Germination and seed storage behaviour in *Pongamia pinnata* L.

Pongamia pinnata is an important non-edible minor oilseed tree[1] that grows in the semi-arid regions. The leaves are a good source of green manure and being leguminous, they enrich the soil with nitrogen. The seeds contain around 30–40% of oil, which has been identified as a source of bio-fuel and has medicinal value. It is predominantly cultivated through seeds and the genetic diversity has been conserved through storage of seeds, the most common conventional and economical method[2,3]. However, the seeds of *P. pinnata* suffer from germination and storage problems. Seed storability varies greatly among tree species and is mainly influenced by the variation in climatic conditions, such as temperature, rainfall and relative humidity, which fluctuate in our country across the year. This makes the storage of seeds difficult for commercial exploitation, cultivation and conservation of plant genetic resources. Differences in storage behaviour are often associated with morphological, physiological, anatomical structure and biochemical composition of the seeds, which affect the desiccation and chilling sensitivity of seeds and thereby the longevity in storage. The present investigation was undertaken to study the germination and seed storage behaviour of *P. pinnata*.

The freshly harvested fruits of var. BAK 49 (IC 430529) were procured from Adilabad District, Andhra Pradesh. Three replications of 25 seeds each were treated with Thiram (0.2%) and were plated between the paper for germination. All the samples were maintained in an incubator at 27 ± 3°C. Germination percentages were recorded according to the ISTA (2003) guidelines[4]. The seedlings were evaluated on the 15th day of plating and the rate of germination was calculated by counting the fresh emergence each day till the final count. The vigour index was calculated by multiplying the germination percentage with root plus shoot length, as described by Abdul-Baki and Anderson[5]. In addition, a quick viability (TTC) test was performed to assess seed viability[6].

Pongamia pods are 4.5 cm long and 1.5–2.5 cm wide, broad, pointed at both ends, yellowish-grey when ripe, and one- or two-seeded. Seeds are elliptical, reniform, compressed, reddish-brown, fairly hard, 2–3 cm long and weigh about 1200 g/1000 seeds, with a thin seed coat. Seeds extracted from fruits showed 14.32% moisture content on fresh-weight basis, with dormancy and poor storability under ambient conditions. The germination of these seeds was 44%, though the results of the TTC test showed 100% viability indicating differential behaviour, either because of differences in physiological maturity and/or dormancy of seeds (Table 1). Various seed treatments were attempted for improving germination percentage. A set of seeds without any treatment was used as control. Of these, hot-water treatment at 60°C for 30 min increased germination percentage significantly from 44 to 100%, showing an increase of 56% over control. Other treatments such as soaking in water overnight and scarification, showed an increase by 32 and 30% respectively. Both vigour index and rate of germination increased with increase in seed viability (Table 1). The hot-water and pre-soaking in water treatments shortened the germination period by 5–10 days along with uniformity of germination, confirming earlier reports[7–9], where artificial softening of the seed coat reduced seed hardness in Fabaceae. Similarly, hot water treatment, and mechanical and chemical scarifications have helped in breaking seed dormancy[10,11].

One of the reasons for poor germination of seeds observed was large size, richness in food (17% starch and 13% mucilage) and high moisture content, which makes the seeds vulnerable to fungal infection, thereby reducing germination. To overcome this problem, the seeds were treated with various fungicides; treatment with Thiram (1.0%) gave the best results (Table 2). Seeds dressed with Thiram and treated with hot-water recorded 60% improved germination over control.

To investigate desiccation and chilling sensitivity, which have been associated with seed storage behaviour, the seeds were desiccated in batches of 50 each in muslin cloth bags over charged silica gel. These seeds were taken out at different intervals to determine the seed moisture content and seed viability through germination test. The desiccated seeds were humidified in mist chambers before conducting the germination test in order to avoid imbibitional injury. Moisture content was determined by low constant temperature oven method. Chilling sensitivity was tested by storing the desiccated seeds (6.0% mc) at +4°C and –20°C for three months, and evaluating the seeds for viability using germination test at 15 days interval. The data were analysed statistically for significance.

