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EFFECTS OF DIFFERENT PRE-TREATMENTS AND GERMINATION MEDIA ON SEED GERMINATION AND SEEDLING GROWTH OF *Parkia timoriana* (DC.) Merr

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Pre-treatments

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ABSTRACT

Parkia timoriana (DC.) Merr. is one of the lesser known multipurpose leguminous tree species found in North East India. Like many other legumes this species too have hard coated seeds which prevent seed germination and thus there is a need to investigate the most appropriate method to break its dormancy. Six pre treatments (tap water, gibberellic acid (GA_3) @ 500 ppm, stratification, sulphuric acid @ 98%, boiling water and nicking) and two media (top of paper (TOP) and sand) were use to evaluate seed germination traits and initial growth parameters of the seedling of this tree species. Along with these pre treatments, seeds sown in TOP media and tap water gave maximum seed germination (72%) and it was followed by seeds exposed to GA_3 for 24 hours (64%) while the least seed germination was reported in seeds treated with sulphuric acid (H_2SO_4) for 5 minutes. Least germination time (Mean Germination Time) was reported from the seeds treated with H_2SO_4 for 1 minute while maximum was reported from the boiling water treatments. Under sand medium, highest germination was reported in control (66.67 %) and it was followed by boiling water treatment (58.33%) and minimum (16.67%) in seeds treated with H_2SO_4 for 5 minute. Seed treated with H_2SO_4 for 5 minutes took minimum germination time too. Interestingly, irrespective to the media, all the seedling growth parameters showed maximum response towards hormonal (GA_3) treatment and minimum towards H_2SO_4 treatment. Significant correlations were found between all the seedling growth parameters except for collar diameter and root-shoot ratio.

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1 Introduction

Parkia timoriana (DC.) Merr (Syn *P. javanica*, family Leguminosae) is one of the promising agroforestry tree species of North East India and other South East Asian countries like Philippines, Malaysia, Thailand, Japan and Vietnam (Salam et al., 1998). It is commonly found in home gardens and Shifting agriculture lands of North East India. It is also reported in wild form in forests having an altitudinal range from 0-60 to ≥1300 metres above sea level (Devi & Das, 2012).

Various plant parts of this species such as, pods, seeds, flowers, young shoots, are consumed by both tribal and non tribal population of North-East Indian states either in raw form or in various preparations such as salads and curries (Salam et al., 2009). The plant provides good economic return (Rocky & Sahoo, 2002), besides, it also serves as an important dietary supplement as-well-as medicinal value for curing various ailments (Devi, 2011).

Normally, many trees exhibit seed dormancy so as to have better chance of survival during unfavourable conditions (Carvalho & Nakagawa, 2000). Like other leguminous trees, *P. timoriana* have hard coated seeds resulting into delay germination, hence the seeds show increase germination time and reduced germination energy. Under natural conditions, the seeds may take a much longer period to germinate thus necessitating use of preteatments as in many other legumes (Doran et al., 1983; Aref et al., 2011) to increases the rate of seed germination.

For obtaining optimum germination and seedling vigour, proper conditioning of seeds is nevertheless essential. The importance of pre treatments for many tree seeds, especially the legumes has been emphasized by many workers (Doran et al., 1983; Tietema et al., 1992; Sahoo, 2007). Several pre treatments such as stratification, acid treatment, nicking etc. have been used to trigger germination while some inorganic substrates such as sand, filter paper, vermiculture etc. have been found supporting seed germination (Rawat, 2009) and seedling growth of trees.

Some species seems to have distinctive preference for a particular media (Bahuguna et al., 1987) while others may grow well in a variety of media. However, the information pertaining to effect of pre treatments and media on seed germination and initial growth parameters of *P. timoriana* are limited. Therefore, present study has been undertaken to find out the effect of pretreatment and germination media on the germination of *P. timoriana* seeds.

