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Studies on Seed Biology of *Cinnamomum tamala* Nees (Lauraceae): A Valuable Multipurpose Tree'

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Cinnamomum tamala is an economically important multipurpose tree. Owing to its high medicinal value and being an important ingredient of the spices, the species is being extracted from its natural habitat and entered in vulnerable category. During the present study it was found that seeds of C. tamala could not be stored beyond 4 days both at room temperature as well as low temperature (4^oC). Besides this, the seeds exhibited desiccation sensitive and loss to seed viability was recorded when desiccated below 80% (fresh weight basis). The freshly harvested seeds registered ~72% germination under 50% shading of light in the seed beds and converted into seedlings.

Ker words: *Cinnamomum tamala*, seed biology, desiccation sensitivity, recalcitrant seeds.

A number of different plant species produces seeds considered as recalcitrant, as they are shed from the mother plant with high moisture content and are desiccation sensitive. They generally directly pass from development to germination (Chin *et al*., 1984; Berjak & Pammenter, 1994; Tommasi *et al*., 2006). Again there are recalcitrant seeds with different desiccation tolerance and their behaviours intermediate between orthodox and recalcitrant (Berjak & Pammenter, 1994). For few recalcitrant seeds, there is consistent literature on some aspects of seed development, on the basic physiology and response to desiccation as well as ecology (Farrant *et al*., 1993; Finch-Savage *et al*., 1996; Pammenter & Berjak, 1999; 2000; Daw *et al*., 2004; Pritchard *et al*., 2004). However many questions are still open, for example concerning the life-span of seeds, long-term storage, temperature during storage, developmental stage of the seeds at which they should be harvested (Pammenter *et al*., 1994). There are some reports available on low temperature sensitive of recalcitrant seeds and the storage life span (Chin & Roberts, 1980; Pammenter & Berjak, 1999). The germination of seed and seedling establishment in the forest floor or in seed bed is greatly affected by different conditions like light regime in the seed bed (Davies, 2001), moisture content in the seed, low temperature treatment of the seeds before sowing etc. Recruitment of tree species on the forest floor is governed by various factors including the seed traits, developmental stages of the embryos, desiccation tolerance of the embryos, microhabitat (Kitajima, 2007).

Cinnamomum tamala commonly called as '*tejpat*' is an evergreen species distributed in the lower Himalayan zone. Leaves are aromatic and traded as spice (Anonymous, 2006) and also as a source of various Ayurvedic formulations (Sarin, 2008). It flowers during March-May and usually pollinated by small insects such as honey bees. The fruits are ellipsoidal drupe. Ripe fruits are dark purple in colour and contain single seed (Fig. 1a). Its leaves and bark are aromatic and traded as a spice (Dhar *et al*., 2002; Anonymous, 2006). The essential oil from bark is pale yellow, and contains 70-80% cinnamic aldehyde. The leaf oil of *C. tamala* resembles cinnamon leaf oil. It has been reported the crude drug from unripe fruit of *C. tamala* is being sold under the name '*nagkesara*' in different parts of India (Vaidya, 1971). Parts of *C. tamala* are also used in many *Ayurvedic* preparations e.g. *sudarshan choorna* and *chandraprabhavati*. The leaf extracts are used as clarifiers in dyeing procedures with myrobalans. Traditionally green dye has been extracted from its leaves (Gaur, 2008).

Owing to its high medicinal value and being an important ingredient of the spices the demand of *C. tamala* is increasing day by day and the species is being exploited from its natural pockets illegally and entered in vulnerable category throughout the Himalayan range (Samant *et al*., 2001, Sharma & Nautiyal, 2009).

Therefore, there is a need to raise high quality individuals in large scale to fulfil the increasing demand on the one hand and help the conservation of the species on the other. However, due lack of information on standard agro-technique, seed characters, seed germination behaviour, conservation efforts have not succeeded completely (Rawat *et al*., 2009; Sharma & Nautiyal, 2009). In view of the above, present study was aimed to study the desiccation tolerance of the seeds, storage temperature and post harvest storage duration, effect of different light condition in the seed bed on seed germination and seedling morphology.

Materials and Methods

Site description: The study was conducted during 2008-2010 in the Department of Botany, Nagaland University, Lumami located in Zunheboto district of Nagaland, India (26°12′37″ N altitude and 95°29′28″ E longitude; altitude 1150-1200 m asl). For the present study three different types of seed beds were prepared viz. 1. Seed bed with normal day light, 2. Seed bed in the poly shade with 50% filtered light and 3. Seed bed in the poly shade with 75% filtered light. All the seed beds were prepared by mixing humus (decomposed forest litter), soil, sand, decayed wood powder at 1:4:1:1 ratio respectively. The mixture was sundried before putting them in the seed bed (polybags).

