

DEVELOPMENT OF *AQUILARIA AGALLOCHA* ROXB. SEEDS: ACQUISITION OF GERMINABILITY, DESICCATION SENSITIVITY AND STORAGE RESPONSE

MAITREYEE KUNDU^{1*} AND RUPNARAYAN SETT²

¹Division of Silviculture & Joint Forest Management,

²Division of Forest Ecology & Rehabilitation, Tropical Forest Research Institute (ICFRE),
Mandla Road, P.O. RFRC, Jabalpur - 482 021, M.P.

Received on 7 Jan., 2005

SUMMARY

Studies were undertaken on the development of the recalcitrant seeds of *Aquilaria agallocha*. Continuous increase in seed dry weight was observed till the time of fruit shedding. The seed acquired germinability at 50 DAA and full germinability was attained at 59 DAA. With the progress of maturity, desiccation tolerance increased, though the tolerance to water loss was not varied so much after the attainment of full germinability. At very early stage of development (41 and 50 DAA) slight reduction in seed moisture content induced germination. Only fully matured seeds were capable to retain viability for few months, if stored at shedding moisture content.

Key words: *Aquilaria agallocha*, desiccation-sensitivity, storage, seed development.

INTRODUCTION

Aquilaria agallocha Roxb. (syn. *A. malaccensis* Lam.), common name agar (family Thymelaeaceae), a native of an Indo-Malaysian region and South China is found in the hills of Northeast India. The mature tree is generally 18 to 21 m in height and 1.5 to 2.5 m in girth with a moderately straight and often fluted trunk. The most remarkable feature of this tree is that certain fungi, which cause large, irregular dark-colored resinous masses to develop in the center of the bole, infect the wood; these are termed as "agar". Agar emits a fragrance for which this wood has been prized from ancient times for perfumery and fixative. *Aquilaria agallocha* fruits are bi-valve capsules, ovoid, usually measuring 2.5-3 cm containing two seeds or rarely one. After maturation, the pericarp opens longitudinally into two halves, and the seeds swing by a thread-like structure from the pericarp. The seeds are comparatively small in comparison with those of some other recalcitrant species, and measure about 1.6 cm long at the mature

stage. The seed coat is white and soft before maturity but turns into hard, brown and brittle when it matures.

Chin and Roberts (1980) described the differences in storage characteristics between orthodox (desiccation-tolerant seeds of low moisture content, which can be successfully stored at low temperature) and recalcitrant (desiccation-sensitive seeds of high-moisture content which cannot be dehydrated and similarly stored) seeds. At maturity, recalcitrant seeds are metabolically active, and lose viability after shedding at relatively high-water content when dehydrated (Farrant *et al.* 1993). There is a limited number of studies on development of recalcitrant seeds: *Avicennia marina* (Farrant *et al.* 1993); *Quercus robur* (Finch-Savage 1992); *Acer pseudoplatanus* (Hong and Ellis 1990). Studies on physiological status, desiccation sensitivity and storage potential at different maturity levels of seeds of other species may provide further insight on the phenomenon of recalcitrance possibly also facilitate identification of the most appropriate seed maturity stage and time for

* Corresponding author, e-mail: spalliwest@yahoo.co.in, ruppuran2001@yahoo.co.in

collection for immediate sowing, or storage. *Aquilaria agallocha* seeds are desiccation sensitive (Kundu and Kachari 2000) and difficult to store. The objective of the present study is to investigate the developmental stages of this recalcitrant species in relation to its sensitivity to drying and storage capacity.

MATERIALS AND METHODS

Ten trees of *Aquilaria agallocha* were marked at Titabar district of Assam for fruit collection for these experiments, which were performed at the Rain Forest Research Institute, Jorhat, Assam. Agar trees flower every year during May to June and fruits mature from July to August. The flowers from ten trees, which started flowering at about the same time, were labeled during fruit set to follow fruit development. Fruits were harvested at regular intervals between 7 and 70 days after anthesis (DAA) and brought to the laboratory for testing.

After fruit collection, the pericarp was removed manually and carefully, mature and immature seeds were extracted, and data on mean fruit and seed length, seed diameter, fresh weight, dry weight, water content and germination percentage were recorded for each sample. For determination of the water content, the seeds were dried in a forced-draft oven for 17h at 103°C. Five replicates of five seeds each were used at each time point. Water content was expressed on fresh weight basis. For evaluation of germination, 25 seeds in three replicates were placed on moist papers in petriplates and germination percentage was recorded 30 days after sowing. Desiccation sensitivity of seeds at each maturity level was tested. Seeds were kept over regularly regenerated silica gel in a desiccator at 25±5°C and 100 seeds in each sample were drawn at intervals to determine the water content and germination percentage. Control seeds were treated with 0.2% Bavistin (fungicide) and stored over water in closed glass containers. For evaluation of storage potential, seeds at each maturity level were stored in closed polythene bags at 15°C at their initial moisture content and also after drying over silica gel. Seeds were sampled after 3 and 5 months for evaluation of viability in terms of germination capacity.

