

Effect of Duration of Accelerated Ageing on Physiological Dormancy in Teak (*Tectona grandis*) Seeds : Preliminary Observations

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ABSTRACT Studies on the duration of accelerated ageing (AA) on physiological dormancy in teak seeds revealed that AA of teak seeds for 6 days would facilitate quicker germination and mobilization of seed proteins with release of physiological dormancy.

Keywords: Teak, *Tectona grandis*, germination, dormancy

Teak (*Tectona grandis* L.f) is the most sought after timber commonly propagated using seeds. Large scale production of teak seedlings, however, is limited by various factors such as low seed yield per tree and poor germination in nurseries [1]. The causes for the major constraint of poor germination are emptiness of drupes, prolonged and protracted germination, irregular dormancy cycles, plus innate problems in seeds themselves [2, 3]. Masilamani and Dharmalingam [4] reported barriers which prevent teak seed germination can be physiological, physical or morphological. Although rapid progress is being made in some areas, a comprehensive understanding of the biophysical, physiological and biochemical determinants of germination and dormancy is needed [5]. Protein metabolism has been implicated in the breaking of seed dormancy as seed storage proteins play a pivotal role, providing reduced nitrogen, carbon and amino acids to growing tissues [6, 7]. Accelerated ageing (AA), recognized as a stress test for seed vigour by the Association of Official Seed Analysts [8] has been shown to enhance teak seed germination [4]. In *Pinus banksiana* and *Quercus rubra* seeds, changes in isozymes, amino acids and proteins were associated with AA, along with improved germination [9]. The objective of the present work was to study the effect

of accelerated ageing on breaking teak seed dormancy and to determine the resultant changes in protein and amino acids levels.

MATERIALS AND METHODS

Mature seeds were collected from 20 trees in seed production area at Tholpatty (11° 39'N; 76°16'E), Kerala state, India in mid December, 2001. Random samples were collected from each tree and bulked. A portion of the bulk sample was stored in paper bags at ambient temperature (30 to 32° C) for one year (natural ageing). A randomized complete block design, with four replications was used for all the experiments. The data were subjected to analysis of variance (ANOVA) following arc sine transformation and the significance of mean differences determined using Duncan's Multiple Range Test [10].

The fresh seeds were subjected to AA in a desiccator. Water was added to the desiccator up to the level of the perforated porcelain disc. Filter papers dipped in water were placed along the walls of the desiccator, ensuring availability of water vapour/ moisture throughout the chamber. The seeds were placed in the desiccator in thin plastic net bags. The tightly closed desiccator was maintained at 40°C and the relative humidity was found to be $98 \pm 2\%$ which

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Table 1. Viability and germination tests for teak seeds after different periods of ageing

Treatment	Germination % (in sand bed)	TZ result
Fresh seeds (Control)	42.5 ^b	86.36 ^a
1 year old fruits (natural ageing)	46.0 ^b	73.58 ^b
6 days of AA	51.0 ^a	65.70 ^c
10 days of AA	9.5 ^c	-
15 days of AA	4.0 ^c	-
20 days of AA	4.0 ^c	-

(Means with the same letter in a column do not differ significantly as per DMRT at 5 % level of significance)

Table 2. Effect of accelerated ageing on germination, soluble storage protein and free amino acids of teak seeds

Treatment	Germination (%)	Soluble storage protein (mg/g)	Total free amino acids (mg/g)
Fresh seeds (Control)	15.25 ^c	87.99 ^a	1.72 ^b
3 days of AA	19.75 ^b	80.72 ^{ab}	2.40 ^a
6 days of AA	32.75 ^a	70.51 ^b	2.57 ^a

(Means with the same letter in a column do not differ significantly as per DMRT at 5 % level of significance)

was measured using the dial hygrometer placed inside the ageing chamber. After 6, 10, 15 and 20 days, samples were taken to determine seed viability by staining (TZ test) and germination tests. Fresh and one year old seeds (natural ageing) were used as control treatments.

