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Effect of priming treatments on seed germination and seedling growth in bamboo [*Dendrocalamus strictus* (Roxb.) Nees]

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ABSTRACT

Instances of flowering of bamboo species *Dendrocalamus strictus* are few and far between which are taken as opportunity by nurserymen to collect seeds for propagation. Germination of seeds is reported to be poor. Therefore, different seed priming treatments were applied to *D. strictus* seeds collected from Ranchi in order to obtain uniform and high germination. Under laboratory conditions, dehusking of seeds before sowing ensured cent percent germination. Seed priming with KNO_3 1% solution resulted in 80.4% increase in germination followed by hydropriming by 16 h (73.1% increase). In field conditions, dehusked seeds gave 23.0% germination without any priming treatment. Priming treatment with KNO_3 1% gave the highest rise in germination (39.1%) followed by hydropriming for 16 h (26.1%). Seeds with their seed coats intact could give germination of 9.5% when germinated without any treatment. A rise of 115.8% in germination was obtained by priming with KNO_3 1% (final germination count 20.5%). The next best treatment was hydropriming for 16 h (final germination 18.5%, a rise of 94.7%). KNO_3 1% also induced the earliest and the most rapid germination. When seedlings germinated in laboratory were transferred to soil, all seedlings from all treatments established successfully without any mortality whatsoever. Therefore, it is recommended that seeds should be primed for 8 h with 1% KNO_3 and germinated in laboratory or in farm house under normal atmospheric condition before transplanting the seedlings to soil.

1. Introduction

Bamboo is traditionally used in almost all parts of India as fuel, as a source of materials for making houses, shelter, fences and innumerable other purposes. In industry, it is in demand for making paper, construction materials, panels, *etc*. Owing to expansion of agriculture, forest fire and over-exploitation bamboos are dwindling from natural flora in many regions. Because of low frequency of flowering and low viability of seeds, its natural expansion is precarious. Appropriate documentation of its artificial propagation through seeds is of vital importance for nurserymen in order to enable them to make use of the precious scarce seeds whenever they can be harvested. Instances of flowering of bamboo species *Dendrocalamus strictus* are sporadic in time but whenever it takes place, it is gregarious. Gregarious flowering of bamboo (*Dendrocalamus strictus*) has been reported earlier by several authors [10,13,21,23,27–29,31,34] and also reported in other species like *Drepanostachyum falcatum* [19] at various geographical regions in India. The usual propagation of bamboo is by stem cuttings, rhizomes and rarely by tissue culture. All the methods are cumbersome, at the same time success rate has been reported to be low [7]. Seeds are available only when flowering takes place which is rare in nature. When flowering occurs, nurserymen tend to collect seeds and use for propagation. However, success rate is still low. Instances of erratic behavior during germination in many forest trees have been recorded by several workers. Authors [16,17] have reported slow and irregular germination in untreated drupes of *Acacia auriculiformis*. The seeds with hard, solid, inflexible seed coat were reported to recover germination with pre-sowing treatments [16,18,22]. Seed dormancy is known to occur in many tropical tree species. Several authors [3,33] stated that seed pretreatment are species specific and that no one type of treatment has been reported to be universally effective. Therefore, breaking the seed dormancy by softening the seed testa to allow water imbibition is crucial for any afforestation program [4]. The enhanced performance of seed lots after priming treatments has been attributed to completion of pre-germination metabolic activities during seed priming, making the seed ready for soon germination after planting compared with unprimed seeds [12,26]. Different approaches of breaking seed dormancy, in order to enhance germination rate and to increase germination process have been proposed by many authors [2,5,6,36] depending upon predominant causes of dormancy. However, breaking of seed dormancy varies from species to species. Therefore, it is very important to determine which method and condition is suitable for each plant species. In order to obtain maximum recovery of seedlings certain

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interventions like seed priming treatments and dehusking of seeds were tried, the results of which are presented in this article.

