



## Investigating Seed Germination and Storage Behaviour of *Premna latifolia* Roxb

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### Abstract

**Objective:** An investigation was carried out to discover seed germination behaviour of *Premna latifolia* by giving dormancy- breaking seed pre-treatments and to determine seed storage behaviour of *P. latifolia*. **Methods:** Different pre-treatments (cold water soaking 48 hrs, hot water soaking 24 hrs, GA<sub>3</sub> 500 ppm and 800 ppm for 48, 72 hrs) and a control (without any treatment) were given to the seeds. Seed storage was done at two moisture content levels (8% and 5.4%), four temperature levels (ambient room temp., 15°C, 5°C and -5°C) for 24 months. Viability was determined for stored seeds by germination tests at 3 months interval (0 days, 3, 6, 9, 12, 15, 18, 21 and 24 months). **Results:** The result indicated that among all treatments, higher germination (90%) and vigour (507.60) was recorded in seeds treated with GA<sub>3</sub> 500 ppm for 72 hrs. Seeds stored at 5°C temperature and 8% moisture content was able to maintain viability for 24 months. **Conclusion:** GA<sub>3</sub> at 500 ppm for 72 hrs is an effective pretreatment for breaking dormancy and enhancing germination in *P. latifolia* seeds while, storing seeds at lower temperatures could be useful in maintaining viability and vigour of the seeds for longer period.

**Keywords:** *P. latifolia*, Seed dormancy, Seed storage, Seed pre-treatment, Gibberellic acid.

### 1. Introduction

*Premna latifolia* Roxb. syn. *Premna mollissima* Roth. Commonly known as dusky fire brand bark is a small sized deciduous tree about 6-7m tall, belong to family Lamiaceae. It is distributed in southern Asia including Cambodia, China, India, Indonesia, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand and Vietnam (Quattrocchi, 2012). It naturally inhabits the lower and outer sub-tropical Himalayan regions up to 1500 meters in elevation (Kumar, *et al.*, 2011). In the Western sub-Himalayan tract, it is often found in the mixed forest of *Acacia catechu* and *Dalbergia sissoo*, and the final stage of sal forest (Troup, 1921). The genus *Premna* is reported to have about 200 species worldwide (Harley, *et al.*, 2004) and 35 species in India (Kabra, *et al.*, 2015), in which most species are small trees or shrubs, rarely lianas (except *P.*

*trichostoma*). The tree has smooth, grayish-white bark, cordate or ovate, cuneate, rounded leaves, small, greenish-yellow flowers in paniculate corymbs, 4-celled drupes, leaves that are 5-17 cm long, and fragrant when crushed. Fruit ripening begins in mid-June to mid-August, while flowering takes place in April and May. Nearly a globose indehiscent drupe, the fruit is green at first but turns bluish-black and lustrous when it ripens. The outer fleshy layer of the drupe encloses three partly or abortive seeds along with to one fully mature seed. The rectangular, cream-colored seed is 3.6 x 3 mm in diameter (Troup, 1921; Kanjilal, 1969; Kumar, *et al.*, 2018). Numerous medicinal applications have been documented for nearly every component of *P. latifolia*, including the roots, leaves, and bark. As an appetizer and

astringent, the roots can be used to treat abscess, asthma, bronchitis, heart problems, diabetes, diarrhea, inflammations, neuralgia, cancer, rheumatoid arthritis, rhinitis, stomach issues, and as a postpartum tonic for women (Dianita and Jantan, 2017; Virshette, *et al.*, 2020). According to reports, the leaves are diuretic and can be useful with agalactia, allergies, colic, coughing, dropsy, dyspepsia, flatulence, neuralgia, piles, rheumatalgia, and tumors (Quattrocchi, 2012). The stem bark is applied to heal wounds, eczema, ring-worms, boils, skin diseases, itches and to reduce fever (Ram, *et al.*, 2004). The natural regeneration of *P. latifolia* is quite low due to seed dormancy and slow growth of the plant in their natural zone of occurrence. Thapliyal and Phartyal (2005) implicated that, the primary cause of seed dormancy in *P. latifolia* is a stony endocarp that mechanically prevents radicle extrusion. Dormancy has developed as a coping mechanism to prevent germination in situations where seedling survival is anticipated to be low. Dormancy ranges from very minimal to extremely strong (deep), and it can be innate, develop, break, and redevelop in seeds. As a result, there are various forms of dormancy, and occasionally a single seed will exhibit multiple forms of dormancy. Dormancy is disturbed in nature either gradually or by a specific environmental event. The kind of dormancy determines the kind of incident that could end it (Baskin and Baskin, 2004; Schmidt, 2000). One of the key components of a successful seed operation is appropriate seed storage. In addition to providing seeds during the year