2 Materials and Methods

2.1 Seed collection and preparation

The seeds of *P. timoriana* were collected directly from naturally grown standing trees during May to June 2015, from Lunglei district of Mizoram, India (Latitude 23°11′26.909″N and Longitude 92°45′06.665″E), having an altitude of 809 m above mean sea level. Twenty two trees were selected randomly across the terrain for this purpose, and five pods from each tree were harvested manually by using bamboo pole. Seeds were extracted from the pods successfully by using secateurs. All the infected and diseased seeds were screened manually and were discarded. All healthy and intact seeds were air dried and stored at room temperature (28±2°C). Size and weight variation of the intact seeds were examined; the seeds that weigh greater than the average weight were all bulked and from this a representative sample was taken for the germination experiments. The soil of the seed collection site is acidic (pH 4.64), and having organic carbon of 1.05%.

2.2 Seed treatments and scarification

Prior to seed germination study, the seeds were subjected to six major pre treatments *viz*., (1) seeds were soaked in tap water inside a 100 ml beaker for 24 hours at room temperature (28±2 °C) and then rinsed with distilled water and air dried; (2) seeds were soaked with 500ppm GA_3 in a 100 ml beaker and kept for 12 and 24 hours, which then finally rinsed with distilled water and air dried; (3) pre-chilled the seeds at 5°C for 5 days; (4) scarified manually by cutting 1mm of the seed coat at the opposite site of the helium by secateurs; (5) seeds soaked in concentrated sulphuric acid (98%) for 1 and 5 minutes, after which the seeds were thoroughly rinsed in tap water and distilled water, and finally air-dried; (6) seeds soaked in boiling distilled water at 100°C and left for 2, 5 and 10 minutes respectively inside a 500 ml flask. A control set of experiment (without any pre treatments) was also used to compare the result with the treated seeds and for each treatment, 100 seeds were used, replicated five times.

2.3 Germination experiments

Treated seeds were sown in two types of media i.e. sand and top of paper (TOP). For sand media, properly graded and sterilized sand free from impurities and toxic chemicals were placed in glass Petri dishes (100 mm diameter). Gradation and sterilization were done by passing through 0.05 mm sieve and oven drying the sand. Whatman No 1 filter paper was used for TOP media. Five replicates with 20 seeds for every treatment was used; totalling 2200 seeds (20 seeds x 5 replicate x 11 sub-treatments x 2 media). Six major treatments having subdivision of varying treatment time, temperature and along with one control, represents the 11 sub-treatment. Both the substrata were kept moisten by adding distilled water whenever needed throughout the duration of the experiments. The experiment was laid out in a completely randomized block design with two factors (seed treatment and germination medium). Petri dishes were kept in a growth chamber at a constant temperature (30±2°C) and 12/12 hr light and darkness. Germination was monitored daily and recorded. Seeds were considered germinated when healthy white radicle was seen emerged through the integument (ISTA, 1976).

As the seed breaks dormancy and seedlings emerged, the initial growth parameters were estimated using ISTA (1999) guidelines. The percent germination (GP), mean germination time (MGT), germination energy (GE), germination index (GI) and seedling vigour (SV) was calculated as follows:

a) Germination percentage (GP): the number of germinated seeds as a percentage of the total number of the tested seeds (Tanaka-Oda et al., 2009).
   GP = (germinated seeds/total tested seeds) x 100 %
b) Mean germination time: Mean germination time was estimated by formula give by Scott et al. (1984) as;
   (MGT days): = $\Sigma T_i N_i$ / S
   Where T_i is the number of days from the beginning of the experiment, N_i the number of seeds germinated per day and S is the total number of seeds germinated.
c) Germination Index (GI):
   GI = $(G_1/1) + (G_2/2) + \ldots\ldots\ldots + (G_x / x)$
   Where G is the germination day 1, 2…, and x represents the corresponding day of germination (Esechie, 1994).
d) Germination energy (GE): the percentage of seed germination obtained at maximum daily germination speed.
e) Seedling Vigor (SV):
   SV = Sh x GP, Where Sh is the seedling height and GP is the Germination Percentage (ISTA, 1985).