RESULTS

The length and diameter of the seeds and the diameter of the fruits increased as the seed developed up to 34 DAA, with little change thereafter (Fig. 1). But

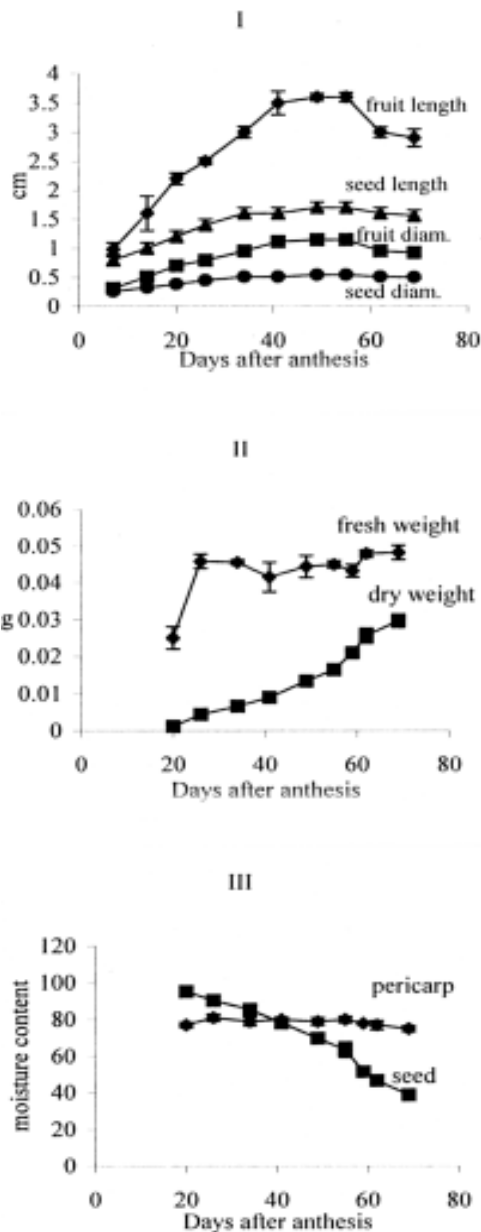


Fig. 1. Changes during development of *Aquilaria agallocha* seed, I mean length and diameter of fruit and seed [\diamond fruit length, \blacktriangle seed length, \blacksquare fruit diameter, \bullet seed diameter], II fresh and dry weight [\diamond fresh weight, \blacksquare dry weight], III moisture content of pericarp and seed [\diamond pericarp, \blacksquare seed]. Bars represents standard deviations of the mean.

the rate of increase of fruit length was much higher due to the development of the pericarp as observed up to 41 DAA. The fresh mass per seed peaked at 26 DAA whereas the dry mass increased steadily from fruit set onwards. The water content of seeds increased up to the first seven days after fruit set and then it continued to decrease with increasing maturity; on the other hand, moisture of pericarp was not changed much and dropped just before the time of shedding to facilitate the sloughing of seeds. Seeds become fully germinable at about 50 DAA and attained full germinability at 59 DAA, when the seed moisture content was found to be reduced to 51%.

Seeds harvested at different maturation stages were tested for their tolerance to drying, and it was observed once they were fully germinable, viability declined upon dehydration to water contents in the range 40-30% (Fig. 2). Mature (70 DAA) seeds appeared slightly more resilient than those that were less mature, although there was no significant difference in tolerance level from 59 DAA till final harvest. The lowest safe water content of seeds under the conditions of dehydration presently used, also increased with the development and it reached maximum at 70 DAA. It was important to note that at very early stages of development (41 DAA), seeds having 78% moisture content did not germinate, however, reduction in moisture content to 60% induced 28% to

germinate. Similarly, the germination capacity of seeds harvested at 50 DAA increased from 16%-53% after desiccation, though further drying resulted in loss of viability.

Seeds harvested at four maturation levels were stored in 2 sets, one at the initial water content and the other after slight desiccation. 46% seeds remained viable after 3 months when collected at 52% initial moisture content, but the seeds failed to germinate after 5 months of storage (Table 1). 91% seeds were viable after 5 months if collected at 34% moisture content after full maturity. Storability increased with maturity and the seeds collected at 46 to 50% initial moisture content achieved moderate storability. However, drying the seeds even above lowest safe water content failed to maintain their viability for the 5 months period irrespective of the stage of development.