with low seed output, proper seed storage helps preserve seeds for a long time. The complex physiological and biochemical process of seed degeneration during storage results in the loss of viability. Temperature and other storage factors affect how quickly seeds deteriorate (Walters, *et al.*, 2005). By keeping seeds dry or cold and with a moisture content of less than 5%, seed viability can be prolonged (Huang, *et al.*, 2003). Because damage from low temperatures occurs more slowly in cold storage, the effects of aging are lessened (Spano, *et al.*, 2007). Thus, seed needs to be preserved between harvest and the next crop's sowing to reduce the pace of degradation and preserve seed physiological quality. The available literature lacks information regarding suitable seed germination behaviour and dormancy breaking protocol to improve germination and seed storage behaviour of *Premna latifolia*. Therefore the present study was carried out (1) to investigate seed germination behaviour of *P. latifolia* by standardizing dormancy breaking protocol (2) to determine seed storage behaviour of *P. latifolia*.

## 2. Material and Methods

The study was carried out at Forest Tree Seed Laboratory, Silviculture and Forest Management division of ICFRE-Forest Research Institute, Dehradun.

### 2.1 Seed Collection

The mature fruits were collected during June, 2022 from 10 trees, at least 100 m apart. The details of the seed sources are as below:

**Table 1:** Details of seed sources of *P. latifolia*

S. No.	Location	GPS Coordinates	Altitude (msl)
1.	Garhi cant.	29°55'54.93"N 78°10'35.67"E	725
2.	Kuthal village	30°24'20.73"N, 78°05'01.70"E	967
3.	Raipur	30°20'33.57"N, 78°00'18.26"E	701

### 2.2 Seed Processing

Seeds were extracted from freshly harvested mature fruits by rubbing under tap water and then shade dried on top of towel paper. Then seeds were separated from dried fruit pulp by hands (Thapliyal and Phartyal, 2005). After extraction seeds were subjected to

germination and moisture test as per ISTA, 2010.

### 2.3 Experimental details

All the treatments with untreated seeds [control] were kept for germination test. The seeds were counted as germinated when radical emergence was about 0.25cm.

Following pre-treatments were given to the seeds:

- T1: Control (No treatment)
- T2: Cold water soaking for 72 hrs
- T3: Hot water soaking for 24 hrs
- T4: GA<sub>3</sub> 500 ppm for 48 hrs
- T5: GA<sub>3</sub> 500 ppm for 72 hrs
- T6: GA<sub>3</sub> 800 ppm for 48 hrs

T7: GA<sub>3</sub> 800 ppm for 72 hrs

Thus, there were total seven treatments with four replications and 100 seeds in each replication. The seeds were kept for 30 days in seed germinator and following observations were recorded.



**Figure 1:** Seed extraction

**2.3.1 Germination Percentage:** 100 seeds in four replication were kept for germination test on germination paper. The germination test

was conducted in seed germinator at 25±1°C. The germination percentage was calculated as per ISTA (2010):

$$\text{Germination \%} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds kept for germination}} \times 100$$

**2.3.2 Moisture Content (%):** Moisture percent was determined by using hot air oven on fresh weight basis. The seeds were oven dried

at a temperature of 103±2°C for 17±1 hrs (ISTA, 2010) and then reweighed. Moisture content was determined by the formula:

$$\text{Moisture content \%} = \frac{\text{Fresh seed weight} - \text{Dry seed weight}}{\text{Fresh seed weight}} \times 100$$

**2.3.3 Mean Germination Time (MGT):** It is an expression of total germination at the end of test period with time taken to complete germination (Bonner, 1983).

**2.3.4 Germination Energy:** It is the percent by number, of seeds in a given sample which germinate upto the time of peak germination. When peak germination is the highest number of germination in a particular day (Willan, 1985).

$$GE = \frac{\text{No. of seeds germinated upto peak time of germination}}{\text{No. of seeds sown}} \times 100$$

**2.3.5 Germination Value:** Germination value (GV) is the index combined speed and completeness of seed germination. Daily

germination counts were recorded and calculated as per Djanvanshir and Pourbeik (1976).

$$GV = (\sum DGS/N) \times \frac{GP}{10}$$

**2.3.6 Vigour Index:** For calculating vigour index, total length of seedling (length of

hypocotyls + length of radicle) was multiplied by total germination percent (Abdul-Baki and Anderson, 1973).