The initial seedling growth parameters such as, length of the radical or coleoptiles, length of root, fresh and dry mass of seedlings, the number of leaves, collar diameter were assessed after 45 days from the two leave stage. Dry weight of the seedling was estimated by oven drying at 60˚C for 48 hours.

2.4 Statistical analysis

Analysis of Variance (ANOVA, 2-way) was carried out using Microsoft Excel to test the effect of different treatments on seed germination and Least Significant Difference (LSD) was used for mean separation. Coefficient of correlation among different plant parameters like root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, number of leaves, collar diameter, root-shoot ratio, biomass and seedling vigor, were also assessed.

3 Results

3.1 Seed Germination

Germination of seeds in *P. timoriana* was greatly influenced by various pre treatments (Table 1). Among these pre treatments, tap water gave maximum seed germination (72%) and it was followed by seed exposure to GA_3 for 24 hours (64%) and stratification (52%) under TOP medium. The seeds treated with tap water and GA_3 for 24 hrs, showed significantly ($P<0.05$) higher germination over control. The duration of seeds exposure to either boiling water or concentrated H_2SO_4 also influenced the germination percent significantly ($P<0.05$). Inverse relation between seedling germination and the duration of treatment was followed in all the treatments (Table 1). Nicking though tended to enhance seed germination but it was not significantly different from control.

Table 1 Effect of different pre treatments on germination traits of *P. timoriana* under TOP and Sand media.

	TOP media				Sand media			
Treatments	GP	MGT	GE	GI	GP	MGT	GE	GI
tap water	72.00	6.50 ± 0.24^{ef}	72.00	9.50 ± 0.15^{c}	50.00	22.20 ± 0.31^{c}	33.33	4.61 ± 0.01^{bcde}
GA_3 12 hour	48.00	5.10 ± 0.22^{g}	28.00	13.23 ± 0.11^{a}	41.67	15.00 ± 0.25^{ef}	16.67	2.78 ± 0.01^{de}
GA_3 24 hour	64.00	9.30 ± 0.25^{d}	44.00	11.55 ± 0.17^{b}	50.00	16.20 ± 0.22^{e}	25.00	6.75 ± 0.02^{a}
Stratification	52.00	9.10 ± 0.33^{d}	52.00	6.49 ± 0.07^{d}	50.00	24.00 ± 0.34^{c}	41.67	3.46 ± 0.01^{cde}
H_2SO_4 1 minute	20.00	5.60 ± 0.25^{g}	20.00	1.81 ± 0.04^{f}	33.33	19.50 ± 0.38^{d}	33.33	0.50 ± 0.01^{f}
H_2SO_4 5 minute	16.00	8.80 ± 0.37^{d}	16.00	1.06 ± 0.02^{f}	16.67	13.50 ± 0.23^{f}	8.33	1.20 ± 0.01^{ef}
boiling water 2minute	40.00	18.30 ± 0.43^{a}	32.00	7.16 ± 0.04^{d}	58.33	22.60 ± 0.29^{c}	16.67	5.50 ± 0.01^{ab}
boiling water 5 minute	36.00	11.10 ± 0.42^{c}	32.00	4.42 ± 0.05^{e}	58.33	18.60 ± 0.26^{d}	41.67	5.28 ± 0.02^{abc}
boiling water 10 minute	20.00	15.80 ± 0.57^{b}	16.00	4.25 ± 0.02^{e}	41.67	27.00 ± 0.39^{b}	25.00	3.12 ± 0.01^{de}
nicking	24.00	6.70 ± 0.28^{f}	24.00	2.08 ± 0.04^{f}	50.00	23.80 ± 0.36^{c}	41.67	4.11 ± 0.01^{bcde}
control	20.00	8.00 ± 0.27^{de}	20.00	1.75 ± 0.04^{f}	67.77	35.10 ± 0.46^{a}	66.67	5.21 ± 0.01^{abc}
LSD ($P<0.05$)		1.69		1.33		2.17		1.93

MGT = Mean germination time, GI = Germination index, GP = Germination percentage, GE = Germination energy, values are pooled means ±SEM, n=5.Within columns means followed by the same letter are not significant (P<0.05).

