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Germination of pre-treated *Morus laevigata* Wall. Seed under laboratory conditions at Pasighat in Arunachal Pradesh, India

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Abstract
An investigation was carried out during April-May, 2019 with an objective to determine the effective pre-sowing treatment for seed germination of *Morus laevigata* Wall., an economically important valuable tree species belonging to the family, Moraceae, known for its quality timber especially for furniture and indoor structures. The experiment was laid out in a completely randomized design in laboratory conditions subjected to 17 pre-treatments with four replications each. The depulped seeds pre-treated with gibberellic acid (GA_3) application of 250 ppm, 500 ppm and 1000 ppm for 12 h resulted significant higher germination in T_8 (60.25%), T_9 (74.25%) and T_{10} (87.50%) respectively, which accelerated with increase in concentration of gibberellin amongst the treatments. In conclusion, the application of GA_3 was effective in increasing seed germination percentage and germination energy for production planting stock, raising plantations and reforestation, subsequently.

Keywords: *Morus laevigata*, pre-sowing treatments, dormancy, gibberellic acid

Introduction
Morus laevigata Wall. is an economically important valuable tree species belonging to the family, Moraceae, known for its quality timber especially for furniture, indoor structures and rarely used for rearing silkworms because of its thick, rough and leathery leaves (Jain and Kumar 1989; Vijayan *et al*. 2011) [20, 47]. In mulberry, a total of 150 *Morus* species were recognized but only few are use in silkworm rearing such as *M. alba*, *M. indica*, *M. latifolia*, *M. nigra* and *M. multicaulis* (Shahina *et al*. 2019) [35]. In India distribution of two wild mulberry species, *viz*., *M. laevigata* Wall. and *M. serrata* Roxb. Has been reported by Hooker (1885) [18] and Parkinson (1923) [27]. *M. laevigata* is distributed in the tropical and sub-tropical Himalayas, from the Indus to Assam, Arunachal Pradesh and also in Bangladesh and Myanmar. It is also found in deciduous and mixed forest of North Eastern region and outer Himalayas from Kumaon eastwards to Assam and also in cultivated forms upto 600-1500 msl in India (Brandis 1906; Kanjilal *et al*. 1940; Tikader and Dandin 2005; Tikader 2011) [8, 22, 36, 41]. *M. laevigata* is commonly known as long mulberry and locally as '*bola*' in and around Arunachal Pradesh and Assam. The tree is deciduous in nature, attaining a height of about 25-30 m with a girth of 4.5 m at maturity, flowering usually occurs during the month of March-April and the fruit ripens late in May or during June (Troup 1921) [44]. The species displays dioecious flower, the length of female inflorescence varied from 5.00-12.00 cm; male inflorescence from 3.72-11.30 cm and the lengthy inflorescence denotes a key character to identify the species. The fruit colour is usually green when young and turns pale yellow when ripen (Ravindran *et al*. 1997; Vijayan *et al*. 2011; Abbasi *et al*. 2014; Tikader *et al*. 2000) [31, 47, 1, 39]. The timber is reported as best furniture wood, termite resistant and used in sport goods (Kanjilal *et al*. 1940; Vijayan *et al*. 2011; Naik *et al*. 2015) [22, 47, 25]. The wood of *M. laevigata* is hard and valued for high grain quality timber placed under class A-I (Anon. 2018) [3]. *M. laevigata* is found wild in forest area and people are using this tree for multi-purpose use other than sericulture. However, this precious species is vanishing rapidly for its slow process of natural regeneration, over exploitation for demand of quality wood and lacking of its endozoochoric seed dispersal birds and arboreal squirrels due to biotic reasons (Hilaluddin *et al*. 2005; Dollo *et al*. 2010; Selvan *et al*. 2013) [17, 12, 33]. Numerous studies have been conducted on birds and squirrels establishing essential role in the regeneration of forests around the world through their seed dispersal activities left in their faeces (Traveset 1998; Traveset *et al*. 2007; Datta and Rawat 2008; Vijayan *et al*. 2011; Kumawat *et al*.