**2.4 Seed Storage:** The seeds were desiccated to two moisture level at 8% and 5% and kept for storage in four different temperature levels i.e. ambient room temperature (control), 15°C, 5°C, and -5°C. The viability of the stored seeds was monitored by germination tests, according to the International Seed Testing Association procedures at intervals of 3 months for 24 months. The germination parameters recorded for seed storage trials were germination percent, mean germination time and vigour index.

### 2.5 Statistical analysis

The data pertaining to standardization of seed pre-treatment and seed storage protocol was analysed using ANOVA technique using General Linear Model in SPSS version 16.0 for windows (SPSS Inc. 1989). To determine the effect of moisture content, storage period, and temperature and their interaction on seed germination percent, mean germination time (MGT) and vigour index. Means were

compared using Tukey's HSD test at 5% level of significance.

## 3. Result

### 1.1 Effect of different seed pre-treatments on germination behaviour of *P. latifolia*.

Estimates of the final germination parameters are presented in Table 2. Critical perusal of the ANOVA revealed that different pre-sowing treatment had significant effect ( $p < 0.05$ ) on various germination parameters of seed. The maximum germination (90%) was recorded in the T5 (GA<sub>3</sub> 500ppm 72 hrs) followed by T7 (GA<sub>3</sub> 800ppm 72 hrs) (89%), whereas minimum seed germination (8%) was recorded in T1 (Control). Mean germination time was found maximum (12.86 days) in T1 and minimum (6.31 days) in T3 followed by T7 (6.32 days). Germination value was recorded highest (93.79) in T7 and minimum (0.84) in T1. Highest values of germination index (3.98) and vigour index (507.60) were observed in T5 whereas minimum (0.23) and (43.77) in T1 respectively. Germination energy was observed maximum 48.0) in T4 and minimum (5.0) in T1.



**Figure 2:** Seed germination

**Table 2:** Effect of different pre-treatments on germination attributes of *P. latifolia*

Treatment	Germination (%)	Mean Germination Time (days)	Germination Value	Germination Index	Vigour Index	Germination Energy
T1- Control	8.0 (0.08) <sup>a</sup>	12.86 <sup>b</sup>	0.84 <sup>a</sup>	0.23 <sup>a</sup>	43.77 <sup>a</sup>	5.0 <sup>a</sup>
T2- Cold water soaking 48 hrs	23.0 (0.23) <sup>a</sup>	9.81 <sup>ab</sup>	2.51 <sup>a</sup>	0.62 <sup>a</sup>	115.92 <sup>a</sup>	10.0 <sup>a</sup>
T3- Hot water soaking 24 hrs	46.0 (0.48) <sup>b</sup>	6.31 <sup>a</sup>	6.70 <sup>a</sup>	0.96 <sup>a</sup>	240.12 <sup>b</sup>	13.0 <sup>a</sup>
T4- (GA <sub>3</sub> 500 ppm 48 hrs)	85.0 (1.02) <sup>c</sup>	7.56 <sup>a</sup>	66.16 <sup>b</sup>	2.96 <sup>b</sup>	443.70 <sup>c</sup> <sub>d</sub>	48.0 <sup>c</sup>
T5- (GA <sub>3</sub> 500 ppm 72 hrs)	90.0 (1.12) <sup>c</sup>	6.38 <sup>a</sup>	86.40 <sup>b</sup>	3.98 <sup>c</sup>	507.60 <sup>d</sup>	42.0 <sup>bc</sup>
T6 - (GA <sub>3</sub> 800ppm 48 hrs)	80.0 (0.95) <sup>c</sup>	6.48 <sup>a</sup>	73.83 <sup>b</sup>	3.24 <sup>b</sup>	410.40 <sup>c</sup>	33.0 <sup>abc</sup>
T7 - (GA <sub>3</sub> 800ppm 72 hrs)	89.0 (1.11) <sup>c</sup>	6.32 <sup>a</sup>	93.79 <sup>b</sup>	3.80 <sup>c</sup>	441.44 <sup>c</sup> <sub>d</sub>	58.0 <sup>c</sup>
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

\*Figures in parentheses are arc sine transformed values

\*Values in the same column with the same letter do not differ significantly (p<0.05)

### 3.2 Effect of Different Seed Storage Period, Temperature, and Moisture Content (%) On Germination Parameters of *P. latifolia*

Table 3 shows the summary of ANOVA for effect of moisture content, storage period and storage temperature on seed quality attributes of *P. latifolia*. The result indicate that, separate effect of moisture levels, storage period and storage temperature as well as interaction effect of moisture content x storage period, moisture x temperature and storage period x

temperature were found to be significant (P <0.05) on seed germination %. However, interaction effect of moisture x period x temperature had non-significant effect on germination percent. All the treatments and their interactions had significant effect on mean germination time except moisture content. Whereas all the treatments and their interactions had significant effect on vigour index.