However, under sand medium, germination was highest in control (66.67 %) and it was followed by boiling water treatment (58.33%) and showed fairly similar values when exposed to tap water, stratification and GA_3 (50%). Minimum seed germination (16.67%) was attained with acid treatment for 5 minute while the seeds exposed to 1 minute acid provided two fold increase in germination (33.33).

3.2 Mean germination time

In case of Mean germination time (MGT), least MGT (5.1 days) was reported from the seeds treated with GA_3 for 12 hours and this was followed by seed treated with H_2SO_4 for 1 minute (5.6 days) and tap water (6.5days) under TOP medium. Among various tested treatment, boiling water treatments took the longest time (18.3, 11.1 and 15.8 days) at 2, 5 and 10 minute exposure respectively and significantly increased MGT compared to the control. On the other hand, H_2SO_4 for 5 minute (8.8 days), stratification (9.1 days) and GA_3 24 hour (9.3days) treatments also showed some influence on the seed MGT.

Under sand media, minimum MGT (13.5 days) among seed treatments was found in H_2SO_4 for 5 minute, followed by seeds exposed to GA_3 for 12 hour (15days) and 24 hour (16.2days) respectively. Unlike TOP media, maximum MGT (35.1 days) was found in control and all the pre treated seeds reduced MGT significantly ($P<0.05$) compared to control (Table 1).

3.3 Germination energy

The percentage of germination obtained at daily germination speed was found maximum (72%) in case of tap water, followed by cold treatment or stratification (52%) and GA_3 24 hrs (44%) under TOP medium. Equal value of GE was recorded in the corresponding treatments: boiling water for 2 minute and 5 minute (32%), H_2SO_4 1 minute and control (20%), and H_2SO_4 5 minute and boiling water 10 minute (16%) respectively.

On the contrary, GE was found highest under control (66.67) and lowest (8.33) in H_2SO_4 treated seeds subjected to five minute duration, under sand medium. Unlike TOP stratified and exposed to GA_3 24 hours and nicked gave fair and better results (Table 1).

3.4 Germination index

Germination index (GI) showed fairly similar trend under both the media. Under TOP media, the GI was in the order of treatment with GA_3 for 12 hour > GA_3 for 24 hour > tap water > boiling water for 2 minute and least when treated with concentrated H_2SO_4 for 5 minute. Moreover between the two media, the GI values was higher under TOP medium for tap water, GA_3 (12 and 24 hour), scarified seeds, seeds stratified and exposed to H_2SO_4 for 1min and boiling water treatment for 2 and 10 minute. The reverse was found true for other treatments under sand media.

3.5 Initial growth parameters

The effect of different pre treatments on initial growth parameters of *P. timoriana* seeds under TOP and sand medium is shown in Table 2 and 3 respectively. Seeds treated with GA_3 for 12 hrs gave the highest result for the studied parameters such as root length, shoot dry weight, number of leaves, collar diameter, total biomass and seedling length, under TOP media. Similarly, GA_3 treatment for 24 hrs was most favourable in increasing the seedling vigour, while, boiling water found most favourable for increasing seedling length and root dry weight. Control gave the highest Root-Shoot ratio under this media. However, under sand media GA_3 treated for 12 hours gave the highest shoot dry weight, number of leaves, collar diameter and total biomass. GA_3 treatment for 24 hrs also gave maximum increase in other parameters like, root length, root-shoot ratio and seedling length. Seeds of *P. timoriana* treated with boiling water for 2 minutes gave the highest seedling length and seedling vigour, whereas, maximum root dry weight was found when the seeds were treated for 5 minutes.

In both the media, Pearson coefficient of correlation showed significant relationship between all the growth parameters except for collar diameter and root-shoot ratio (Table 4 and 5).