2013; Bobadilla *et al*. 2016) [42, 43, 11, 47, 24, 7]. For the domestication of this tree, it is necessary to develop the nursery techniques and also supplement by redeeming the species in nature. The existing information revels physiological dormancy in most species of the Moraceae family. The dormancy of seeds must be broken to induce germination by various methods depending on the plant species and type of dormancy (Koyuncu 2005; Barbour *et al*. 2008) [23, 5]. Pre-sowing treatment facilitates breaking seed dormancy to ensure satisfactory maximum germination (Alamgir and Hossain 2005) [2]. Most of the mulberry are propagated vegetatively, but *M. laevigata* reported poor in rooting with survival of 12% (Tikader and Thangavelu 2006[40]; Tikader and Kamble 2008) [38]. Therefore, it is very important to determine which method and condition is suitable for production of planting stock for making efforts towards the conservation of *M. laevigata* Wall. before they extinct from their own habitat (Naik *et al*. 2015; Tikader and Dandin 2007) [25, 37]. Apparently, there is limited investigation and paucity of suitable technique for germination response of *M. laevigata* seeds, hence the objective of this study was to determine the effect of different pre-sowing treatments on seed germination enhancement.

Materials and Methods

The present investigation was carried out in laboratory conditions of Horticulture and Forestry, Central Agricultural University, Pasighat, East Siang District, Arunachal Pradesh, India situated at 28°04′43″ N latitude and 95°19′26″ E longitude with an altitude of 153 msl. The mature fruits of *Morus laevigata* Wall. Was collected during the month of April 2019, from Tajum village, (28°2′28″N latitude; 95°20′5″E longitude at 131 msl) East Siang District, Arunachal Pradesh. The fruits after collection were processed manually through depulping, separation with a sieve, rinsing with tap water and cleaned. Seeds that floated were considered non-viable and were discarded. For germination parameters the seeds were subjected to seventeen treatments with four replications each and a hundred seeds per replication was laid out in a completely randomized design (CRD) using standard methods. Except for control, all the fresh depulped seeds were subjected to the following treatments: T_1 -control (untreated fresh seed with pulp); T_2 - depulped seed; T_3 -drying for 2 days in shade at ambient temperature; T_4 -drying for 1 week in shade at ambient temperature; T_5 -soaked in 0.5% hydrogen peroxide for 12 h; T_6 -soaked in 1% hydrogen peroxide for 12 h; T_7 -soaked in 1.5% hydrogen peroxide for 12 h; T_8 -soaked in 250 ppm gibberellic acid (GA_3) for 12 h; T_9 -soaked in 500 ppm gibberellic acid (GA_3) for 12 h; T_{10} -soaked in 1000 ppm gibberellic acid (GA_3) for 12 h; T_{11} -dipped in luke warm water and left to cool at ambient temperature for 12 h; T_{12} - soaked in boric acid 500 ppm for 24 h; T_{13} -soaked in boric acid 1000 ppm for 24 h; T_{14} -soaked in boric acid 2000 ppm for 24 h; T_{15}- soaked in NaCl solution (10 mM) for 24 h; T_{16} - soaked in NaCl solution (20 mM) for 24 h and T_{17} -soaked in NaCl solution (40 mM) for 24 h. The plastic trays (48×35×8 cm) were filled with a mixture of soil, sand and farm yard manure in the ratio 3:1:1 and the seeds were sown in the laboratory condition in the month of April, 2019. The trays were monitored and watered daily to maintain adequate moisture content as per requirement. Germination started 5 day after sowing and the seeds were considered germinated when visible shoot was seen emerged on the surface. The data was recorded on daily basis until all germination process was over. The various germination parameters at the end of the experiment were calculated using the following equations:

Germination percentage: The germination percentage was calculated using the formulae (ISTA, 2003) [19]:

$$\text{Germination Percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \text{ x } 100$$

Peak value, germination value and germination speed

Peak value was calculated as the maximum mean daily germination (MDG) reached at any time during the period of test. Germination value is a composite value combining both germination speed and total germination providing an objective means of evaluating the results of germination test was calculated using the formula of Czabator (1962) [10].

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

$$\text{Germination Speed} = N_1/d_1 + N_2/d_2 + N_3/d_3 + \ldots\ldots\ldots\ldots + N_n/d_n$$

Where
N- Number of germinated seeds,
d- Number of days.

Germination energy and energy period: Germination energy (GE) was calculated on the basis of percentage of total number of seed that had germinated when germination reached its peak, and the Energy Period was taken up to the day of peak germination (Seward, 1980[34] and Willan, 1987) [48].

$$\text{GE} = \frac{\text{Number of seed germinated up to the time of peak germination}}{\text{Total number of seeds sown}} \text{ x } 100$$

Data analysis: The data obtained for germination parameters were statistically analysed using the analysis of variance (ANOVA) procedure for the significance of the treatments and the differences between the means were compared by Fisher's least significant difference test at 0.05 level following the model suggested by Panse and Sukhatme (1985) [26]. Result data lying beyond the range (in per cent) were transformed to arcsine-square-root-transformation values before statistical analysis, and the means of the germination energy was tested using Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) [15].