**Table 3:** ANOVA for the effect of moisture content level, storage period and storage temperature on seed quality attributes of *P. latifolia*

Source	DF	Germination %	Mean Germination Time	Vigour Index
Moisture	1	47.43*	6.25 ns	97.27*
Period	8	89.38*	24.99*	122.66*
Temperature	3	66.67*	87.09*	122.66*
Moisture x Period	6	2.67*	4.18*	19.52*
Moisture x Temperature	3	10.37*	3.31*	14.29*
Period x Temperature	24	1.82*	7.86*	4.68*
Moisture x Period x Temperature	24	1.43 ns	2.13*	3.01*

\*Significant at 5 % probability level, ns -not significant

### 3.3 Effect of Moisture Content (%) on Germination Parameters

The final data is summarized in Table 4, the ANOVA indicated that moisture content (%)

had significant effect (p<0.05) on germination percent and vigour index but non- significant effect on mean germination time. Overall



maximum germination (25.02%), MGT (7.64 days) and VI (97.27) was found in M1 and

lower values of germination (18.33%), MGT (7.04 days) and VI (58.55) were found in M2.

**Table 4:** Individual effect of moisture content (%), Storage period (months) and storage temperature on germination attributes of *P. latifolia*

Moisture content (%)	Germination %	MGT	VI
M1	25.02	7.64	97.27
M2	18.33	7.04	58.55
<b>Storage period (months)</b>			
P1	52.0 (0.51) <sup>e</sup>	10.13 <sup>d</sup>	186.14 <sup>e</sup>
P2	30.75 (0.36) <sup>d</sup>	9.47 <sup>d</sup>	117.04 <sup>d</sup>
P3	28.50 (0.27) <sup>d</sup>	8.72 <sup>cd</sup>	109.47 <sup>d</sup>
P4	21.62 (0.24) <sup>c</sup>	7.45 <sup>bc</sup>	86.10 <sup>c</sup>
P5	16.37 (0.16) <sup>bc</sup>	7.39 <sup>bc</sup>	43.73 <sup>ab</sup>
P6	15.37 (0.16) <sup>bc</sup>	6.58 <sup>ab</sup>	45.88 <sup>b</sup>
P7	11.12 (0.11) <sup>ab</sup>	5.41 <sup>a</sup>	31.29 <sup>ab</sup>
P8	11.25 (0.11) <sup>ab</sup>	5.58 <sup>a</sup>	30.06 <sup>ab</sup>
P9	8.12 (0.08) <sup>a</sup>	5.31 <sup>a</sup>	23.19 <sup>a</sup>
<b>Storage temperature</b>			
T1	9.77 (0.17) <sup>a</sup>	4.0 <sup>a</sup>	34.68 <sup>a</sup>
T2	25.77 (0.18) <sup>b</sup>	8.16 <sup>b</sup>	88.91 <sup>b</sup>
T3	25.72 (0.26) <sup>b</sup>	8.63 <sup>b</sup>	88.20 <sup>b</sup>
T4	25.44 (0.26) <sup>b</sup>	8.57 <sup>b</sup>	86.29 <sup>b</sup>

\*Figures in parentheses are arc sine transformed values

\*Values in the same column with the same letter do not differ significantly ( $p < 0.05$ )

### 3.4 Effect of storage period on germination parameters

The data in Table 4 indicates that, the storage period significantly influence germination parameters. The germination %, MGT and VI were found to be decreasing with the advancement of storage period. The maximum value for germination (52%), MGT (10.13 days) and VI (186.14) was recorded in P1 (i.e. fresh seeds), which was declining with increasing time of storage. The lowest values of germination (8.12%), MGT (5.31 days) and VI (23.19) were recorded in P9 (i.e. after 24 months of storage).

### 3.5 Effect of temperature on germination parameters

The data presented in table 4 indicates the significant difference in seed quality parameters between seeds stored in ambient room temperature and lower temperatures. However, there was no significant difference in seed quality parameters between -5°C, 5°C and 15°C storage. Among all the temperatures, maximum germination (25.77%) and VI

(88.91) was recorded in T2. Whereas, maximum MGT was recorded in T3 (8.63 days). Minimum values of germination (9.77%), MGT (4.0 days) and VI (34.68) were recorded in T1.