4 Discussions

Seed germination may be influenced by several external and internal factors. Adaption to the prevailing environment cause different species to evolve differently against dormancy. This allows seeds to germinate only when conditions are likely to favour the establishment of a new plant (Bewley, 1997; Hilhorst, 1995; Vleeshouwers et al., 1995; Li & Foley, 1997; Baskin & Baskin, 2004). The cause and nature of the seed coat impermeability, however, are not fully understood in some plants, but it has been found that under natural conditions and after most pre-treatments the first site at which water penetrates is the stophioles (Harper, 1977). This could be seen as a small raised area close to hilum and is the weakest and the reinforced area of the seed coat. However, variation in the timing and germination percentage of the seeds seen in many species could be attributed to several factors such as the relative position of the seed on the parent plant, micro-environment, quantity of reserve food content and provenance (Gutterman, 1982; Gray & Thomas, 1982; Owoh et al., 2011).

Table 2 Effect of pre treatments on various initial growth parameters of *P. timoriana* grown under TOP media.

Treatments	RL(cm)	SL(cm)	RDW(g)	SDW(g)	NL	CD(mm)	R/S	TB(g)	TSL(cm)	SV	VI
tap water	3.73±0.04^{cde}	12.3±0.72^{cde}	0.071±0.01^{bc}	0.567±0.01^{a}	4±0.33^{b}	2.96±0.195^{d}	0.30±0.002^{cd}	0.638±0.021^{b}	16.03±0.77^{bcd}	1154.16±55.2^{ab}	HV
GA_3 12 hour	7.5±0.51^{a}	18.2±1.01^{ab}	0.082±0.007^{ab}	0.598±0.009^{a}	5±0.33^{a}	3.41±0.038^{a}	0.41±0.009^{a}	0.68±0.014^{a}	25.37±1.45^{a}	1233.6±69.7^{a}	HV
GA_3 24 hour	4.39±0.16^{bcd}	17.1±2.65^{abc}	0.076±0.007^{ab}	0.451±0.014^{c}	4±00^{b}	3.02±0.042^{cd}	0.26±0.007^{e}	0.527±0.013^{def}	21.49±2.59^{ab}	1375.36±165.7^{a}	HV
stratification	4.12±0.3^{bcd}	10.75±2.32^{def}	0.036±0.005^{de}	0.497±0.01^{b}	4±0.33^{b}	3.35±0.004^{a}	0.38±0.006^{b}	0.533±0.012^{de}	4.87±2.3^{bcd}	773.24±119.5^{c}	HV
H_2SO_4 1 minute	1.9±0.28^{ef}	7.75±1.18^{ef}	0.032±0.004^{ef}	0.067±0.007^{d}	3±00^{c}	3.23±0.055^{abc}	0.25±0.01^{e}	0.099±0.009^{g}	9.65±1.18^{de}	193±23.7^{de}	LV
H_2SO_4 5 minute	1.5±0.25^{f}	5.3±0.33^{f}	0.015±0.003^{f}	0.055±0.005^{d}	3±0.33^{c}	3.17±0.029^{bcd}	0.28±0.006^{d}	0.07±0.002^{g}	6.8^{e}±0.58	108.8±9.4^{e}	LV
boiling water 2 minute	2.17±0.44^{ef}	10.96±0.58^{def}	0.053±0.005^{cd}	0.444±0.012^{c}	3±0.33^{c}	3.20±0.1^{abcd}	0.20±0.006^{g}	0.497±0.015^{ef}	13.13^{cde}±0.94	367.64±37.6^{de}	LV
boiling water 5 minute	3.25±0.38^{cdef}	11.4±1.59^{cde}	0.088±0.005^{ab}	0.463±0.012^{c}	3±00^{c}	3.27±0.056^{abc}	0.29±0.006^{d}	0.551±0.011^{cd}	14.65^{bcd}±1.95	293±70.2^{de}	LV
boiling water 10 minute	4.6±0.87^{bc}	20.7±2.96^{a}	0.09±0.003^{a}	0.573±0.008^{a}	5±00^{a}	3.29±0.035^{ab}	0.22±0.003^{f}	0.664±0.01^{ab}	25.3^{a}±3.83	910.8±76.6^{bc}	HV
nicking	2.6±0.1^{def}	15.57±0.81^{abcd}	0.041±0.004^{de}	0.446±0.006^{c}	4±00^{b}	3.32±0.061^{ab}	0.17±0.007^{h}	0.487±0.004^{f}	18.17^{bc}±0.73	436.08±17.4^{d}	LV
control	5.8±1.25^{ab}	13.8±2.05^{bcd}	0.042±0.005^{de}	0.475±0.008^{bc}	4±0.33^{b}	2.95±0.05^{d}	0.42±0.005^{a}	0.517±0.005^{def}	19.6^{abc}±3.29	392±65.4^{d}	LV
LSD (p<0.05)	1.83	5.8	0.019	0.032	0.84	0.26	0.02	0.04	7.04	265.75	

