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Effect of Different Pre-sowing Treatments and Tetrazolium Test in *Phoebe goalparensis* Hutch. Under Eastern Himalayas, India

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A B S T R A C T

The experiment was conducted to study the effect of different pre-sowing treatment and tetrazolium viability test in seeds of *Phoebe goalparensis* Hutch. subjected to completely randomized design with eight treatments and four replications carried out in the laboratory and nursery bed in research field of Department of Silviculture and Agroforestry, College of Horticulture and Forestry, Pasighat, Arunachal Pradesh. Significant differences in germination were observed across the treatments. The study revealed that that highest germination percentage in lab (81.5%) and nursery bed (70.0%) was recorded in treatment T_8 (depulped, nicking and overnight soaking in 0.05% gibberellic acid) significantly enhanced seed germination. The tetrazolium test for stained viable seeds (84.33%) approached amicable value with the highest germination percentage recorded in T_8 that can be used as a rapid and effective method in seed testing. Economical production of seedling by adopting pre-sowing treatments of depulped, sun drying for 8 hours (T_4) or depulped, soaking in ambient water for 24 hrs (T_5) could be used for cultivation of *P. goalparensis* seedling.

Introduction

Phoebe goalparensis Hutch. is a tropical rainforest species belonging to slow growing timber species of the family *Lauraceae*. The tree grows on soils varying from sandy loam to clay and attains a height of approximately 36 metres and a girth of 3.8 metres and is distributed in North-East India in the foot-hills of Eastern Himalayas up to an altitude of 1250 m. It is commonly known as bonsum, nikahi or Assam teak. The rich brown heartwood hardiness can be compared as about 70% that of teak. It is one of the commercial important timber species of Northeast India (Kundu *et al.*, 2012). Because of its desirable characters, there is a huge demand for quality planting stock of this species in the forestry and private sectors. The natural regeneration of this species is negligible and scanty due to production of less number of viable seeds and inhibiting pericarp for germination. Further, it has been observed that the populations of this species are decreasing alarmingly in its natural habitat, largely because of timber operation for wood based industries. The conservation of this species is necessary for future availability of the source through seedling production and their ascertained afforestation programming. The pre-sowing treatment is especially important in forest seeds which

take much longer time to germinate or exhibit varying types of dormancy (Gupta and Raturi, 1975). The effect of pre-sowing treatments on seed germination of some tropical forest tree species have been reported by few authors Azad *et al.,* (2011); Merou *et al.,* (2011); Mwase and Mvula (2011), and Khan (2015). Ensuring the speedy test of seed viability, tetrazolium test can be applied as an effective and reliable method for this species. Considering the facts the study was under taken to investigate viability test and effects of pre-sowing treatment on seed germination of *Phoebe goalparensis* in the laboratory and nursery condition.

Materials and Methods

Mature fruits of *Phoebe goalparensis* Hutch. were collected during the month of September (2016) from Ruksin Forest Range (27°50′N latitude; 95°13′E longitude, at 131 m elevation), East Siang District, Arunachal Pradesh. Seed processing was done manually to get the depulped and one day shade dried seeds for the effects of eight different pre-treatment methods on the germination rates and viability test using Tetrazolium solution (Peters, 2000). (i) *TTZ test*: Four hundred seeds with 100 seeds per replicate were used for viability test and different staining category was recorded subjected to analysis of variance (Table 1). The seeds were soaked in water for 24 hours and longitudinally sectioned. The sections were immersed in a 1% aqueous solution of tetrazolium (pH 6.5) for 24 hrs at room temperature (26ºC) under dark conditions. The tetrazolium solution was drained and sections were rinsed 2-3 times with water. The topographical staining pattern of the embryos (plumule and radicle) and cotyledons were studied under a dissection microscope (ISTA, 2003). (ii) *Pre-sowing treatments*: A total of 400 seeds in four replicate with 100 seed per replicate were taken separately for each treatment for germination experiment in both nursery and field conditions. Each replicate of seeds were subjected to the following treatments: Control with pericarp, provided no treatment (T_1); depulped seed (T_2); depulped and dipping in 2% hydrogen peroxide overnight (T_3); depulped and sun drying for 8 hours (T_4); depulped and soaking in ambient water for 24 hrs (T_5); depulped and soaking in luke warm water for 24 hrs (T_6); depulped, nicking and soaking in ambient water for 24 hrs (T_7); depulped, nicking and overnight soaking in 0.05% gibberellic acid (T_8). Immediately after the pre-treatments, seeds were sown in nursery beds provided with mulch and separately in laboratory condition in germination trays filled (rearranged at random every 2 days) with mixture of sterilized soil, sand and farm yard manure in the ratio 3:1:1 respectively at a depth of 2.5 cm placed on a table at the room temperature ranged from 23°C to 26°C. Watering was carried out regularly as per the requirement and observed daily for initiation and progress of germination until no more germination was observed. The germination data was recorded from the 28th day after sowing was expressed as days to initial germination and was taken as visible signs of successful germination.