### 3.6 Interaction effect of moisture content (%), storage period and storage temperature on germination parameters

The interaction effect of moisture content (%), storage period and storage temperature is presented in table 5. The data indicates that germination percent, MGT and VI decrease with increasing storage period. The data on germination percentage revealed that, among four storage temperatures, seeds stored at 5°C maintained comparatively higher viability after 24 months in both moisture levels. The seeds maintained viability (15%) upto 24 months when stored at lower temperatures (5°C, 15°C and -5°C) as compared to ambient room temperature. Speedy decline in viability was observed in seeds stored at ambient room temperature and lost complete viability in 9 months in 8% moisture content and in 12

months at 5.4% moisture content. The minimum time (4.75 days) taken to complete seed germination was recorded in -5°C temp and 5.4% MC followed by 6.33 days in 15°C temp storage with 5.4% MC at 18 months of storage. Whereas maximum time (12.05 days) taken to complete germination was observed in 5.4% moisture content, ambient room temperature and 3 months of storage. The values of vigour index of the seeds stored at 8% moisture content (252.60) and 5.4%

moisture content (119.68) were reduced significantly with increasing storage period. Maximum value of vigour index was maintained by the seeds stored at 5°C with 8% moisture content after 24 months of storage (42.0) followed by the seeds stored at -5°C with 8% moisture content (37.12) as compared to seeds stored at ambient room temperature which lost their viability completely.

**Table 5:** Effect of storage temperature, moisture content (%) and storage period on germination attributes of *P. latifolia*

Storage period										
8.0% Moisture content										
Storage temperature		0 day	3 months	6 months	9 months	12 months	15 months	18 months	21 months	24 months
Ambient room temp.	GP	60.0	14.0	4.0	1.0	2.0	0	0	0	0
	MG T	10.60	9.45	8.50	2.0	6.50	0	0	0	0
	VI	252.60	42.0	8.80	3.40	6.70	0	0	0	0
15°C	GP	60.0	45.0	39.0	29.0	24.0	17.0	18.0	15.0	11.0
	MG T	10.60	7.64	7.64	8.94	9.61	7.93	8.13	5.39	7.87
	VI	252.60	190.80	197.34	58.87	68.40	32.30	58.68	39.90	36.40
5°C	GP	60.0	46.0	43.0	29.0	24.0	21.0	21.0	15.0	15.0
	MG T	10.60	8.44	8.38	7.35	9.78	9.76	7.30	8.76	8.15
	VI	252.60	199.64	138.03	138.62	52.0	49.46	71.19	35.70	42.0
-5°C	GP	60.0	41.0	43.0	38.0	29.0	25.0	20.0	19.0	13.0
	MG T	10.60	11.17	9.50	7.41	9.51	10.09	8.89	9.47	9.0
	VI	252.60	172.61	141.04	172.90	93.96	75.50	47.50	54.25	37.12
5.4% Moisture content										
Ambient room temp.	GP	44.0	24.0	19.0	8.0	0	0	0	0	0
	MG T	9.66	12.05	6.98	6.37	0	0	0	0	0
	VI	119.68	66.48	82.17	42.40	0	0	0	0	0
15°C	GP	44.0	22.0	32.0	30.0	20.0	26.0	8.0	15.0	9.0
	MG	9.66	10.06	7.10	8.55	7.18	11.17	6.33	6.71	6.33

	<b>T</b>									
	<b>VI</b>	119.68	71.28	152.32	96.42	51.55	88.66	16.93	49.25	19.05
<b>5°C</b>	<b>GP</b>	44.0	29.0	23.0	18.0	19.0	18.0	15.0	13.0	10.0
	<b>MG T</b>	9.66	7.59	11.08	12.0	8.75	6.80	7.91	6.52	6.43
	<b>VI</b>	119.68	125.57	73.83	86.40	53.01	55.26	40.27	37.18	35.20
<b>-5°C</b>	<b>GP</b>	44.0	25.0	25.0	20.0	13.0	16.0	7.0	13.0	7.0
	<b>MG T</b>	9.66	9.37	10.56	7.03	7.78	6.86	4.75	7.78	4.75
	<b>VI</b>	119.68	68.0	82.25	89.80	24.26	65.92	15.82	24.26	15.82

