds sown without shade showed least germination percentage (63.8%) and poor overall performance in growth parameters as compare to seeds sown under SLPS (75.0%) and DPLS (98.0%) with maximum germination percentage (**table 3)**. The growth and development was also dynamic under DLPS with longest plumule and radical length measured as 13.1 cm and 26.7 cm respectively followed by SLPS (12.9 cm & 26.3 cm respectively) and control (12.2 cm & 23.93 cm respectively). The height and leaf area of plants in DLPS were measured as 39.8 cm and 7.9 cm, followed by SPLPS (39.2 cm & 7.4 cm) as compare to control (36.1 cm & 6.5 cm). In two way ANOVA of seed germination values, seed moisture contents in relation to storage periods characteristics and effect of shade on seed germination and establishment of *A. malaccensis* were recorded as significant at $P<0.05$ (n=5).

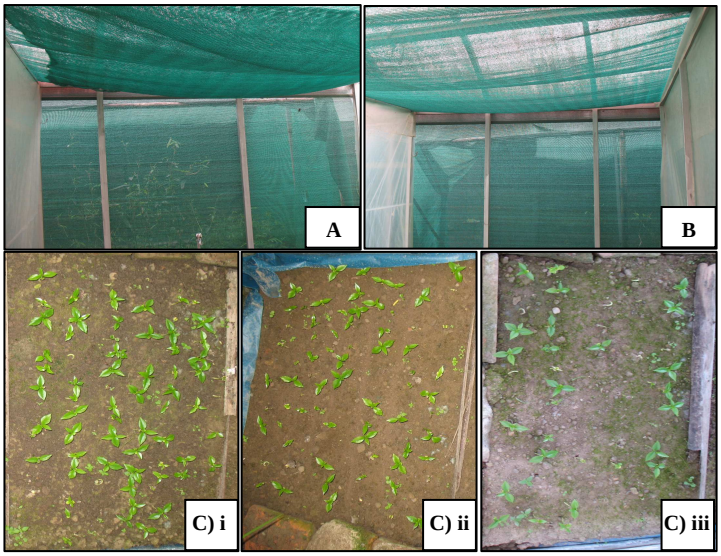
Table 3: Effect of Shades on seed germination and establishment of *A. malaccensis* in Arunachal Pradesh, India

Conditions		3 DAG		30 DAG	
	Germination (%)	Plumule length (cm)	Radical length (cm)	Plant height (cm)	Leaf area (cm)
No layer	63.8±7.0	12.2±2.2	23.93±4.0	36.1±3.2	6.5±1.8
Single layer	75.0±7.7	12.9±1.4	26.3±1.2	39.2±1.7	7.4±1.7
Double layer	78.0±9.8	13.1±1.2	26.7±4.6	39.8±5.6	7.9±4.5

DAG=Days After Germination, Significant at P<0.05 (n=4)

A. malaccensis is reported as a shade tolerant medium size tree found growing underneath tall trees in the tropical and sub-tropical evergreen forest in nature [21]. Shade house and lab experiments help in determining the relative importance of factors influencing seed germination processes [22]. The seeds sown under DLPS (**table 3, figure 3)** have shown approximately absolute germination rate (98.0 %) as compare to SLPS (75.0 %) and control (63.8 %) with healthier and taller plants. This may be attributed to low soil moisture evaporation rate due to shades that minimized it and other supportive abiotic factors, which in turn aided in profuse seed germination. The profound influence of light intensity (however not measured) was recorded. The seeds sown under direct sunlight showed the least germination percentage and poor performance in growth parameters such as seedling height, leaf area, plumule and radical length. The polynet system of shading might be one of the factors that changes the light requirements of photoblastic seeds [23]. This has established the fact that *Aquilaria* is rather a shade demander tree than shade tolerant. However, a general trend has been considered for shade tolerators having large seeds and light demanders having small seeds [2] usually not fitting for *Aquillaria* bearing medium sized seeds but a shade tolerant species. The proposed shade tolerance index [6] constitutes a new quantitative approach which can be used to understand and predict succession of forested ecosystems and biogeography patterns. They correlated shade tolerance driven succession to climatic variables and considered as a primary driving factor of forest dynamics.

Fig 2: A) Double-Layer-Polynet Shading (DLPS); B) Single-Layer-Polynet Shading (SLPS); C) *A. malaccensis* seed germination under i) DLPS; ii) SLPS and iii) No shade or control conditions



The germination capacity of seeds is not only influence by storage period and availability of shaded condition but also with abiotic and other reproductive factors like phenology, pollination, fertilization etc. which induct low reproductive potential[8]. In our experiments, the seed germination and emergence from soil counted up to 30 days after sowing. The earlier workers reported germination of *Aquilaria* seeds from 37 to 45 days [14, 16, 24] from sowing day. Some seedling died even after emergence which may be due to physiological disorder, fungal infection and insect attack which usually prevail during sowing season. Usually, seed germination time coincides with monsoon period and greatly enhances damping-off of seedlings in this tree species. Proper mycorrhizal symbiosis may also be an appropriate solution.