2. Materials and methods

The experiment was conducted at Ranchi (the capital of Jharkhand state) located at 23°15′ and 23°18′ North latitude and 85°25′ East longitude and at an elevation of 625 m amsl (above mean sea level) in eastern plateau and hills region (EPHR) under field conditions. The experiment was followed in split plot design with four replications in 2016–18. Two sets of the seeds were prepared. One set was subjected to dehusking *i.e.*, removal of seed coat; in the other set seed coat was kept intact. Samples from both sets were subjected to ten priming treatments and one sample was kept as unprimed control in both sets as presented in Table 1.

For applying the priming treatments, seeds were kept on filter paper moistened to saturation with respective solutions and kept at 25 ± 1 °C in B.O.D. incubator for 8 h in dark. Four replications of 100 seeds each were kept for each treatment. After 8 h of treatments seeds were dried in shade. The dried seeds were put for germination in laboratory in B.O.D incubator at a constant temperature of 25 ± 1 °C. One subset from both sets was kept for germination in soil. Final germination as percentage of total seeds, speed of germination, total seedling length, leaf length, leaf width and seedling dry weight were recorded. Germination test was according to ISTA [37]. Seedling dry weight was recorded by keeping ten seedlings from each replication for drying in oven at 80 °C overnight (8 h). Seeds were considered to have germinated normally when radical and two leaves were visible. The experiment was carried out in split plot design using priming treatments as main plots and seed coat intact and dehusked seeds as sub plots. The mean germination time was calculated using the formula [11]:

Mean germination time $= \Sigma\, g_i t_i / g_i$, where:
g_i = number of seeds newly germinated on ith day of observation; and
t_i = number of day on which observation is taken.

Statistical analysis of data has been done using Systat-12 software [35], which was used for computation of descriptive statistics (mean, standard deviation, standard error, critical difference, *etc.*) and to compute the effect of different priming treatments on seed germination and seedling growth behaviour.

Table 1
Different priming treatments on seeds of *Dendrocalamus strictus*.

Treatments		Sub-treatments
T1	Soaked in 0.5% KNO_3 for 8 h	With seed coat Without seed coat
T2	Soaked in 1.0% KNO_3 for 8 h	With seed coat Without seed coat
T3	Soaked in 100 ppm GA_3 for 8 h	With seed coat Without seed coat
T4	Soaked in 250 ppm GA_3 for 8 h	With seed coat Without seed coat
T5	Soaked in 200 ppm Ethrel for 8 h	With seed coat Without seed coat
T6	Soaked in 500 ppm Ethrel for 8 h	With seed coat Without seed coat
T7	Solid matrix priming (SMP) of seeds for 8 h	With seed coat Without seed coat
T8	Solid matrix priming (SMP) of seeds for 16 h	With seed coat Without seed coat
T9	Hydropriming for 8 h	With seed coat Without seed coat
T10	Hydropriming for 16 h	With seed coat Without seed coat
T11	Control (Untreated)	With seed coat Without seed coat

3. Results and discussion

Flowering of bamboo has been observed as an extraordinary event [32] simply because it happens rarely over time and space. In the spring of 2016, sporadic flowering in groves of *Dendrocalamus strictus* was observed in Ranchi which later on was found to be widespread over several districts (*viz.*, Ramgarh, East Singhbhum, Lohardaga and Khunti) around Ranchi during March to May 2016. Seeds were collected from Bargawaan village in Namkum Community Development Block in Ranchi.