The seed moisture content declined steadily with desiccation time (Table 3). The decline was highly significant on the first day, i.e. about 42% over control followed by a slow decline of 17–18% in

Table 1. Effect of various treatments on seed germination of *Pongamia pinnata*

Treatment	Germination (%)	Vigour index	Rate of germination (*d*)	Viability (TTC staining pattern)
Control	44 ± 1.23	1063 ± 0.98	2.96	100
Pre-soaking in water (24 h)	68 ± 2.10	2326 ± 1.12	3.00	100
Scarification	70 ± 1.17	2244 ± 1.08	4.13	100
Hot water (30 min)	100 ± 1.20	2810 ± 0.94	4.97	100

Table 2. Effect of fungicide on the survival of *Pongamia* seeds

Treatment	Concentration (%)	Survival (%)
Fresh seeds		40
Captan	0.5	85
Captan	1	90
Thiram	0.5	85
Thiram	1	95
Sodium hypochlorite (for 10 min)	0.2	80
Sodium hypochlorite (for 10 min)	0.5	85

Table 3. Effect of desiccation on seed germination of *P. pinnata*

Period (days)	Moisture content (%)	Seed viability: Germination (%)	Seed viability: TTC staining pattern	Vigour index*	Days for final count
Control	14.32	100.0 ± 2.21	100	2698	15
2	8.32	100.0 ± 2.17	100	2584	18
7	6.95	95.7 ± 1.89	95	2549	20
15	4.97	93.2 ± 2.21	92	2288	22
20	3.67	90.0 ± 2.10	90	2223	28
28	3.39	90.0 ± 2.21	90	2200	30

*Vigour index = Germination % × (root length + shoot length).

Table 4. Effect of chilling on seed viability of *P. pinnata*

Storage temperature	Storage duration (months)	Germination (%)
Control (fresh seeds; mc 14.3%)	–	100
Desiccated seeds (mc 6.0%)	–	95
+4°C	3	90
–20°C	3	90

mc = moisture content.

the subsequent days. Seed viability of 90–95% was maintained at all the desiccation levels. The seeds took about 28 days to achieve lowest moisture content of 3.39% (critical moisture), at which viability was maintained up to 90%. The vigour index followed a similar trend (Table 3). The patchy and less TTC staining pattern appears to correlate with progressive decline in seed viability and seed deterioration indicated by loss in seedling vigour with the advancement of desiccation, reflecting the efficacy of this technique in assessment of seed viability and quality of *Pongamia* seeds (Table 2). This result is in conformity with earlier observations[12], suggesting that the seed deterioration is associated with the loss of vigour. On desiccation, the germination period was extended to 22–30 days compared to 15 days in the case of control, indicating induction of dormancy as the seeds progressed to maturity under desiccation. The dried seeds maintained 80–100% viability when exposed to low temperature (4°C and –20°C) for different durations (Table 4). No significant adverse effect of desiccation and chilling on viability was observed (90% germinability) even at 6.0% moisture and at –20°C, confirming the orthodox nature of *Pongamia* seeds (Table 4).

Higher seed moisture content (40–60%), large seed size and weight have often been associated with recalcitrant storage behaviour of seeds (weight of 1000 seeds exceeding 500 g)[13]. However, *Pongamia*, despite having tropical/subtropical habitat and large and heavy seeds with moisture content of about 14% on fresh-weight basis, is not sensitive to desiccation and chilling. Therefore it can be stored normally with drying of seeds to 5–10% moisture level under low temperature conditions according to the objectives.

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Secondary emissions from spectrofluorimeters

Heisenberg's uncertainty principle states that when a wave of frequency λ is generated in a finite space, it is accompanied by its overtones (2λ, 3λ, 4λ, etc.) and undertones ($\lambda/2$, $\lambda/3$, $\lambda/4$, etc.). According to eigenfunctions, the intensity of these harmonics dies out exponentially. As a result, only the first or second harmonics of several-fold lower intensity are of significance[1,2]. A description of the harmonics obtained from a two-channel digital real-time oscilloscope (Model Tektronix TDS 220) shows that when a wave of 2555 Hz is generated, it is accompanied by frequencies of 5110 and 7665 Hz, which represent the first and the second overtones respectively. The ratio of their intensities is 100 : 1.73 : 0.27 (Figure 1). Thus the uncertainty in generating monochromatic light cannot be solved by improving instrumentation.