

Amplification of Seed Germination and Seedling Growth Attributes of a Multipurpose Tropical Tree *Parkia timoriana* (DC.) Merr. Through Different Pre-sowing Seed Treatments

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ABSTRACT: *Parkia timoriana* (DC.) Merr. is highly underutilized, multipurpose and sustainably harvested legume tree of north east India. It is mainly propagated through seeds, but due to over exploitation, insect-pest infestation, climatic disruption and various other threats its population is declining from the wild. Further due to innate seed coat dormancy, the germination percentage and seed vigour is very less which further resulted into poor natural regeneration. Keeping this view, we attempted to investigate phenotypically superior trees based on scoring scale from wild and screened out best pre-sowing treatment for enhancing germination and growth attributes. Study started with selection of 21 healthy populations of *P. timoriana* from northeast India through field survey, assortment of phenotypically superior trees through scoring scale method and tested different pre-sowing treatments viz., Growth regulators, Acid Scarification and Organics for enhancing seed germination and seedling growth attributes. Study observed that, seeds pre-soaked in water for 24 hour, treated with GA₃ (100 ppm, 24 hour) or cow dung slurry (1:1) for 12 hour, required less germination time (2-3 days), having higher germination percentage (90-98%), germination energy (76.41-80.43), germination index (15.4-19.5) and required minimum mean germination time (6-7 days) as compare to unprocessed seeds. This study recommends the use of organic treatments i.e. water soaking for 24 hour, Cow dung slurry (1:1) for 12 hour and growth regulator i.e. GA₃ 100 ppm for 24 hour, for enhancing seed germination and growth attributes in *P. timoriana*.

Keywords: Seed coat dormancy, growth regulators, germination percentage, pre-sowing treatments, scoring scale

Parkia timoriana (DC.) Merr. Syn. *P. roxburghii* G. Don from Fabaceae family (subfamily Mimosoideae tribe Mimoseae) commonly known as tree bean is an important tree legume of old world tropics [1, 2]. The name was derived after Mungo Park (1771-1805), a well-known Scottish explorer of West Africa. Out of the twelve known species of *Parkia* found in Indo-Pacific region, *P. timoriana* is most prevalent species in Southeast Asian countries viz. India, Bangladesh, Myanmar, Java, Thailand, Egypt and the Malaysian region [3, 4]. In India, it is confined to North eastern states (i.e. Mizoram, Nagaland, Manipur and Assam) in evergreen primary and disturbed rain forest, moist mixed deciduous forest and dry evergreen forest, at an altitudinal range of 0-600 m asl [5-7]. It is locally known as 'Yongchak' in north-east India, 'Petai' in Indonesia, Malaysia and 'Sator' in Thailand.

Tree bean flourishes well in sandy, loam, podzolic soil and in close proximity of river banks [8, 9]. The most

favourable temperature required for its proliferation is 24°C. It is generally found growing under semi-wild condition or in homestead backyard gardens [10]. *Parkia* tree is a popular non-conventional source of dietary, therapeutic and recreational values among the ethnic communities of Eastern Himalayan region of India [11-13]. Its immature tender pods and seeds have unique taste and flavour which are consumed during developmental stages by the local people [14-17]. In North East India, it is considered as highly pride and costly vegetable, fetching Rs. 50-70/- per pod during off-season and Rs. 15-20/- per pod during peak season [18-21].

However, in the recent past, tree bean plants are under severe threat in North East India. Unsustainable harvesting, insect-pest infestation, climatic disruption, mobile radiations and various other threats have resulted a vast population decline as well as poor natural regeneration in the wild [22, 23]. Moreover, lack of

organized plantation and absence of appropriate government policies, has created demand-supply imbalance of tree been in North Eastern States [24]. Hence to fill this shortfall, considerable volume of tree been seeds is being imported from neighbouring country Myanmar [25]. Nevertheless, the presence of innate seed coat dormancy, the seed needs prolong germination time and have reduced germination energy which further necessitate for conditioning of seeds [26]. However, the information pertaining to effect of pre-treatment and media on seed germination and initial growth parameters of *P. timoriana* are limited. Thus, the present study has been undertaken to screen out phenotypically superior *P. timoriana* population from different growing region of north east India and to explore suitable pre-sowing seed treatment which can amplify seed germination and enhance initial growth attributes under natural conditions.