3.1. Germination

All priming treatments were found better than control in terms of effect on germination (Figs. 1 & 2). Seeds primed with KNO_3 1% solution increased the germination from 48.40% (in control) to 87.30% (a rise of 80.40%) followed by hydropriming for 16 h which raised the germination from 48.40% to 83.80% (*i.e.*, a rise of 73.10%). While KNO_3 1% solution resulted in a rise of 80.40% in germination, KNO_3 0.5% solution gave a rise of 52.10%. Hydropriming for 16 h increased the germination by 73.10% while the same priming treatment for duration of 8 h increased the germination by 75.00%. Similar effects of hydropriming on the germination rate has been observed with *Acacia tortilis* [24] and *A. coriacea* [30]. GA_3 100 ppm solution enhanced the germination by 41.70% while GA_3 250 ppm enhanced the germination by 18.40% only. Ethrel 500 ppm solution was able to raise the germination by 59.90% while ethrel 200 ppm raised the germination by 34.70% only. Solid matrix priming for 8 h duration was more effective than 16 h duration. The shorter duration increased the germination by 56.20% *i.e.*, from 48.40 to 75.60% while, priming in solid matrix for longer duration resulted in increase of 42.36%.

When seed coats were manually removed, there was cent per cent germination irrespective of the treatment. However, removal of seed coat resulted in significant difference in parameters like early germination, uniformity of emergence, seedling dry weight between treatments. When seeds were germinated with their seed coats intact, the total seedling length was more in all corresponding priming treatments as well as in control. Similarly, seedling dry weight was found to increase by all priming treatments as compared to control. Further, it was observed that by removing the seed coat although the germination and total seedling length increased, there was a decrease in seedling dry weight except in case of solid matrix priming for 8 h and priming with 500 ppm solution of ethrel.

3.2. Speed of germination

Soaking of seeds in 100 ppm solution of Gibberellic acid (GA_3) for 8 h resulted in most rapid germination when seeds were germinated under

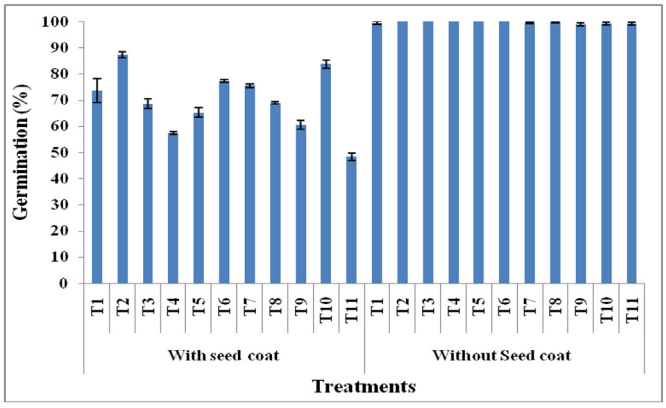

Fig. 1. Effect of different priming treatments on percent germination in seeds of *D. strictus*.

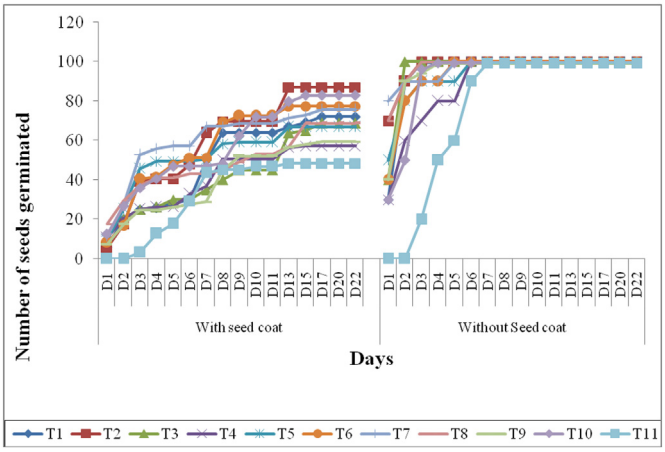

Fig. 2. Effect of different priming treatments on number of seeds germinated under laboratory conditions.

field condition in polybags, with as well as without seed coat (Table 2). This treatment, in terms of speed of germination was followed by priming of seeds with 1% KNO_3 solution for 8 h which in turn was followed by hydropriming of seeds for 16 h for both conditions *i.e.*, with as well as without seed coat. In laboratory conditions, the response was different from that in field conditions. Fastest germination was observed when Solid matrix priming treatment was given to seeds for 8 h, with seed coat intact as well as when seed coat was removed. Under laboratory conditions, it was found that when seed coats were removed, solid matrix priming for 16 h and seed priming for 8 h with KNO_3 1% solution produced similar results. It was also observed that mean time taken in germination was lesser when seed coat was removed. However, interestingly, it was observed that, in polybags *i.e.*, under field conditions, germination was faster when seed coat was intact.