The number of days on which the last seedling emerged was recorded and expressed as days to final germination, 90 days (lab condition) and 95 days (nursery bed) from the date of sowing. The various germination parameters such as germination percentage, peak value and germination value were recorded at the end of the experiments. Each treatment and the control was laid in Completely Randomized Design. Values of germination percentage were transformed (arcsine-square-root transformation) prior to analysis and were backtrans formed for tabular presentation. The obtained data was analyzed using the analysis of variance procedure (ANOVA) variance following the

model suggested by Panse and Sukhatme (1985). At the end of the germination period, the germination percentage, peak value, germination values were calculated using the following equations:

Germination percentage

The germination test was carried out in which four replicates of 100 seeds each were used (ISTA, 2003). The germination percentage was calculated using the formulae as:

$$\text{Germination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds used}} \times 100$$

Peak value

Peak value was calculated as the maximum mean daily germination (MDG) reached at any time during the period of test (Czabator, 1962).

Germination value

Czabator's formula (1962), which quantifies germinative energy by combining speed and completeness of germination, was used for evaluation of the treatments.

Germination Value= Final DGS X Peak value; where DGS is (Daily Germination Speed)

Results and Discussion

Variation in viability percent across different category description of staining seeds through Tetrazolium solution as depicted in Table 1 shows stained (84.33%) and unstained (15.67%) embryo's being significant ($p < 0.05$) different for all the 11 categories. Tetrazolium test of stained viable seeds has shown a close agreement with germination test results of laboratory and nursery beds compared with T_8 (depulping, nicking and overnight soaking in 0.05% gibberellic acid). The non-viability seeds of *P. goalparensis* as determined by the tetrazolium test exhibiting unstained embryo category for Tv_6,(2.33%), Tv_7(3.33%), and Tv_8(10.00%) respectively.

Hence, the Tetrazolium test can be applied as a reliable method for determining the potential of viable seeds of *P. goalparensis* seeds quickly, without processing for time taking germination tests.

The present findings are in agreement with those reported by Bisht *et al.,* (2001); Fogaca *et al.,* (2006); Singh and Murtem (2009); Aslam *et al.,* (2010); Costa and Santos (2010), and Zoghi *et al.,* (2011).

Effect of different pre-sowing treatments on seed germination values of *P. goalparensis* was observed for the corresponding mean performance values for nursery (germination beds) and laboratory conditions (germination trays) presented in Table 2 and 3 respectively. The data reveals that in lab and nursery condition the germination percentage for treatments ($p < 0.05$) varied significantly. The maximum germination percentage (81.5%) in lab condition and (70.0%) in nursery beds was recorded in the treatment T_8 for both condition, respectively. Gibberellic acid increased seed germination, thus confirming its role as a stimulatory agent (Cetinbas and Koyuncu, 2006; Negi and Sharma, 2011). The highest germination value (T_8) was followed by T_7 (70.75%) which was at par with T_4 (69.75%), T_5 (68.50%) in lab condition and similar trend was recorded in nursery bed for the treatments. The control (T_1) seeds without any treatment were lowest in germination in lab (3.25%) and nursery bed (2.50%) respectively, indicating scanty natural regeneration due to the presence of some inhibiting substances in the embryo by enveloping layers.

Table.1 Code category description of seeds staining Viability test through tetrazolium were as follows

Code	Category Description	% of seed Viability	Arcsine Value	(Percent)
Tv_1	Embryo completely stained	100	12.00	(4.33)
Tv_2	Embryo with radicle completely stained, cotyledons stained more than half.	100	34.45	(32.00)
Tv_3	Embryo with radicle completely stained, cotyledons stained less than half.	100	26.56	(20.00)
Tv_4	Embryo with cotyledon stained more than half.	100	12.88	(5.00)
Tv_5	Embryo with cotyledon stained less than half.	100	12.46	(4.67)
Tv_6	Unstained embryo, cotyledon stained more than half.	0	08.74	(2.33)
Tv_7	Unstained embryo, cotyledon stained less than half.	0	10.40	(3.33)
Tv_8	Unstained embryo, cotyledon and radicle.	0	18.42	(10.00)
Tv_9	Embryo with radicle stained.	100	08.13	(2.00)
Tv_{10}	Embryo with radicle stained, cotyledons and periphery stained less than half.	100	15.68	(7.33)
Tv_{11}	Embryo with radicle stained, periphery stained less than half.	100	17.44	(9.00)
	Mean ± SE		16.11 ± 0.910	
	(Range)		(8.13 - 34.45)	
	M.S.S.		193.17	
	F- Test		155.43*	
	C.D. (5%)		1.89	
	C.V. (%)		6.92	

*Significant at the 0.05 p level.