MATERIALS AND METHODS

Study area

Extensive field surveys were undertaken in Eastern Himalayan region where *Parkia* population is declining

and at the same time efforts are also being made to explore healthy population for germplasm collection and conservation. Twenty one populations representing four states of Northeast India were selected i.e. 9 from Manipur, 4 from Nagaland, 4 from Tripura and 4 from Mizoram; information on morpho-physiological characters of selected population and physiographical features of each location were recorded. From each selected populations, five trees of superior phenotypic characters and bearing prolific pods (at least 100 m apart) were selected based on scoring scale method [18]. The selected phenotypes were considered for further phonological studies and collection of seeds (Figure 1 and Table 1).

Seed collection and preparation

Ten mature black colour pods were harvested manually from each selected phenotypes either by climbing and / or using bamboo pole during April-May months. Pods were then split open and de-pulped from yellow powdery mass to extract dark brown or black colored seeds. The seeds were sun dried for 3-4 hour before storing at room temperature ($28 \pm 2^\circ\text{C}$). Size, thickness and weight

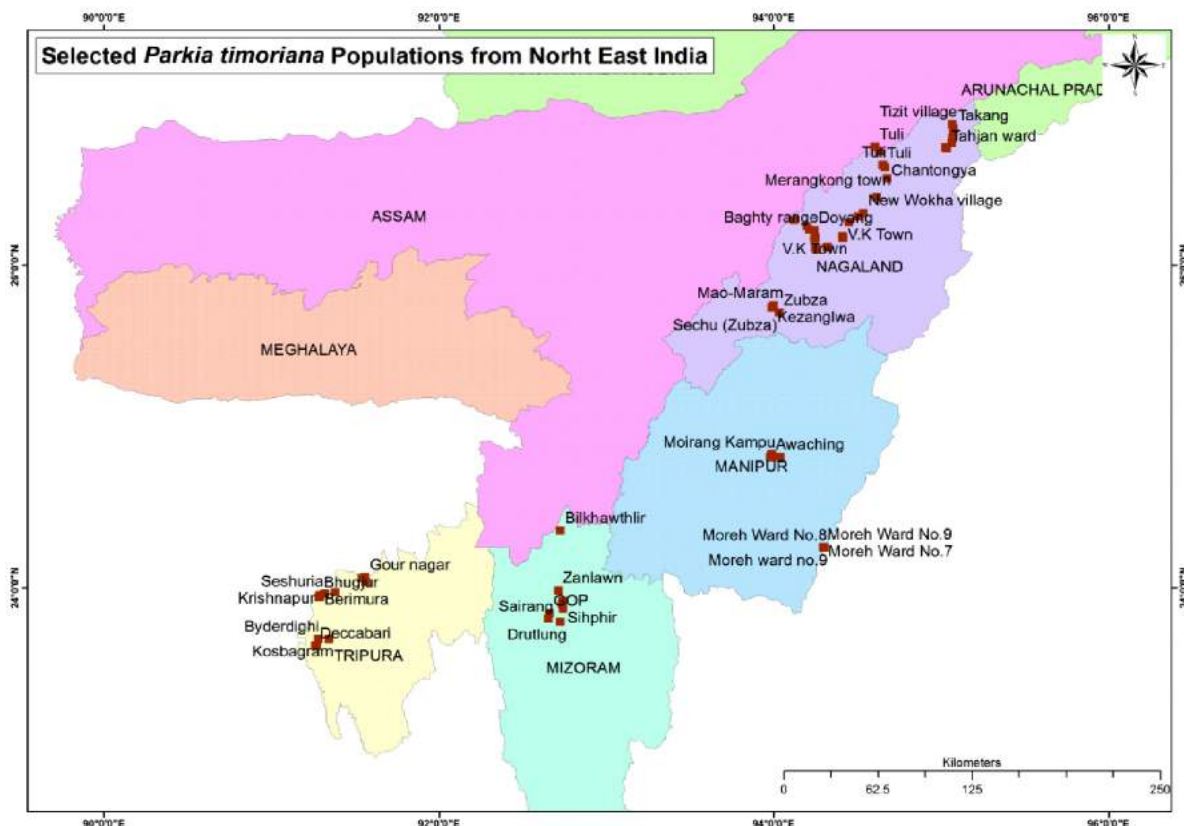


Figure 1. Population of *P. timoriana* selected form different growing region of North East India

Table 1. Selection of superior phenotypes based on scoring scale

Weights for categories and subcategories out of 100		
Characteristics	Performing Traits	Scores
Total Height (5)	10-15	1.0
	15-20	2.5
	20-25	1.5
No. of branches (11)	0-5	0
	5-10	2.0
	10-15	3.0
	>15 m	6.0
Insects & Pest Infestation (4)	present absent	04.0
Pod Size (10)	<25 cm	2.0
Length	25-30 cm	3.0
	>30 cm	5.0
Pod Size	<2 cm	2.0
Width (10)	2-4 cm	3.0
	>4 cm	5.0
Seed Length (cm) (12)	Small (<1.6)	2.0
	Medium (1.6-1.8)	4.0
	Large (>1.8)	6.0
Seed Breadth (cm) (10)	<1.0	2.0
	1.0-1.5	3.5
	>1.5	4.5
Seed Weight (g) (8)	0-3	2.0
	3-6	2.5
	6	3.5
Pod Per Cluster (16)	<5	0
	5-10	2.0
	10-15	3.0
	15-20	5.0
	>20	6.0
No. of seed per POD (14)	<10	2.0
	10-15	4.0
	15-20	8.0

variation of the intact seeds were examined. All the infected and diseased seeds were screened manually and were discarded.

Test of viability