Table 2
Summary of different priming treatment's effects on Mean speed of germination.

Sub-treatments	Mean speed of germination (in days)		
	Priming Treatments	Under laboratory conditions	Under field conditions
With seed coat	T1	5.90	10.06
	T2	5.80	7.56
	T3	6.84	7.30
	T4	5.19	8.52
	T5	4.16	9.74
	T6	4.86	9.34
	T7	4.00	9.06
	T8	5.31	9.16
	T9	5.93	11.96
	T10	5.72	8.27
	T11	6.00	13.58
	C.D.	0.598	0.862
	SE(m)	0.201	0.29
	SE(d)	0.285	0.41
	C.V.	6.424	5.289
Without Seed coat	T1	1.49	11.15
	T2	0.70	9.50
	T3	1.20	9.37
	T4	2.50	11.35
	T5	1.50	10.71
	T6	1.60	10.86
	T7	0.68	10.74
	T8	0.69	10.77
	T9	1.33	11.33
	T10	1.92	9.59
	T11	4.78	11.91
	C.D.	0.458	0.697
	SE(m)	0.154	0.235
	SE(d)	0.218	0.332
	C.V.	15.947	3.814

3.3. Seedling Length

As far as the effect of priming treatments on seedling growth, ethrel 500 ppm solution was found to reduce the total seedling length (Table 3). In this case a significant reduction in root length was observed while there was an increase in shoot length as compared to control. In all other treatments, there was an increase in root length as well as shoot length and consequently in total seedling length. Increase in shoot length and decrease in root length was observed when primed with GA_3. GA_3 treatment was given with two concentrations *viz.*, 100 ppm solution and 250 ppm. This trend of response with respect to seedling shoot and root length was observed with both concentrations. The total seedling length was highest as a result of priming with GA_3 100 ppm. The length of seedlings produced from seeds treated with KNO_3 1% solution was next to GA_3 100 ppm. In some earlier work, Gibberellin has been reported to have stimulatory effects on respiration in cucumber seeds and seedlings [14,20]. Exogenous GAs are thought to stimulate germination through promotion of hydrolytic and proteolytic enzyme activity, which acts to mobilize food reserves in the cotyledons or endosperm [1].

3.4. Shoot: root length

Highest shoot: root length ratio was produced by 100 ppm GA_3 priming treatment (Fig. 3). As compared to 0.64 in control without seed coat, it increased the shoot: root length ratio to 1.57 and compared to 0.79 in control with seed coat intact it increased it to 1.55. In dehusked seeds, all priming treatments resulted in increased the shoot:root length ratio while in seeds with seed coat intact, two treatments resulted in reduction in ratio of shoot: root length *viz.*, 0.5% KNO_3 and SMP for 16 h. The SMP for 8 h had no effect on shoot: root length while rest of the treatments resulted in increased shoot: root length ratio.

3.5. Leaf length/width

In seeds with seed coat removed, it was found that leaf length and width were significantly affected by priming treatments (Table 4). Priming with KNO_3 at both concentrations *viz.*, 0.5% and 1.0% resulted in increased leaf length (37.35 mm and 34.93 mm, respectively) as compared to control (29.45 mm). Another priming treatment which resulted in remarkable increase in leaf length was solid matrix priming for both durations *viz.*, 8 h and 16 h (37.75 mm and 36.31 mm). Interestingly, GA_3 at 100 ppm for 8 h reduced the leaf length significantly

Table 3
Summary of different priming treatment's effects on shoot length and root length.