CONCLUSION

The artificial regeneration and plantation have been encouraged in both the states to promote conservation of *Aquilaria* species at large, however; people face difficulty to overcome from associated abiotic and biotic stressors and achieve higher survival rates. The present findings study may be helpful for raising large scale planting stock from freshly collected seeds of *Aquilaria* tree under nursery conditions here following this protocol. Owing to capability of *Aquilaria* tree to grow in dark shades with improved performance, large scale plantation programmes may now be initiated in the state of Arunachal Pradesh for this treasure species for enormous economic benefits.

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REFERENCES

[1] T.-P. Lin, M. Chen, *Annals of Botany*, **1995,** 76, 381-387.
[2] R.P. De Souza, I.F.M. Valio, *Biotropica*, **2001,** 33(3), 447-457.
[3] M.D. Swaine, T.C. Whitmore, Vegetatio, **1988,** 75: 81-86.
[4] C.K. Kelly, A. Purvis, *Oecologia*, **1993,** 94, 356-360.
[5] D.R. Drake, *Biotropica*, **1993,** 25, 461-467.
[6] M. Saikia; Karuna Shrivastava, and S. S. Singh, *Asian Journal of Biological Sciences*, **2013,** 6(2), 96-105.
[7] T. Soehartono, A.C. Newton, *Biological Conservation*, **2001a,** 97: 29-41.
[8] M.L. He, S.Y. Qi, L.J. Hu, He et al./ *J Zhejiang Univ SCI*, **2005,** 6B(8): 849-852.
[9] G.U. Ahmad, P. Gogoi, In: Ahmed GU, Gogoi, P. (Eds.), Seminar on 'Scope and Dimension of Agar (*Aquilaria spp.*) Plantation in NE India', **2000,** Hojai, Assam, India, 28-33.
[10] International Union for Conservation of Nature (IUCN), Red List of Threatened Species, version 2012.2; www.iucnredlist.org (retrieved on 11th August, **2014**).

[11] S. Oldfield, C. Lusty, A. Mackinven, The Word List of Threatened Trees, World Conservation Press, Cambridge, **1998**.
[12] K. Chakrabarty, A. Kumar, V. Menon, Trade in Agarwood. WWF-India/TRAFFIC-India, New Delhi, **1994**.
[13] U. Shankar, *Current Science*, **2012**, 102(4), 596-604.
[14] P.K. Gupta, Soil, Plant, Water and Fertilizer Analysis. Agrobios, Jodhpur, India, **2004.**
[15] P. Saikia, M.L. Khan, Phenology, *The Indian Forester*, **2012**, 138(3), 289-295.
[16] Q.L. Tran, Q.K. Tran, K. Kouda, N.T. Nguyen, Y. Maruyama, I. Saiki, S. Kadota, *J. Traditional Med.*, **2003,** 20(3): 124-131.
[17] N. Adelina, Seed leaflet: *Aquilaria malaccensis* Lam. Forest & Landscape, Denmark, **2004,** 103, 1-2
[18] S.C. Nath, J. Kalita, N. Saikia, *Indian Perfumery*. Essential Oil Association of India, **1999**, 43(3), 159-163.
[19] S. Kumar, J. Radhamani, A.K. Singh, K.S. Varaprasad, *Current Sci.*, **2007**, 93(7), 910-911.
[20] U.S. Mohammed, S.A. Mukul, M.A.S. A. Khan, M. Alamgir, M.Y. Harun, M.S. Alam, *Small-scale Forestry*, **2008**, 7, 139–149.
[21] M.D. Swaine, V.K. Agyeman, B. Kyereh, T.K. Orgle, J. Thompson, E.M. Veenendaal, Ecology of forest trees in Ghana, ODA Forestry Series 7, University of Oberdeen, London, **1997**.
[22] P.F. Wareing, I.D.J. Phillips, The control of growth and differentiation in plants. Pergamon Press, Oxford, **1978**.
[23] M. Saikia, Ph.D. Thesis. NERIST (Deemed University), (Itanagar, Arunachal Pradesh, India, **2014**).
[24] T. Tabin, A. Arunachalam, Karuna Shrivastava, K. Arunachalam, Tropical *Ecology*, **2009**, 50(2), 243-248.