Before pre-treatment and seed sowing, the seeds were subjected to viability test for determining the seed vigour. Batch of 20 seeds from each seed lot were peeled and then immersed in 1% 2,3,5-triphenyl tetrazolium chloride (TTC) for 7 hours at 30°C in the dark.

Seed treatment and scarification

For amplification of seed germination, the seeds were first surface sterilized with 5% sodium hypochlorite solution for 1 minute so as to free from contamination if any and then rinsed thoroughly in distilled water for 2-3 minutes. *Parkia* seeds were then subjected to three major

pre-sowing treatments viz., Growth regulators, Acid Scarification and Organics before germination. These three major treatments further having subdivision of varying treatment, time, temperature and along with one control, represent in total 13 sub-treatments. In each treatment, 20 seeds were used, replicated three times. A control set of experiment (unprocessed seeds) was also used to compare the result with the treated seeds.

- (1) Growth regulators: T1 - GA₃ (100 ppm, 24 hour); T2 - GA₃ (200 ppm, 24 hour); T3 - Cytokinin (20 ppm, 24 hour); T4 - Cytokinin (30 ppm, 24 hour). Use same pattern for hour
- (2) Acid Scarification: T5- HCl (conc.) for 2 min; T6 - HCl (conc.) for 4 min; T7 - H₂SO₄ (conc.) for 2min; T8 - H₂SO₄ (conc.) for 4 min.
- (3) Organics: T9–Water soaking (12 hour); T10– Water soaking (24 hour); T11 - Cow dung slurry (1:1) for 12 hour; T12 - Cow dung slurry (1:1) for 24 hour; T13–Control (Unprocessed)

Germination experiments

Treated seeds were sown in polybags of size of 8 × 6 inches which were filled with mixture of sand, soil and well rotten FYM in the ratio of 1:1:1 w/w respectively. To facilitate aeration and proper drainage, nine perforations were made to the polybags before filling them with prepared media mixture. After seed treatment, one seed per polybag was dibbled and then covered with a thin layer of sterilized soil. The soil sterilization was carried out though autoclaving the soil for 1 hour at 121°C and at 15 pound pressure. The experiment was laid out in a completely randomized block design. Germination was monitored daily in the morning and seed were considered germinated with the appearance of when healthy white radical from the integuments [27].