Seed collection and transport: The details of collection and transport of different types of seeds for experimental purpose may materially affect result. For the present study, the intact mature fruits of about 16 weeks old (Fig. 1a) were harvested randomly from the trees from two trees available in Mokokchung town, Nagaland, India during 2007-2009. The collection was completely randomized without screening the size and colour of the fruits except the damage by the insects, microorganisms or birds. The collected seeds were transported to the Laboratory within 24 hours of collection in the plastic bags. The seeds were cleansed by rubbing the fruits with soft cloth towels, briefly surface cleansed with soft Laboratory detergent (1%, v/v, Extron a commercially available detergent, make: Merck, India) and stored in plastic bags at 25°C. The time leg between removal of the seeds from the fruits and initiating experiments was 1-2 days. The seeds were made into different sub lots/groups for different experiments. In each lot there were 50 seeds.

***Experimental process*:** The processed seeds were sowed immediately after harvest in the seed bed while rests of the seeds were treated differentially as described below:

A. A set of freshly processed seeds were sowed in three light conditions viz. normal light (5000 lux), poly house with 50% shade (2500 lux) and poly house with 75% shade (1250 lux); B. To test the desiccation tolerance to the embryos/seeds, part of the seeds were desiccated/ dehydrated at various levels (100 to 30%, fresh weight basis). The rapid drying of the seeds was achieved by exposing to cool blowing air and slow drying was achieved by exposing to the normal sunlight before they are sowed to the seed beds and C. After processing one sub lot of seeds were stored without desiccating for various periods (0-18 days) at 4°C (in the refrigerator) and 25°C (in the Laboratory) in sealed polybags before they were sowed in the seed beds.

To study the emergence, survival and growth of seedlings of *C. tamala* under each condition, three replicates of 50 seeds each were used. In each experimental condition 50 polybags (15 cm long and 7.5 cm diameter) were used. In each polybag the soil mixture was packed. In each poly bag only one seed was sowed. The seed beds were watered weekly. The experimental design was completely randomized. The data was collected daily basis for seed germination, seedling morphology, per cent response etc.

The seedlings were maintained in the respective polybag and watered at regular interval and studied the seedling morphology for six months. Once the seedling showed normal functioning like formation of normal leaves, seedling growth etc., the seedling were transferred to the nature.

Results

Seeds sowed in the experimental seed beds started germinating and emergence of radicals within 10 days. The seed germination rate, germination time, morphology of seedlings was greatly influenced by illumination in the seed beds, level of desiccations, temperature of post harvest storage and duration. There was a significant variation in time taken for initial response (10 to 15 days) and germination rate/seedling emergence (52.2 to 72.2%) across the seed beds with different light intensities (Fig. 2). Seeds sowed in the seed bed under higher illumination (5000 lux) under performed as there was delay in initial time taken for germination and overall germination rate (15 days and ~52% respectively). Compared to normal irradiation, seeds sowed under lower irradiations performed better. Of the different light conditions studied in the present study, optimum response was achieved from seeds sowed under 50% shade (2500 lux) where within 10 days of sowing radical started emergening and registered 72.2% seed germination (Fig. 2).

Seeds sowed under normal light condition delayed germination and leaves were curly and exhibited stunted growth of the seedlings, while, seeds sowed under 75% shaded condition, seedlings were healthy but comparatively etiolated in comparison to 50% shaded condition.

Besides light requirement for seed germination, seeds were also tested for their desiccation tolerance, post harvest storage and temperature tolerance during storage. In the present study with *C. tamala*, the seeds sowed immediately after harvest exhibited highest germination frequency (72.2%) after 10 days of sowing (Table 1).

Table 1: Effect of desiccation of mature seeds on seed viability and seed germination of *Cinnamomum tamala* in the seed bed.

Desiccation level (%) (fresh weight)	Time for first sign germination (days)	% germination (±SE)*	Type of response
100	10	72.2 (±1.5)[a]	Healthy seedlings with healthy roots
90	16	70.5 (±2.0)[a]	As above
80	19	71.5 (±1.5)[a]	As above
70	25	58.3 (±1.0)[b]	Delayed germination and seedling were stunted in growth
60	32	42.5 (±1.5)[c]	Dwarf seedlings with shorter leaves and poor rooting
50	35	35.2 (±1.5)[d]	As above
40	37	12.0 (±2.0)[e]	Seedlings with shorter leaves and roots but degenerated subsequently
30	-	-	No germination

* Standard error from mean.