Results

The summary results for germination percentage, peak value, germination value and germination speed of *Morus laevigata* Wall. at laboratory conditions are presented in Table 1. There was significant variation ($p \leq 0.05$) among treatments for the germination parameters. The mean germination percentage varied from 12 to 87.50%. Seeds soaked in 1000 ppm gibberellic acid for 12 h (T_{10}) exhibited highest germination percentage (87.50%), followed by T_9 seeds soaked in 500 ppm gibberellic acid for 12 h (74.25%) and T_8 seeds soaked in 250 ppm gibberellic acid for 12 h (60.25%) was significantly higher in contrast with others. Amongst the treatment with boric acid, hydrogen peroxide, luke warm water and untreated depulped seeds, the germination recorded within the range of

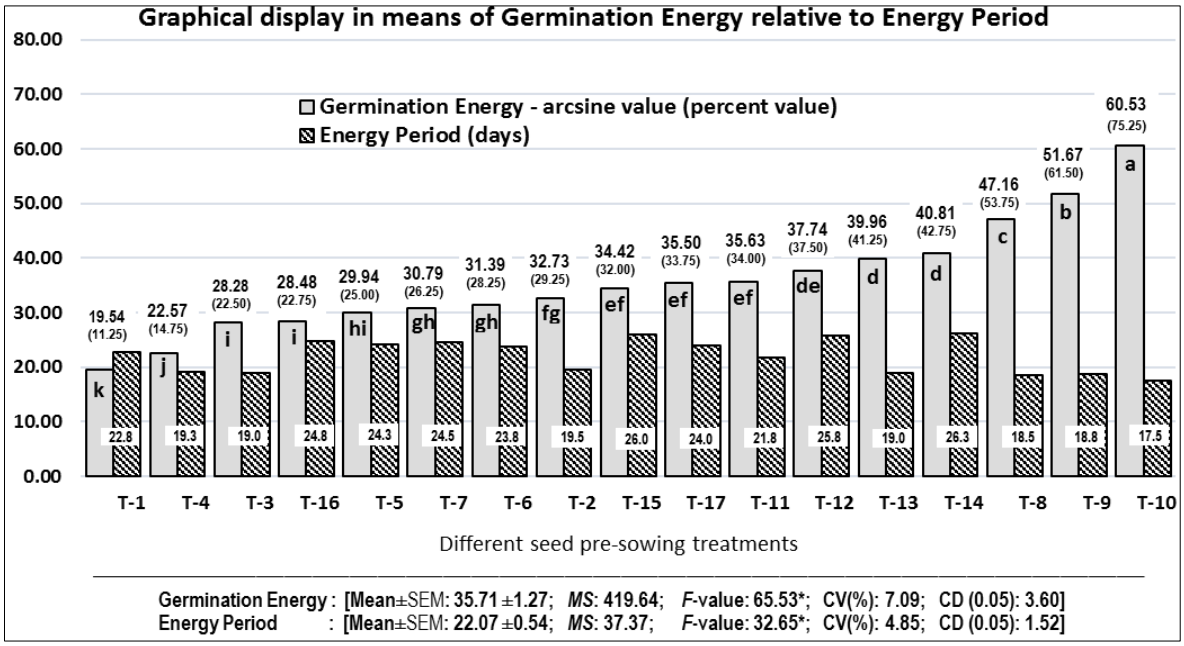
46.75-25.25% when compared with drying treatments T_3 (30.65%) and T_4 (16.25%). The percentage germination for control T_1 (untreated seed with pulp) resulted the lowest of 12%. Peak value, germination value and germination speed for treatments T_{10}, T_9, and T_8 were significantly greater than other treatments, and bestowed with gibberellin for fastest seed germination, which may be related to survival and capable of producing vigorous seedling in field conditions.

Table 1: Effect of seed pre-sowing treatment on germination parameters of under seventeen treatments (laboratory conditions)