## 4. Discussion

### 4.1 Effect of different pre-treatments on germination attributes of *P. latifolia*

Between 50 and 90 percent of wild plants worldwide yield seeds that are dormant when they reach maturity; the precise inactive characteristics depend on a number of variables, including genetics, growth form, geographic distribution, and climatic conditions (Baskin and Baskin 2014). An evolutionary adaptation that can aid in long-term survival in intact natural environments is seed dormancy (Willis, *et al.*, 2014). But in the restoration context, where quick plant reestablishment is essential to stop more deterioration, dormancy can be a major obstacle (Turner, *et al.*, 2013). In the present study, different pre-treatment had significant effect on the germination parameters in which, treatment GA<sub>3</sub> 500 ppm for 72 hrs resulted in obtaining maximum germination (90%) followed by GA<sub>3</sub> 800 ppm for 72 hrs (89%), whereas minimum seed germination (8%) was recorded when intact seeds were kept for germination. The improved germination rate in GA<sub>3</sub>-treated seeds may be the consequence of endogenous auxin and gibberellin-like chemicals diffusing, which breaks seed dormancy and promotes early and accelerated germination (Gurung, *et al.*, 2014; Singh, *et al.*, 2016). Similar results are reported by Kumari, *et al.*, (2007), where GA<sub>3</sub> enhanced seed germination in *E. officinalis*, which reduces the effect of inhibitors. Treatment of gibberellic acid was effective in increasing seed germination irrespective of concentration and duration, when compared

with the control. Hot water treatment for 24 hours gave 46% germination which was also effective pre -treatment as compared to control. Perhaps the outer layer of seeds became thinner, which enhanced water absorption but could not accelerate germination. Study conducted by Santos, *et al.*, (2016) also support the present study in which, the highest initial growth in *Passiflora* spp. was observed after immersion of seeds in GA<sub>3</sub> solution. GA<sub>3</sub> also improved germination value, germination energy and vigour of the seed and seedlings. This could possibly be outlined by active amylase, which broke down the available carbohydrates into simpler sugars, making it easier for the faster-growing seedlings to get nutrition and energy. It has been established that pre-treating seeds with GA<sub>3</sub> encourages plant growth (Taiz and Zeiger, 2010; Dillip, *et al.*, 2017).

### 4.2 Effect of storage temperature, storage period and moisture content (%) on germination attributes of *P. latifolia*

The present study indicated that seeds of *P. latifolia* can survive in low moisture content, low temperature for about 24 months in which seeds lose viability with advancement of storage period. In this study 8% moisture content with 5°C storage temperature was observed suitable for maintaining around 15% viability for 24 months of storage. Whereas seeds stored at ambient room temperature lost complete viability in 9-12 months of storage. Chauhan and Nautiyal (2007) reported much faster loss of seed viability at



room temperature (10– 35°C) and retaining of seed viability for more than two years (storage at 0–4°C in refrigerator) in *Nardostachys jatamansi*. Germination in stored seeds of *Arbutus xalapensis* decreased from 93 to 52% between the periods studied, reducing by approximately 10% every six months (Calderón, *et al.*, 2023). Onyekwelua and Fayose (2007) claimed that the seeds can be kept in airtight, sealed containers to prevent freezing injury from ice formation, which is likely the reason that they cannot be stored at below-freezing temperatures. The germination percent and/or seed viability gradually declines with increase in storage period as reported by Yilmaz and Aksoy (2007), irrespective of different storing conditions. The production of free radicals and lipid peroxidation, which damages membranes and produces harmful byproducts, is usually responsible for the decline in seed viability. Seed degradation is most likely caused by oxidative damage to proteins and DNA. Loss of viability is also likely to result from a failure of the repair processes in cells, which are made up of a complex system of "enzymatic and non-enzymatic" antioxidant defenses to guard against the negative effects of activated oxygen species (Umarani, *et al.*, 2015). Ajiboye, *et al.*, (2009) studied the storage behaviour of *Tamarindus indica*, *Prosopis africana*, *Parkia biglobosa* and *Albizia lebeck* and observed that the 5 weeks cold storage treatments at 4°C gave 80% germination in *Albizia lebeck* and 70% in *Tamarindus indica* compared to untreated seeds served as control.