RL= root length(cm), SL= shoot length(cm), RDW= root dry weight(g), SDW= shoot dry weight(g), NL= no. of leaves, CD= collar diameter(mm), R/S= root shoot ratio, TB= total biomass (g), TSL= seedling length(cm), SV= seedling vigor, VI= vigor index, HV= high vigor, LV= low vigor, values are pooled means ±SEM, n=5. Within columns means followed by the same letter are not significant (P<0.05).

Table 3 Effect of pre treatments on various initial growth parameters of *P. timoriana* grown under Sand media.

Treatments	RL(cm)	SL(cm)	RDW (g)	SDW(g)	NL	CD(mm)	R/S	TB(g)	TSL(cm)	SV	VI
tap water	5.75±0.72^{bc}	20±3.18^{bcd}	0.146±000^{ab}	0.877±0.012^{bc}	4±00^{b}	3.16±0.21^{b}	0.29±0.01^{bc}	1.023±0.012^{b}	25.75±3.9^{bc}	1287.5±194^{bc}	LV
GA_3 12 hour	6.35±0.49^{bc}	25±0.58^{abc}	0.13±0.015^{bc}	0.947±0.013^{a}	5±00^{a}	3.91±0.14^{a}	0.25±0.01^{cd}	1.077±0.028^{a}	31.35±1.07^{ab}	1306.36±44.5^{bc}	HV
GA_3 24 hour	9.5±0.21^{a}	26±2.65^{ab}	0.109±0.001^{de}	0.451±0.005^{g}	4±0.33^{b}	2.32±0.08^{c}	0.37±0.02^{a}	0.56±0.006^{g}	35.5±2.85^{ab}	1775±142.7^{a}	HV
stratification	6.15±0.65^{bc}	18.7±0.43^{cd}	0.109±0.002^{de}	0.78±0.001^{d}	4±00^{b}	3.74±0.18^{ab}	0.33±0.01^{ab}	0.889±0.001^{d}	24.9±1.08^{bc}	1245±54.1^{bc}	LV
H_2SO_4 1 minute	2.5±0.58^{d}	8.75±1.3^{e}	0.052±0.003^{g}	0.317±0.002^{h}	3±0.33^{c}	3.63±0.41^{ab}	0.29±0.04^{bc}	0.369±0.002^{h}	11.25±1.88^{d}	374.96±62.5^{d}	LV
H_2SO_4 5 minute	2.7±0.23^{d}	9.5±0.32^{e}	0.02±000^{h}	0.115±0.033^{i}	4±0.33^{b}	3.47±0.09^{ab}	0.28±0.01^{bcd}	0.135±0.033^{i}	12.2±0.55^{d}	203.37±9.1^{d}	LV
boiling water 2minute	6.5±0.15^{bc}	28.3±1.56^{a}	0.09±0.003^{f}	0.573±000^{f}	5±0.33^{a}	3.57±0.07^{ab}	0.23±0.01^{d}	0.664±0.004^{f}	34.8±1.71^{ab}	2029.88±99.7^{a}	HV
boiling water 5 minute	5.25±0.26^{c}	17.3±3.58^{d}	0.161±000^{a}	0.915±0.003^{ab}	5±0.33^{a}	3.69±0.26^{ab}	0.30±0.01^{bc}	1.076±0.004^{ab}	22.55±3.32^{c}	1315.34±193.6^{b}	HV
boiling water 10 minute	3.15±0.61^{d}	18.95±2^{cd}	0.096±0.062^{ef}	0.861±0.003^{c}	3±0.33^{c}	3.66±0.01^{ab}	0.17±0.01^{e}	0.957±0.003^{c}	22.1±2.6^{c}	920.91±108.3^{c}	LV
nicking	3.6±0.03^{d}	23.55±0.84^{abcd}	0.082±000^{f}	0.651±0.003^{e}	4±0.33^{b}	3.72±0.1^{ab}	0.15±0.02^{e}	0.733±0.003^{e}	27.15±0.84^{bc}	1357.5±41.9^{b}	HV
control	7.1±0.06^{b}	21.5±0.4^{bcd}	0.129±0.003^{c}	0.911±0.002^{ab}	5±00^{a}	3.15±0.1^{b}	0.33±0.02^{ab}	1.04±0.001^{ab}	28.6±0.35^{abc}	1906.76±28.9^{a}	HV
LSD (P<0.05)	1.478	6.44	0.016	0.04	0.905	0.634	0.059	0.047	7.323	368.36	