Data represents the mean of three replicates.

In the same column, figures followed by the same letter were statistically identical to the threshold of 5% (Newman-Keuls, ± standard error).

The germination rate decreased considerably as the storage period increased exhibited <50% germination after 6th day of storage and after 16th day no seed germinated. As the storage period increased, germination period also delayed significantly. The seeds were stored in two different temperatures (4^{o}C and 25^{o}C). There was no significant difference in germination period as well as germination rate when compared in both the temperatures though experiments conducted at 4°C performed showed slightly better (Table 2).

In the present investigation *C. tamala* seeds exhibited desiccation intolerance. The seeds sowed immediately after harvest without desiccation registered highest germination within minimal period of time. With desiccation, the viability decreased and registered poorer germination rate (Table 2).

Table 2: Effect of post harvest storage of mature seeds at 4°C and 25°C on seed viability and germination in the seed bed.

Storage period (days)			Time taken for	
germination	% germination (±SE)*			
	(days) from seeds stored at		from seeds stored at	
	4°C	25°C	4°C	25°C
0	10	10	72.2 (±1.5)[a]	72.2 (±1.5)[a]
2	10	14	71.5 (±1.5)[a]	69.5 (±1.0)[a]
4	15	16	65.2 (±2.0)[b]	55.0 (±1.5)[b]
6	15	17	50.5 (±1.0)[c]	51.3 (±2.0)[b]
8	18	21	35.5 (±1.0)[d]	33.5 (±1.5)[c]
10	30	35	22.2 (±2.0)[e]	21.5 (±1.5)[d]
12	30	36	10.5 (±1.5)[f]	10.0 (±1.0)[e]
14	45	50	07.5 (±2.5)[g]	04.5 (±1.5)[f]
16	47	50	5.0 (±0.5)[g]	03.0 (±0.5)[f]
18	-	-	-	-

* Standard error from mean.

Data represents the mean of three replicates.

In the same column, figures followed by the same letter were statistically identical to the threshold of 5% (Newman-Keuls, ± standard error).

It was found that when the moisture content reduced to <60% (fresh weight basis) the germination rate decreased below 35% and abnormal seedling morphology was recorded which ultimately affected the seedling establishment in the seed beds as well as in the nature. The seedlings developed from the seeds desiccated up to 70% exhibited normal seedling morphology and normal growth in nature (Fig. 1b). The seeds were desiccated following two methods i.e., rapid desiccation and slow desiccation. It was found that the seeds desiccation achieved by rapid method performed better in comparison to slow method. The seedlings formed from the seeds desiccated by rapid method were healthier and seedlings established better in the seed bed as well as in the nature (Fig. 1c).

Figure - 1

Figure 1: a. Mature seeds of *C. tamala*; **b**. Healthy seedlings in polybags germinated from the seeds, **c.** An established seedling converted into young plant in the field.

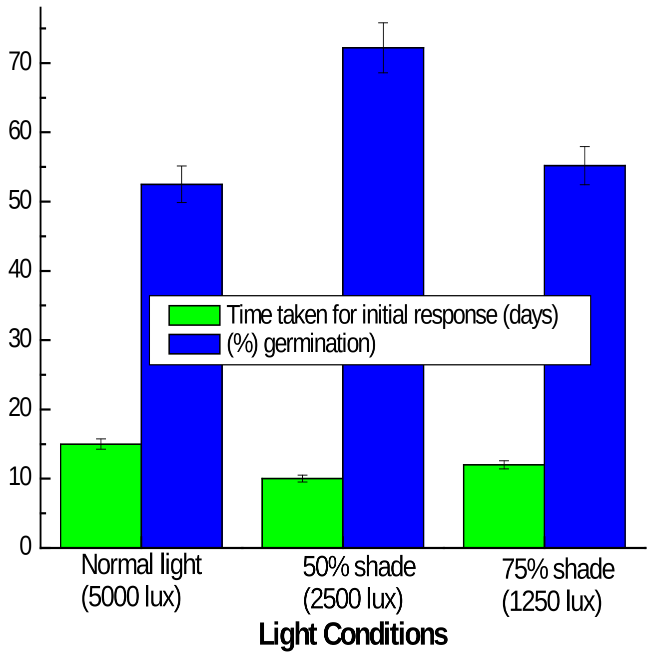

Figure 2: Effect of different microclimatic conditions (light intensities) on seed germination of *Cinnamomum tamala* in the seed bed

Discussion

The practice of seed preservation is as old as agricultural practices but systematic collections and storage facilities have been a development of the 20th century. Presently there is an estimate over 1500 seed or gene banks around the world containing over 6 million seed accessions. For some plant species, using relatively fresh seeds gives superior germination over stored seeds. Viability of a seed lot declines over time and though old seed may germinate, the resulting seedlings may have reduced vigor and fail to establish as well as seedlings from fresh seed (Walters, 2004).