DISCUSSION

In *Aquilaria agallocha*, the dry mass of seeds increased up to the completion of their development as found for other recalcitrant species (Farrant et al. 1992), and therefore there was no maturation-drying phase like the orthodox species. The fresh mass increased rapidly until the fourth week of seed development, after which no remarkable change in fresh weight occurred. The rapid increase in dry mass with proportionate decrease or replacement of seed water content results in the stability of fresh weight of seed in subsequent stages of development.

At 59 DAA, seeds acquired full germinability. After this period, the developmental stage did not affect the desiccation sensitivity of the seeds, though the dry mass accumulation continued. During the earlier stages of seed development, drying induced some germination, which was also reported by Fu *et al.* (1994) in *Clausena lansium* seeds. Drying-induced germination is common in orthodox seeds, where it stimulates expression of certain developmental changes inducing germination of the premature seeds, but it occurs after physiological maturity in the maturation drying stage (Kermode *et al.* 1986). In recalcitrant seeds, the basis of the induction of germination capacity at relatively early developmental

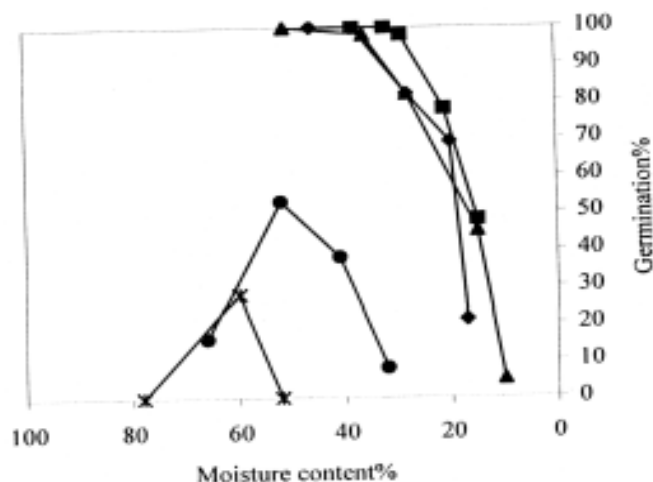


Fig. 2. The relationship between seed moisture content and germination percentage during different developmental stages. -x- 41 DAA; -●- 50 DAA; -▲- 59 DAA; -◆- 63 DAA and -■- 70 DAA.

Table 1. Viability (assessed as germination) after storage of *Aquilaria agallocha* seeds collected at different developmental stages.

Developmental stages	Initial moisture content	Moisture content after desiccation	Viability(%)±s.d.		
			after 0 months	after 3 months	after 5 months
50 DAA	65%		18.6±5.03	0	0
		52%	50.1±7.1	0	0
		40%	36.8±4.0	0	0
59 DAA	52%		100	45.9±3.0	0
		36%	98.0±1.8	20.5±6.2	0
63 DAA	45%		100	72.5±4.6	70.5±8.2
		36%	100	88±5.2	0
70 DAA	34%		100	93.8±2.5	90.6±3.0
		24.40%	71.8±2.8	62.0±8.1	0

stages, where only half of the total dry mass has been accumulated, is not known. However, as histodifferentiation occurs at early stage of development, it appears that the germination potential is achieved before reserve accumulation starts. Drying of the immature seeds above the respective critical moisture content appears somehow to induce developmental changes that trigger germination.

After full attainment of germinability, the seeds are tolerant to a certain degree of dehydration, which is not affected by the stage of maturation. Farrant *et al.* (1993) made a similar observation on *Avicennia marina* seeds, although an increase in desiccation tolerance of *Quercus robur* with progressive development was recorded by Finch-Savage (1992). On the other hand, Fu *et al.* (1994) have shown that maturing seeds of *Clausena lansium* and *Litchi chinensis* are more desiccation tolerant than when fully mature. The difference in response in the recalcitrant seeds may appear due to the difference in progress of the developmental events. But in over-mature seeds, the less tolerance than the maturing seeds might be the result of physiological deterioration rather than the desiccation damage.

It is important to note that the seeds of the agar tree are sensitive to drying long before full maturity, therefore, inhibition in germination processes may not be the only cause of desiccation sensitivity in this case as opined by Farrant *et al.* (1988). Storage of *Aquilaria agallocha*

seeds for short periods can appear to be possible only after full maturity. However, even these seeds cannot be stored for a similar period after a measure of drying. It can be concluded that seeds of this species are highly recalcitrant and although before completion of dry mass accumulation, the seeds can be induced to germinate and to tolerate some desiccation; total accumulation of reserve materials is necessary for short-term storage.

ACKNOWLEDGEMENTS

We thank Mrs. Jonaki Kachari for excellent assistance in the experiments.

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