RL= root length(cm), SL= shoot length(cm), RDW= root dry weight(g), SDW= shoot dry weight(g), NL= no. of leaves, CD= collar diameter(mm), R/S= root shoot ratio, TB= total biomass (g), TSL= seedling length(cm), SV= seedling vigor, VI= vigor index, HV= high vigor, LV= low vigor, values are pooled means ±SEM, n=5. Within columns means followed by the same letter are not significant (P<0.05).

Table 4 Coefficient of correlation between various initial growth parameters of *P. timoriana* grown under TOP media.

	RL	SL	RFW	RDW	SFW	SDW	NL	CD	R/S	TB
SL	.722**	-								
RFW	.911**	.858**	-							
RDW	.583*	.512	.604*	-						
SFW	.729**	.947**	.859**	.684*	-					
SDW	.319ns	.480ns	.359ns	.880**	.659*	-				
NL	.550*	.529*	.526*	.512ns	.561*	.393ns	-			
CD	-.630*	-.232ns	-.522*	-.101ns	-.135ns	.228ns	.035ns	-		
R/S	.588*	-.129ns	.301ns	.235ns	-.049ns	-.109ns	.210ns	-.095ns	-	
TB	.358ns	.491ns	.396ns	.908**	.672*	.998**	.415ns	.054ns	.220ns	-
SV	.789**	.823**	.785**	.611*	.856**	.527*	.654*	-.099ns	.233ns	.546*

RL= root length(cm), SL= shoot length(cm), RFW= root fresh weight(g), RDW= root dry weight(g), SFW= shoot fresh weight(g), SDW= shoot dry weight(g), NL= no. of leaves, CD= collar diameter(mm), R/S= root shoot ratio, TB= total biomass(g), TSL= seedling length(cm), SV= seedling vigor, VI= vigor index, **significant at P<0.01, *significant at P<0.05, ns not significant.

In present study it was reported that simple tap water yielded highest seed germination when soaked for 24 hour duration. Seeds composed mainly of hydrophilic polymers, with little amount of osmotically active compound (Obroucheva, 2012) and therefore when the seeds are soaked in water, the water firstly binds to hydrophilic compounds in the cell walls and cytoplasm and when hydration level reaches 22% (approx), the respiration rate increases, glycolysis and Kerbs cycle are activated, and metabolism of amino acid starts. Further increase in water content (50%) activates protein and mRNA syntheses as well as hydrolysis of stored proteins and starch begins. Hence, when hydration reaches 50-60% all the necessary physiochemical and biochemical activities leads to seed germination (Obroucheva & Antipova, 1994).