RESULTS

Seed germination

Germination Period

Germination of seeds in *P. timoriana* was greatly influence by various pretreatment (Figure 2). Days for initial seed germination varied from 2 to 8 day after sowing, the fastest seed germination (*i.e.* 2nd day & 3rd day), were observed with water soaking (24 hour) from organic treatment and GA₃ (100 ppm, 24 hour) from growth regulator treatment respectively, whereas, seed pre-

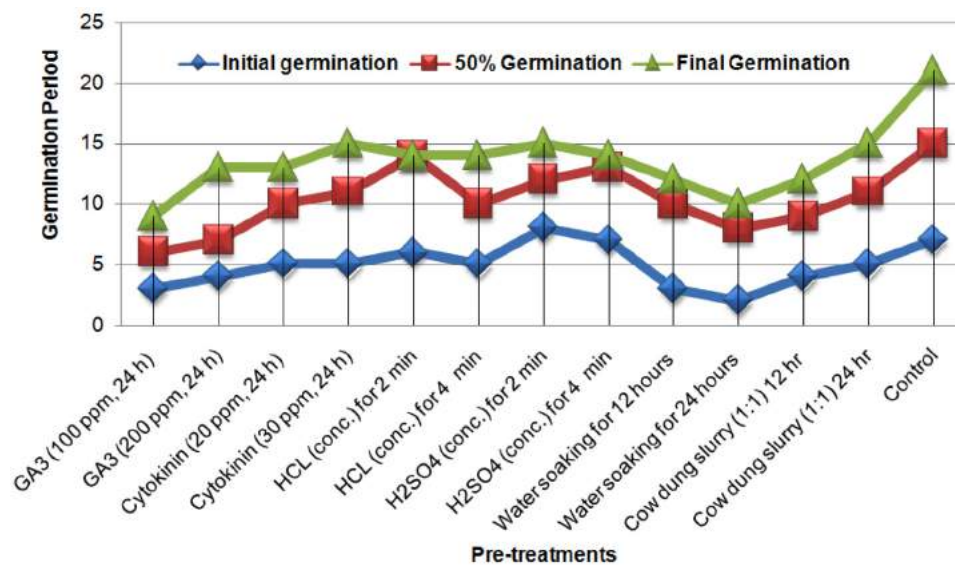


Figure 2. Influence of pre sowing seed treatments on seed germination

treated with conc. H₂SO₄ (2 min.) took maximum time for germination. In case of 50% germination, GA₃ (100 and 200 ppm, 24 hour) treatment outperformed than other pre-treatments. Similarly, for final germination, GA₃ (100 ppm, 24 hour) and water soaking for 24 hour seed treatment were found most suitable, whereas, H₂SO₄ (4 hour) treatment was found in appropriate for seed pre-treatment. Among various categories of pre-treatment tested, organic treatments were found best suitable followed by growth regulators and least suitable recorded was acid treatments.

Germination percentage

Germination percentage in *P. timoriana* was significantly affected by various pre-treatments (Table 2). Among various pre-treatments, Water soaking for 24 hour gave maximum seed germination percentage (98%), followed by GA₃ 100 ppm for 24 hour and Water soaking for 12 hour for 95% each. Seed treatment with Cytokinin 20 ppm for 24 hour (90%) and cow dung slurry (1:1) for 12 hour (90%) also showed significantly higher germination percentage than control. Among various categories of pre-treatments tested, germination percentage was recorded maximum with growth regulator, followed by organic treatment and slightest by acid treatments. However, acid seed treatment both with conc. H₂SO₄ (2 min. and 4 Min.) for 24 hours and with conc. HCl (2 min. and 4 Min.) for 24 hours, depicted some positive result over control.