## Conclusion

In the present study, among different pre-sowing treatments, seed germination attributes were improved by treatment of GA<sub>3</sub> at different concentrations and durations. However GA<sub>3</sub> 500ppm for 72 hrs performed significantly well than other treatments. Therefore, GA<sub>3</sub> 500ppm for 72hrs should be applied for improving seed germination *P. latifolia* for successful regeneration programmes. Seed storage was significantly influenced by storage temperature, storage

period and moisture content. In this study seeds started to lose viability rapidly in storage. Seeds can be stored for more than one year at lower temperatures (5°C and -5°C) for maintaining viability. Our study recommend storing seeds at 8% MC and 5°C temperature. However, in terms of desiccation, seeds can tolerate drying till 5.4 % MC after which they lose viability and vigour. The seeds of *P. latifolia* can be categorized as "Intermediate" due to the ability to tolerate desiccation to low moisture content but deterioration of seed in hermetic condition shows sensitivity to low temperatures.

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## References

1. Abdul-Baki, A. A. & Anderson, J. D. "Seed vigour index." *Crop Science*, 13 (1973): 620-632.
2. Ajiboye, P. O., Yussuf, A. D., Issa, B. A., Adegunloye, O. A. & Buhari, O. N. "Current and lifetime prevalence of mental disorders in a juvenile Borstal institution in Nigeria." *Research Journal of Medical Sciences*, 3.1 (2009): 26-30.
3. Azad, S. M., Zedan-Al-Musa, M. & Abdul Matin, M. "Effects of pre-sowing treatments on seed germination of *Melia azedarach*." *Journal of Forestry Research*, 21 (2010): 193-196.
4. Baskin, J. M. & Baskin, C. C. "A classification system for seed dormancy." *Seed Science Research*, 14 (2004): 1-16.
5. Baskin, C. C. & Baskin, J. M. "Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination." 2nd ed. San Diego, CA: Academic/Elsevier, (2014).
6. Bonner, F. T. "Measurement of moisture content in seeds of some North American hardwoods." *Proceedings of the International Seed Testing Association*, 37.3 (1972): 975-983.
7. Chauhan, R. S. & Nautiyal, M. C. "Seed germination and seed storage behaviour of *Nardostachys jatamansi* DC., an endangered medicinal herb of high-altitude

- Himalaya." *Current Science*, 92.11 (2007): 1620-1624.
8. Dianita, R. & Jantan, I. "Ethnomedicinal uses, phytochemistry and pharmacological aspects of the genus *Premna*: A review." *Pharmaceutical Biology*, 55.1 (2017): 1715-1739.
  9. Dillip, W. S., Singh, D., Moharana, D., Rout, S. & Patra, S. S. "Effect of GA concentrations at different time intervals on seed germination and seedling growth of rangpur lime." *Journal of Agroecology and Natural Resource Management*, 4.2 (2017): 157-165.
  10. Djavanshir, K. & Pourbeik, H. "Germination value: A new formula." *Silvae Genetica*, 25.2 (1976): 79-83.
  11. Gurung, N., Swamy, G. S. K., Sarkar, S. K. & Ubale, N. B. "Effect of chemicals and growth regulators on germination, vigour, and growth of passion fruit (*Passiflora edulis* Sims.)." *The Bioscan*, 9.1 (2014): 155-157.
  12. Harley, R. M., Atkins, S., Budantsev, A. L., Cantino, P. D., Conn, B. J., Grayer, R., Harley, M. M., de Kok, R. P. J., Krestovskaja, T., Morales, R., Paton, A. J., Ryding, O. & Upson, T. "Labiateae." In: Kubitzki, K. (Ed.), *The Families and Genera of Vascular Plants*, vol. VI. Berlin: Springer, 2004, 167-275.
  13. Huang, Z., Zhang, X., Zheng, G. & Gutterman, Y. "Influence of light, temperature, salinity and storage on seed germination of *Haloxylon ammodendron*." *Journal of Arid Environments*, 55.3 (2003): 453-464.
  14. "International rules for seed testing" (ISTA). International Seed Testing Association (ISTA) Zurich, Switzerland. (2010).
  15. Kabra, A., Kabra, R. & Baghel, U. S. "*Premna* species: A review." *Journal of Biological and Chemical Chronicles*, 1.1 (2015): 55-59.
  16. Kanjilal, U. "The Forest Flora of the Siwalik and Jaunsar Forest Division, U.P." *Delhi: The Manager of Publications*, (1969): 395-396.
  17. Kumar, A., Tamta, M. L., Negi, N., Chandrasekhar, K. & Negi, D. S. "Phytochemical investigation and antifeedant activity of *Premna latifolia* leaves." *Natural Product Research*, 25.18 (2011): 1680-1686.
  18. Kumar, B. D., Deepika, D. S. & Raju, S. "On the reproductive ecology of *Premna latifolia* L. and *Premna tomentosa* Willd. (Lamiaceae)." *Journal of Threatened Taxa*, 10.1 (2018): 11105-11125.
  19. Kumari, R., Sindu, S. S., Sehrawat, S. K. & Dudi, O. P. "Germination studies in aonla (*Emblia officinalis* Gaertn)." *Haryana Journal of Horticultural Sciences*, 36.1&2 (2007): 9-11.
  20. Martínez-Calderón, V. M., Sosa-Ramírez, J., Luna-Ruiz, J. D., Pérez-Salicrup, D. R. & Sandoval-Ortega, M. H. "Effect of storage and pre-germination treatments on seeds of *Arbutus xalapensis* from north-central Mexico." *New Forests*, 54 (2023): 1119-1130.
  21. Onyekwelua, J. C. & Fayose, O. J. "Effect of storage methods on the germination and proximate composition of *Treculia africana* seeds." In: *Proceedings of the International Conference on Agricultural Research for Development*; October, University of Kassel-Witzenhausen and University of Göttingen, Tropentag, 2007.
  22. Quattrocchi, U. "CRC World Dictionary of Medicinal and Poisonous Plants: Common Names, Scientific Names, Eponyms, Synonyms, and Etymology." Boca Raton, FL: CRC Press, (2012): 869-870.
  23. Ram, J. A., Bhakshu, L. M. & Raju, V. R. R. "In vitro antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases." *Journal of Ethnopharmacology*, 90 (2004): 353-357.
  24. Santos, C. H. B., Cruz Neto, A. J., Junghans, T. G., Jesus, O. N. & Ginardi, E. A. "Estádio de maturação de frutos e influência de ácido giberélico na emergência e crescimento de *Passiflora* spp." *Ciência Agronômica*, 47.3 (2016): 481-490.
  25. Schmidt, L. "Guide to Handling of Tropical and Subtropical Forest Seed." *Danida Forest Seed Centre*, (2000): 263-303.
  26. Singh, M., John, S. A., Rout, S. & Patra, S. S. "Effect of GA3 and NAA on growth and quality of garden pea (*Pisum sativum* L.) cv. Arkel." *The Bioscan*, 10.3 (2016): 381-383.
  27. Spanò, C., Buselli, R., Ruffini Castiglione, M., Bottega, S. & Grilli, I. "RNases and nucleases in embryos and endosperms from