Table.2 Effect of seed pretreatment on germination parameters of under eight treatments (laboratory condition)

Treatments	**Germination percent (Arcsine value)**	**Peak value**	**Germination value**
T_1	9.05 (2.50)	0.02	0.00
T_2	52.42 (62.75)	0.74	0.52
T_3	52.55 (63.0)	0.74	0.53
T_4	56.74 (69.75)	0.79	0.73
T_5	55.88 (68.50)	0.77	0.69
T_6	53.00 (63.75)	0.74	0.52
T_7	57.32 (70.75)	0.85	0.74
T_8	64.54 (81.5)	0.94	0.87
Mean ± SE	50.19±1.54	0.70±0.03	0.58±0.02
(Range)	(9.05-64.54)	(0.022-0.94)	(0.00-0.87)
M.S.S.	1167.28	0.32	0.28
F- Test	247.31*	239.31*	246.55*
C.D. (5%)	3.19	0.05	0.05
C.V. (%)	4.33	5.22	5.84

*Significant at the 0.05 p level.

Table.3 Estimating seed germination parameters under eight treatments (field condition)

Treatments	Germination percent (Arcsine value)	Peak value	Germination value
T1	10.40 (3.25)	0.04	0.00
T2	50.63 (59.75)	0.65	0.41
T3	49.76 (58.25)	0.64	0.39
T4	52.40 (62.75)	0.65	0.46
T5	53.96 (65.25)	0.73	0.49
T6	48.74 (56.50)	0.61	0.37
T7	55.45 (67.75)	0.73	0.51
T8	56.81 (70.00)	0.77	0.62
Mean ± SE	47.27±1.47	0.60±0.03	0.41±0.03
(Range)	(10.40-56.81)	(0.037-0.77)	(0.00-0.62)
M.S.S.	918.90	0.22	0.13
F- Test	211.65*	137.01*	82.79*
C.D. (5%)	3.06	0.06	0.06
C.V. (%)	4.41	6.68	9.84

*Significant at the 0.05 *p* level.

The removal of pericarp (depulped seeds), treated with nicking, overnight soaking in 0.05% gibberellic acid (T_8), either depulped, nicking, soaking in ambient water for 24 hrs (T_7), or adopting economical method of depulped, sun drying for 8 hours (T_4) or depulped, soaking in ambient water for 24 hrs (T_5) results in earlier onset of enhanced germination (Hossain *et al.*, 2014). Seeds of Bonsum are recalcitrant in nature and the viability declines with time, hence, the results obtained in this study entail the vital role of pre-treating *P. goalparensis* seeds prior to sowing for enhanced germination and domestication of the species. High seed germination percentage for treatments suggests the best method to be applied before sowing *P. goalparensis* seeds. The results reported in this study agreed to those in literature Murugesh (2011); Azad *et al.*, (2012); Vijayalakshmi and Renganayaki (2017).

There were highly significant ($p < 0.05$) differences among the treatments for peak value and germination value (Table 2 and 3) for treatments with higher germination percentages (T_8, T_7, T_4, T_5,) and minimum was observed in T_1 followed the same pattern for both laboratory and nursery conditions, indicates a better measure of seed performance which is a prerequisite for the fast and uniform seed germination that, guarantees rapid and good seedling growth in the field (Shuaibu *et al.*, 2015 and Al-Absi, 2010). The rapid and complete germination of seeds is usually a desirable objective in the production of tree seedlings, hence the findings indicate the need of seed pre-treatment in *Phoebe goalparensis* due to negligible and scanty natural regeneration.

The findings of the present investigation reveals that the seeds of *Phoebe goalparensis* can be treated with depulped, nicking and soaking overnight in 0.05% gibberellic acid (T_8) before sowing for getting maximum germination. And to get cost effective germination for large scale production of seedling with minimum cost, time, and labour, presowing treatments of depulped, sun drying for 8 hours (T_4) or depulped, soaking in ambient water for 24 hrs (T_5) could be adopted by the forest department, nursery owners, farmers, NGOs, and researchers for economic cultivation of this species.

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