Mean germination time

Among various categories of treatments investigated, Mean Germination Time (MGT) was found least for organic treatment followed by growth regulator treatments (Table 2). However, seed treatment with GA₃ 100 ppm for 24 hour outnumbered among all pre-treatments (6.23 days), followed by water soaked seed for 24 hour (6.50 days), water soaking 12 hour (7.81 days) and cow dung slurry (1:1) for 12 hour (7.56 days) showed significant results. Seed treatment with, GA₃ 200 ppm for 24 hour (8.55 days) and Cytokinin 20 ppm for 24 hour (9.26 days), also represented some influence on the seed MGT over control. On the other hand, HCl for 4 min (19.60 days) and conc. H₂SO₄ for 4 min (19.30 days) treatments were found non-significant among various tested pre-treatments.

Germination energy

Germination energy i.e. the percentage of germination obtained at daily germination speed was found maximum (80.43%) in case of water soaking for 12 hours, followed by GA₃ 100 ppm for 24 h (76.41%) and water soaking for 24 hours (71.08%) treatment. On the contrary, GE was found minimum for acid treatments i.e. H₂SO₄ for 4 min (19.45%) and 2 min (21.24 %) and HCl for 4 min. (18.82 %), even lower than control i.e. 34.67 % (Table 2). However, treatment with HCl for 2 min. (50.73%), gave some good result in the present study as it dissolved the impermeable coating which might obstruct water

Table 2. Influence of pre-treatments on germination attributes of *P. timoriana*

Treatments	GP	MGT	GE	GI
GA ₃ (100 ppm, 24 hour)	95.00	6.23±0.14	76.41	17.5±0.20
GA ₃ (200 ppm, 24 hour)	75.00	8.55±0.30	41.57	11.8±0.19
Cytokinin (20 ppm, 24 hour)	90.00	9.26±0.24	34.39	8.61±0.18
Cytokinin (30 ppm, 24 hour)	75.00	11.0±0.37	35.02	6.15±0.19
HCl (conc.) for 2 min	70.00	16.80±0.43	50.73	1.97±0.20
HCl (conc.) for 4 min	79.00	19.60±0.25	18.82	1.49±0.21
H ₂ SO ₄ (conc.) for 2 min	55.00	17.30±0.54	24.24	1.64±0.19
H ₂ SO ₄ (conc.) for 4 min	45.00	19.30±0.42	19.45	0.42±0.20
Water soaking for 12 hour	95.00	7.81±0.57	80.43	12.1±0.32
Water soaking for 24 hour	98.00	6.50±0.28	71.08	19.5±0.18
Cow dung slurry (1:1) 12 hour	90	7.56±0.29	53.82	15.4±0.20
Cow dung slurry (1:1) 24 hour	80	9.02±0.43	36.69	13.5±0.19
Control	35.00	9.23±0.25	34.67	3.26±0.16
LSD (p<0.05)		2.16		1.29

GP= Germination Percentage, MGT= Mean Germination Time, GE= Germination Energy, GI=Germination Index

imbibition. However, prolonging exposure to conc. acid treatments, decreased germination percentage, germination energy and germination index. In case of different categories of pre-treatments, organic treatments topped the list.

Germination index

As observed in MGT and GI, similar trends were observed with Germination Index (GI). Germination index calculated for various pre-treatments follows the order Water soaking (24 hour) > GA₃ (100 ppm) > Cow dung slurry (12 hour) > Cow dung slurry (12 hour) > water soaking (12 hour) > GA₃ (200 PPM) which are highly significant as compare to control. Among various categories of treatments Organic seed pre-treatment was at par followed by growth regulators and bare minimum for acid treatments, even lesser than control.