- naturally aged seeds stored in different conditions." *Journal of Plant Physiology*, 164 (2007): 487-495.
28. Taiz, L. & Zeiger, E. "Plant Physiology." *Sinauer Associates Inc., USA*, (2010).
  29. Thapliyal, R. C. & Phartyal, S. S. "Dispersal and germination syndromes of tree seeds in a monsoonal forest in northern India." *Seed Science Research*, 15 (2005): 29-42.
  30. Troup, R. S. *The Silviculture of Indian Trees*, Vol. II, 1921, 778.
  31. Turner, S. R., Steadman, K. J., Vlahos, S., Koch, J. M. & Dixon, K. W. "Seed treatment optimizes benefits of seed bank storage for restoration-ready seeds: The feasibility of prestorage dormancy alleviation for mine-site revegetation." *Restoration Ecology*, 21.2 (2013): 186-192.
  32. Umarani, R., Aadhavan, E. K. & Faisal, M. M. "Understanding poor storage potential of recalcitrant seeds." *Current Science*, 108.11 (2015): 2023-2034.
  33. Virshette, S. J., Patil, M. K. & Shaikh, J. R. "A review on pharmacological properties and phytoconstituents of indigenous carminative agents." *Journal of Pharmacognosy and Phytochemistry*, 9.3 (2020): 142-145.
  34. Walters, C., Wheeler, L. M. & Grotenhuis, J. M. "Longevity of seeds in a genebank: species characteristics." *Seed Science Research*, 15 (2005): 1-20.
  35. Willan, R. C. "A Guide to Forest Seed Handling with Special Reference to the Tropics." *Food and Agriculture Organization*, (1985): 244.
  36. Willis, C. G., Baskin, C. C., Baskin, J. M., Auld, J. R., Venable, D. L. & Cavender-Bares, J. "The evolution of seed dormancy: Environmental cues, evolutionary hubs, and diversification of the seed plants." *New Phytologist*, 203.1 (2014): 300-309.
  37. Yilmaz, D. D. & Aksoy, A. "Physiological effects of different environmental conditions on the seed germination of *Rumex scutatus* L. (Polygonaceae)." *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 23.1-2 (2007): 24-29.

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