The time for emergence of radicals from the germinated seeds, germination rate, seedling morphology and seedling establishment is influenced by various many factors. Plant species differ greatly in their habit preference, temperature requirement, post harvest storage, specific pre-treatment for seed germination, seedling emergence and survival. A number of tree species exhibit positive as well as negative correlation between canopy cover/light requirements, temperature etc. (Kwit & Platt, 2003; Pages *et al*., 2003). The requirement of light for successful seed germination and healthy seedling morphology appears to be species specific. Seedling survival on the seed beds/forest floor is governed by the availability of light, water and nutrients (Kitajima, 2007). Increase in light and nutrients in mesic gaps stimulate the growth of the competing shrubs and fast growing herbaceous plants. In general, the varying microenvironment had a significant effect on survival of seedlings of French Alps (Pages *et al*., 2003). According to Onen (1999), germination percentage was almost independent from light conditions. In mugwort the germination values obtained from light conditions were similar at the same incubation temperatures. Therefore, it was supposed that presence or absence of light had no effect on both germination percentage and germination speed of mugwort seeds. But, the fresh achenes germinated in room temperature under daily light were 14 per cent higher than that of under the dark condition (Onen, 1999). However, a brief exposure to low intense light was found sufficient to stimulate germination. But as the seeds aged, they became less dependent on light and eventually could germinate also in darkness (Holm *et al*., 1986). In the present study with *Cinnamomum tamala*, there was moderate germination in seeds sowed across the different light conditions. In higher illumination the germination was slightly delayed compare to lower light intensity. From the other experiments, it was found that the seeds of *C. tamala* are recalcitrant in nature and does not go for dormancy. Present result is in agreement with the previous reports which describes that the non-dormant seeds of many species germinate well more or less equally in light and dark (Baskin & Baskin, 1988).

During the present study, investigations were carried out on the relationships among rate of drying, dehydration tolerance, post harvest storage and storage temperatures. From the results obtained in the present study clearly shows that *C. tamala* seeds are desiccation-sensitive. The seed material dried at the two rates used in this study showed considerable difference in seedling morphology and seedling establishment. The seedlings developed from the seeds desiccated by rapid desiccation method were healthy. The present observation is in agreement with Pammenter *et al.* (1991) where they have reported a similar response while working with *Landolphia kirkii*.

To determine seed type the seeds should be tested prior to storage. Seeds that tolerate desiccation to 5% moisture content or below (10-15% fresh weight of seeds) are likely to show orthodox seed-storage behaviour. Seeds that tolerate desiccation to 10-12% moisture content (40-50% of fresh weight), but whose viability is reduced when subjected to further desiccation to a lower moisture content are likely to show intermediate seed storage behaviour. Seeds that are killed by desiccation to 15-20% moisture content ~ 70% fresh weights of the seeds) are likely to be recalcitrant (Rao *et al*., 2006). In *C. tamala* dehydration of seeds below 60% reduced the viability of the seeds and germination. In addition to drying, another form of controlling the biological activities is the use of low temperature. Storing the seeds at 4^oC exhibited slightly higher percentage of viability and germination in comparison to storage at 25^oC. The lowest temperature withstood by the recalcitrant seeds seems to vary with the species (Fu *et al.*, 1990; Oliveira & Valio, 1992; Barbedo & Cicero, 2000; Tommasi *et al*., 2006). *Ginkgo biloba* seeds could be stored at 4^oC for one year but when stored at 25^oC, seeds died after 6 months (Tommasi *et al*., 2006). Present study with *C. tamala*, also exhibited a similar response where seeds stored at 4^oC germinated better over seeds stored at 25^oC.

The findings in the present study reveals that the seeds of *C. tamala* are desiccation-sensitive i.e., they are recalcitrant in nature and the seeds cannot be stored over a period of one week either at room temperature or at low temperature (4^oC). No differences were observed among germination rates of seed lots stored at different temperatures. Besides these, the seeds of this economically important species exhibit optimal germination and seedling establishment under diffused light condition. Further studies on mechanism of seed dispersal, seed storage and temperature tolerance will help in developing the conservation strategies of this economically important spice yielding species.

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