Growth attributes

The effect of different seed treatments on initial growth attributes of young seedlings at 90Days after Showing (DAS) on growth media (sand, soil & FYM in 1:1:1 ratio) is shown in Table 3 and Figure 3. Among the various pre-treatment tested, water soaked treatment for 24 hour (among organic treatment) showed highest result for different parameters such as shoot dry weight, root dry weight, collar diameter, total biomass, total shoot length and seed vigor followed by 12 hour water soaked treatment. However, among growth regulator GA₃ (100 ppm, 24 hour) treatment gave second highest result in terms of total biomass, shoot length and seeding vigor.

Seed soaked in Cow dung slurry (1:1) for 12 hour treatment also found suitable for enhancing initial growth attributes *i.e.* seed vigour and total biomass. Although, no significant differences were observed among 12 hour and 24 hour seed treatment with cow dung slurry. Among the various categories of treatment tested, organic treatments were found most appropriate followed by growth regulators and least by acid treatment (H₂SO₄ and HCl), as they gave non-significant result in comparisons to other treatments.

DISCUSSION

In present study it was reported that seeds pre-soaked in water for 12-24 hours or soaked in cow dung slurry (1:1) for 12 hours germinates early as compare to unprocessed seeds. This may be due to the fact that, soaking increase wall permeability of hard seed coat due to water imbibition, thus solubilised hydrophilic barriers and enhanced gaseous exchange with external environment which favour seed germination. However, variation in the timing and germination percentage of the seeds seen in this species could be attributed to various other factors such as quality of reserve food content, provenance variation, micro-environment etc. [26, 28]. Among growth regulators, GA₃ treatment yielded highest seed germination when treated with 100 ppm for 24 hrs. This is supported by the fact that, gibberelic acid eradicates seed dormancy by mobilization stores food material to the growing embryo and bypass specific light, temperature prerequisite required for early seed germination. [29, 30] also reported that increase in

Table 3: Influence of pre-treatment on seedling growth attributes of *P. timoriana* at 90 days after sowing

Treatments	RL (cm)	SL (cm)	RDW(g)	SDW(g)	NL	CD (mm)	R/S	TB (g)	TSL (CM)	SV
GA ₃ (100 ppm, 24 hr)	6.95±0.51	18.20±1.19	0.072±0.095	0.423±0.031	4±0.34	3.41±0.04	0.41±0.009	0.68±0.014	25.37±1.45	1404.1±49.3
GA ₃ (200 ppm, 24 hr)	5.39±0.21	17.10±2.65	0.076±0.007	0.391±0.020	5±0.22	3.02±0.04	0.26±0.007	0.527±0.013	21.49±2.59	1240.2±34.5
Cytokinin (20 ppm, 24 hr)	4.02±0.13	10.23 ±1.13	0.061±0.076	0.311±0.028	4±0.1	3.02±0.031	0.04±0.015	0.372±0.104	14.25±1.26	1084.5±76.2
Cytokinin (30 ppm, 24 hr)	3.89±0.20	13.59±1.02	0.068±0.084	0.294±0.011	4±0.0	3.11±0.023	0.29±0.020	0.010±0.095	17.48±1.22	1053±12.8
HCL (conc.) for 2 min	2.09±0.19	9.02±1.05	0.053±0.035	0.011±0.0012	2±0.01	3.07±0.05	0.23±0.080	0.064±0.036	11.11±1.24	437.9±3.4
HCL (conc.) for 4 min	2.54±0.04	7.96±0.81	0.074±0.021	0.017±0.0023	2±0.11	3.01±0.03	0.32±0.049	0.757±0.023	10.5±0.85	342±9.9
H ₂ SO ₄ (conc.) for 2 min	1.8±0.32	6.72±1.35	0.025±0.028	0.089±0.005	3±0.09	3.52±0.021	0.27±0.012	0.114±0.033	8.52±1.67	243.9±39.4
H ₂ SO ₄ (conc.) for 4 min	1.4±0.14	5.82±0.25	0.021±0.043	0.071±0.008	3±2.3	3.11±0.019	0.24±0.0043	0.092±0.051	7.22±0.39	203±65.8
Water soaking for 12 hr	4.01±0.22	18.68±2.5	0.009±0.130	0.682±0.006	4±0.33	4.02±0.05	0.21±0.007	0.691±0.136	22.69±2.72	1411±89.1
Water soaking for 24 hr	5.89±0.35	19.68±2.5	0.102±0.003	0.609±0.003	5±0.99	4.23±0.05	0.29±0.014	0.711±0.006	24.57±2.85	1478±15.9
Cow dung slurry (1:1) 12 hr	4.35±0.30	13.75±2.65	0.045±0.006	0.597±0.021	4±0.24	3.45±0.051	0.32±0.005	0.642±0.027	18.1±2.95	1345±11.1
Cow dung slurry (1:1) 24 hr	4.06±0.22	11.63±1.98	0.039±0.004	0.471±0.019	4±0.30	3.31±0.033	0.35±0.007	0.51±0.0023	15.69±2.2	1229±09.2
Control	4.8±1.32	13.8±1.05	0.063±0.005	0.458±0.007	4±0.34	2.74±0.04	0.34±0.004	0.521±0.004	18.6±2.37	375±48.8
LSD (p<0.05)	2.12	7.54	0.017	0.056	0.94	0.53	0.055	0.041	8.19	3.58.23