It was further found that GA_3 and stratification helped in seed germination; germination energy and germination index of *P. timoriana*. Reduction in duration for seedling emergence and seedling growth of this species was also reflected by these two treatments. The literature reveals that GA_3 occurs at relatively high concentration in developing seeds but usually drop to a lower level in mature dormant seeds (Yamauchi et al., 2004). It plays a vital role in seed germination in two different stages; first in the initial enzyme induction and second is in the activation of reserve food mobilizing systems. Hence, seed coat treatment of *P. timoriama* seeds by 500 ppm GA_3 might have favoured these two stages, resulting into better germination. Khan (1980) and Yamauchi et al. (2004), also reported the reduction of ABA, following an increase in synthesis of gibberellin and cytokinin is seeds of some tree species after stratification. Thus, GA_3 and stratification are positively correlated which conforms to our findings. Boiling water treatment also gave good result in the present study up to some extent. Increase in temperature might have dissolved the thin waxy coating in this species that prevents water to imbibe the seeds. However, decreased in germination percentage, germination energy and germination index after prolong treatment, might be due to embryo injury triggered by prolong temperature exposure (Otegbeye & Momodu, 2002; Hossain et al, 2005; Omokhua et al., 2015).

Table 5 Coefficient of correlation between various initial growth parameters of *P. timoriana* grown under Sand media.

	RL	SL	RFW	RDW	SFW	SDW	NL	CD	R/S	TB
SL	.702**	-								
RFW	.944**	.775**	-							
RDW	.534*	.733**	.665*	-						
SFW	.803**	.898**	.912**	.783**	-					
SDW	.701**	.775**	.858**	.726**	.941**	-				
NL	.806**	.863**	.750**	.509ns	.788**	.704**	-			
CD	.048ns	.132ns	.025ns	.069ns	.088ns	.050ns	.190ns	-		
R/S	.702**	.005ns	.549*	.006ns	.250ns	.243ns	.301ns	-.095ns	-	
TB	.701**	.794**	.860**	.783**	.950**	.996**	.701**	.054ns	.220ns	-
SV	.659*	.696**	.640*	.633*	.771**	.676*	.739**	-.099ns	.233ns	.691**

RL= root length(cm), SL= shoot length(cm), RFW= root fresh weight(g), RDW= root dry weight(g), SFW= shoot fresh weight(g), SDW= shoot dry weight(g), NL= no. of leaves, CD= collar diameter(mm), R/S= root shoot ratio, TB= total biomass(g), TSL= seedling length(cm), SV= seedling vigor, VI= vigor index, **significant at P<0.01, *significant at P<0.05, ns not significant

The media to which seeds get exposed also play important role in seed germination and establishment. They also provide not only pre requisites such as contact area, favourable micro environment but also condition the seed for germination (Kumar & Bhatnagar, 1976). The sand media provided better contact area over TOP in the present study by helping imbibitions of water and oxygen diffusion as also argued by Bahuguna et al. (1987). This could be the reason why untreated seeds of *P. timoriana* under sand media excelled in germination and vigor germination index than the TOP media.

In present study the effect of H_2SO_4 was minimal in breaking seed coat as is reflected in poor seedling emergence and other growth parameters. Longer exposure of the seeds to H_2SO_4 might have damaged the embryo of *P. timoriana*. Similar views have been expressed by Aduradola & Adejomo (2005) for *Erythrophleum suaveolens* seeds.

Conclusions

Both physical (exogenous) and physiological (endogenous) inhibition are likely to be the cause of dormancy in the seeds of *P. timoriana*. Present results showed more positive skewed towards physiological dormancy, as tap water, GA_3 and cold treatment (stratification) gave better result. However, acid Scarification, boiling water and nicking affect the germination and initial growth parameters. Our study recommends the use of simple tap water in *P. timoriana* seeds if the growing substratum is TOP, while, GA_3 and stratification should be used for sand substrata for enhancing seed germination and better yield of seedlings.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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