Treatments	Germination percent (Arcsine Value)	Peak value	Germination value	Germination speed
T_1	12.00 (20.19)	0.49	0.22	0.67
T_2	33.25 (35.20)	1.50	2.09	2.16
T_3	26.00 (30.65)	1.18	1.28	1.72
T_4	16.25 (23.76)	0.77	0.52	1.11
T_5	27.50 (31.57)	1.04	1.01	1.41
T_6	29.75 (33.04)	1.19	1.32	1.53
T_7	26.50 (30.95)	1.07	1.06	1.37
T_8	60.25 (50.94)	2.91	7.36	4.22
T_9	74.25 (59.53)	3.28	9.02	5.30
T_{10}	87.50 (70.53)	4.29	14.00	6.48
T_{11}	35.50 (36.53)	1.56	2.26	2.05
T_{12}	39.00 (38.64)	1.46	2.04	2.08
T_{13}	46.75 (43.13)	2.16	4.23	3.08
T_{14}	45.25 (42.26)	1.63	2.48	2.38
T_{15}	34.50 (35.96)	1.23	1.42	1.81
T_{16}	25.25 (30.15)	0.92	0.78	1.35
T_{17}	38.25 (38.20)	1.41	1.80	2.10
Mean ± S.Em	38.31 ± 1.42	1.65 ± 0.08	3.11 ± 0.40	0.67 ± 0.13
(Range)	20.19-70.53	0.49-4.29	0.22-14.00	0.22-6.48
MS	624.51	03.90	54.11	09.66
CV (%)	7.37	10.22	25.45	10.51
F- value	78.19*	136.32*	86.24*	151.57*
CD ($p \leq 0.05$)	4.01	0.24	1.12	0.35

*significantly different at $p \leq 0.05$

In Figure 1. the germination energy and energy period were simultaneously calculated to explore the vigourness and rapid germination. From the statistical analysis, it revealed that germination energy varied from 11.25-75.25% among the treatments and the significant highest value was calculated for T_{10}, T_9 and T_8 and lowest by T_1 depicted graphically for germination energy ascendingly. Energy period ranged between 17.5-26.30 days and minimum energy period (17.5 days) were recorded for the maximum germination energy in T_{10} (75.25%) following the *vice versa* trend for all the treatments for germination energy conversely to energy period.



Note: Bars denoted with the same letter (s) for germination energy are not significantly different at $p<0.05$ probability level according to Duncan's Multiple Range Test (DMRT).Values with * are significantly different at $p<0.05$; ± S.Em, standard error of the mean; *MS*, mean square value; f-value, ratio of variances (*Fisher* analysis of variance); CV, coefficient of variation; CD, critical difference at 0.05 level

Fig 1: Germination energy (*ascendingly*), and energy period for different pre-treated seeds of *Morus laevigata* Wall. Under nursery conditions.