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, HV= High Vigor, LV= Low Vigor, Values are pooled means ±SEM, n=5.

synthesis of gibberellins and cytokinin in seeds of some tree species after pre-treatments.

It was also account that *P. timoriana* seed soaking in water for 24 hour or 100 ppm GA₃ treatment for 24 hour were having highest germination percentage and germination index and required minimum mean germination time. As these treatments may help in initial enzyme induction and mobilization of reserve food material and thus resulting into better germination. Similar, findings was reported in *Flemingia semialata* [31], *Bauhinia rufescens* [32], *P. Timoriana* [26] and *Acacia catechu* [33] and that seed soaked in water for 24 hours have maximum germination rate and vigorous vegetative growth of seedlings. Thus, are positively correlated which conforms to our findings. Treatment with acids (H₂SO₄ and HCl for 2 min) gives some good in the present study as it dissolved the impermeable coating which might obstruct water imbibition. However, prolonging exposure to conc. acid, decreased seed germination and growth attributes might be due to embryo injury [34-36].

Apart from water soaking for 24 hour and GA₃100 ppm for 24 hour pre-treatments, seeds treated with Cow dung slurry (1:1) for 12 hours gave good results in case of seedling growth attributes i.e. shoot length, fresh and dry biomass, vigour index and seedling vigour, as it may provide certain pre requisites such as contact area, favourable micro environment, nutrients vital for initial growth. This finding is in confirmation with the results obtained in case of *Melia azedarach* [37].

In comparison to all pre-treatments tested, the un-processed seeds were slower in breaking seed coat dormancy as reflected though poor seedling emergence and other growth parameters. This also suggested that poor germination in *P. timoriana* is due to seed coat dormancy [38]. Longer exposure of the seeds to acids such as HCl and H₂SO₄ for more than 4 minutes was not effective in inducing germination as it damages the embryo. Similar views have been expressed in *Pterocarpus marsupium* seeds [24] and *Uraria picta* [39].

CONCLUSION

The outcomes of the present study established the positive effects of pre-sowing treatments on seed germination and seedling growth attributes. Growth regulators like GA₃ 100 ppm for 24 hour, organic treatment like water soaking for 24 hours and cow dung slurry (1:1) for 12 hour render enhanced germination and



Figure 3. Influence of pre-sowing treatments on a) Seed Germination and b) Initial growth attributes

seedling vigour than other treatments. Prolonged exposures to conc. acids such as HCl and H₂SO₄ damages the embryo thus not recommended for pre-sowing seed treatment. In particular our study recommends the use of organic treatments i.e. water soaking for 24 hour and Cow dung slurry (1:1) for 12 hour, pre-treatments as they are not only cheap and easy to use and also fulfills all the pre requisites needed for seed germination and growth attributes in seedlings of *P. timoriana*.

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