Following AA for each test periods the seeds were pretreated by alternate soaking in water and sun drying ($31 \pm 1^\circ\text{C}$, 8 hours) for six cycles for germination studies [11]. Germination tests were conducted in sand beds according to International Seed Testing Association [12] Rules with 100 seeds per replication. The data were collected for the following 28 days. The seeds embedded in the stony endocarp were extracted and samples of four replications each with 25 seeds were taken for Tetrazolium (TZ) test, an indirect test for seed viability. The seeds were soaked in 1 % of 2, 3, 5 triphenyl tetrazolium chloride solution at 30°C for 4 hours in the dark and then observed for staining patterns [13]. Only seeds completely stained red were recorded as viable.

A second AA experiment was set for just 6 days, since a steady decline in germination percentage beyond six days of ageing was observed, using the following treatments (i) Fresh seeds (0 days of AA) (ii) 3 days of AA and (iii) 6 days of AA. The seeds were subjected to germination test. Simultaneously the seeds were estimated for proteins and amino acids. The soluble seed storage proteins were determined by the method of Lowry *et al.* [14] and free amino acids by the method of Moore and Stein [15] and both were expressed in mg per g seed.

RESULTS AND DISCUSSION

Of the various treatments in the first experiment, seeds subjected to 6 days AA gave good germination when compared to the control (Table 1). Fresh seeds showed the highest viability (86 %) in TZ test. However, in nursery, germination of the fresh seeds was lesser than one-year-old seeds (46 %) and six days aged seeds (51 %). This suggests that seed viability in teak decreases with ageing (either natural or accelerated). After 10 days AA there was complete loss in seed viability.

In the case of teak, which is universally acknowledged as a seed with complex and multifarious dormancies operating, the above tests play an important role. In fresh seeds, with 86 % viability, germination was only 50 % of the TZ value. Conversely, when subjected to ageing (natural and accelerated), TZ values were 73 % and 65 % with germination of 46 % and 51 % respectively, that is, 60 % of the viable seeds of the naturally aged group and 75 % of the viable seeds of accelerated aged group germinated. This shows that physiological dormancy exists in teak which could be overcome through ageing processes. The process of AA has simulated seed metabolism occurring in naturally aged seeds. The physiological changes occurring during natural (slow) ageing will occur during a short period when seeds are exposed to high temperature and humidity [16]. It is suggested that after-ripening associated with hormone imbalance in teak seeds results in old drupes showing moderate germination compared to fresh seeds [4].

The second experiment showed that germination increased significantly from 15 % in the control to 20 % in 3 days aged seeds and 33 % in 6 days aged seeds, about a 50 % increase over the control (Table 2). Enhancement of seed germination resulting from a short period of AA has been observed in water oak (*Quercus nigra*) [17]. Bonner [18] in his preliminary work on long leaf pine (*Pinus palustris* Mill.) seeds also observed germination enhancement by AA. Masilamani and Dharmalingam [4] have reported that forced ageing in teak enables early germination, which is associated with more seedlings per 100 fruit and better seedling vigour.

Biochemical estimations on soluble storage proteins and total free amino acids indicated a linear reduction in proteins and increase in free amino acids with AA (Table 2). This suggests mobilization of protein reserves for triggering germination. Seed storage proteins accumulate in the late stages of seed development and are mobilized only during germination [19]. Reports of Beardmore *et al.*, [20] reveal that storage protein mobilization in *Populus grandidentata* occurs following seed germination. Increase in the levels of free amino acids confirms that initiation of germination is an amphibolic process, catabolic to provide energy and raw materials for early growth of the seedlings and anabolic to utilize the subunits for biogenesis. Studies that have examined total seed proteins [21] suggest that cross-linking and proteolysis may occur with ageing. Callis [22] suggested that multiple mechanisms are responsible for seed storage protein hydrolysis, involving regulation at molecular level. Fincher [23] reported that the hydrolysis of storage proteins into their constituent amino acids is induced by proteases.

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