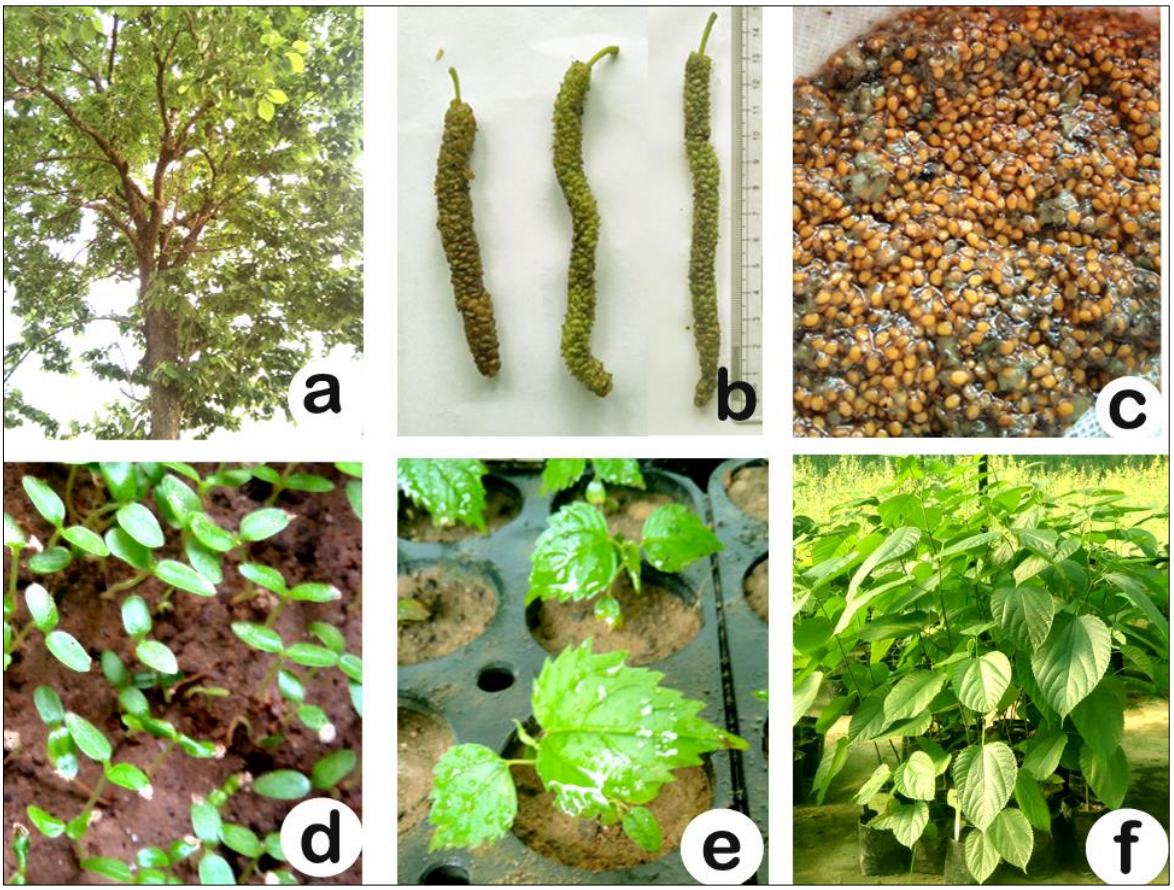


Plate 1: Adult individual of *Morus laevigata* (a), long fruit [Cylindrical syncarpous] (b), depulped seeds (c), germinated seedlings (d), transplanted seedlings (e) well grown seedling in polybag (f).

Discussions

The pre-sowing treatment on seed can improve both germination and speed of germination. The treatments must be adjusted on the base of the status of seed dormancy. In most of the species of Moraceae family physiological dormancy are reported by Baskin and Baskin (2004) [6]; Koyuncu (2005) [23] and Willis *et al*. (2014) [49]. The lowest germination occurred in T_1 - untreated seed with pulp (12%) and in T_2 considerable improvement of 33.25% exhibited when untreated depulped seed germinated, indicated that seeds with pulp likely contain substances that inhibit germination, this agrees with the fact that in some species, dormancy is Overcomed by removing pulp (Johnson and Chirco 2003; Chimera and Drak 2010) [21, 9]. Maximum germination was observed in depulped seeds using different levels of gibberellic acid (GA_3) which exhibited highest germination percentage (87.50%) when seeds soaked in 1000 ppm gibberellic acid for 12 h, followed seeds soaked in 500 ppm gibberellic acid for 12 h (74.25%) and seeds soaked in 250 ppm gibberellic acid for 12 h (60.25%) was significantly higher with comparison to rest of the treatments. The efficacy of gibberellic acid to induce germination depends on the concentration which attributed to the fact that GA_3 treatment supplements maximum seed germination in *M. laeviagata*. Prior studies on related species explored by Gunes and Cekic (2004) [16], Giba *et al*. (1993) [14] and Rai *et al*. (1988) [30] reported that the inhibitory effect of retardants can overcome by gibberellic acid and showed effective regulator in improving seed germination. Effective pre-sowing treatments with GA_3 in the present study was similar to that accorded by Dweikat and Lyrene (1988) [13] and Petkov (1995) [28]. The germination obtained by pre-sowing treatments at different levels followed by boric acid, hydrogen peroxide and luke warm water was significantly lesser to GA_3. The effect of saline medium on seed germination exhibited 34.50%, 25.25% and 38.25% when pre-treated with NaCl solution in T_{15}, T_{16} and T_{17} respectively, suggested reduced percentage in germination through osmotic effects, and similar findings were reported in mulberry and many crops (Ungar 1978 and Vijayan 2004) [45, 46].

Peak value was found maximum in GA_3 treatments of T_{10} (4.29) followed by T_9 and T_8 in contrast with other treatments. The germination value and germination speed followed the similar trend of peak value for all the treatments as the germination value depends on product of mean daily germination and peak value. This is in accordance with the study carried out by Koyuncu (2005) [23] and Rinaldi *et al*. (2022) [32]. The germination energy expressed highest percentage for GA_3 treatments attained early at 17.5-18.8 days of energy period, whereas the prolong energy period and delayed germination give low germination percent. Willan (1987) [48] concluded that germination energy is a measure of speed of germination, it gives an idea of the vigour of the seeds. Similar impressions were quoted with findings of Pourhadian and Khajehpour (2010) [29] and Asiedu *et al*. (2011) [4]. The study revealed that fresh depulped seed soaked in gibberellic acid (250-1000 ppm for 12 h) seemed to be an appropriate method for enhancing germination percentage in *Morus laevigata*.

Conclusion

The results of this present investigation taken for pre-sowing

treatments suggested that depulped seeds and treated with gibberellic acid (250-1000 ppm) could enhance germination from 60.25 to 87.50% which can easily be implemented by the nursery growers, commercial laboratories to raise sufficient planting stock for enriching the forest, commercial plantations and domestication.

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