Treatments	Shoot length (mm)		Root length (mm)	
	With seed coat	Without seed coat	With seed coat	Without seed coat
T1	58.80	56.81	87.47	47.83
T2	78.09	60.53	66.09	57.54
T3	89.30	52.50	57.79	33.38
T4	86.29	34.37	50.71	34.94
T5	72.04	51.62	70.99	37.24
T6	51.44	37.31	40.35	50.50
T7	62.91	51.65	79.21	60.88
T8	53.05	57.31	72.88	56.75
T9	62.42	43.26	58.63	56.42
T10	60.58	42.31	66.29	43.80
T11	42.28	29.31	53.48	45.81
SE(m)	4.69	1.21	5.70	1.79
C.D. for treatments (A)	15.14	3.94	19.01	5.71
C.D. for seed coat factors (B)	10.83		13.75	
C.D. for B at same level of A	11.11		12.59	
C.D. for A at same level of B	10.94		13.92	

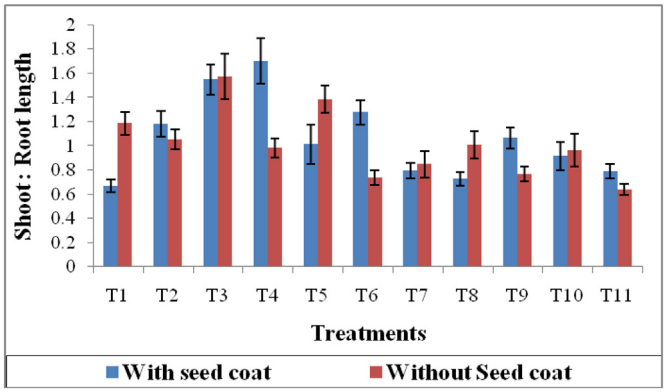

Fig. 3. Effect of different priming treatments on Shoot: Root length in seeds of *D. strictus*.

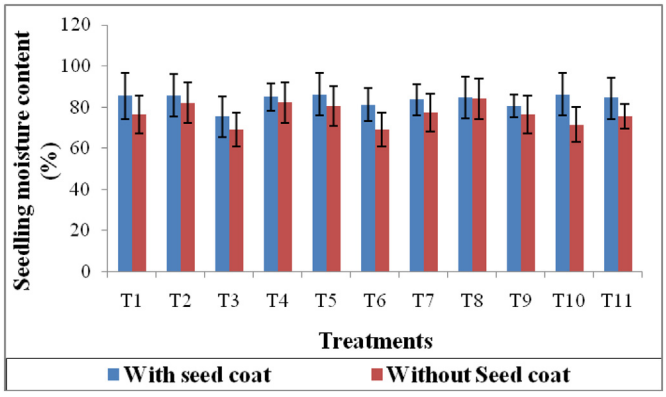

Fig. 4. Effect of different priming treatments on seedling moisture content.

(20.92 mm) while at 250 ppm it had no significant effect on leaf length. Similarly, leaf length was also found to decrease by treatments with 200 ppm and 500 ppm ethrel (25.10 mm and 21.49 mm, respectively). The leaf length in unprimed control with seed coat intact and seed coat removed.

3.6. The seedling moisture content

The seedling moisture content in case of different priming treatments were found to exhibit significant differences (Fig. 4). In case of unprimed control with seed coat removed it was 75.53%. In case of hydropriming for 16 h it was 71.43%, with 100 ppm GA_3 it was 68.93 and 500 ppm ethrel it was 69.16%. On the other hand, in case of seed coat intact treatments, in control it was 84.37% while for 100 ppm GA_3 it was 75.28% and 500 ppm ethrel it was 81.12%. Seeds with seed coat removed and hydroprimed for 16 h and those with seed coat intact hydroprimed for 8 h also exhibited less seedling moisture content (71.43% and 80.52% respectively) as compared to their corresponding controls (75.53% and 84.37%, respectively).

3.7. Dry matter content of seedlings

In general there is clear cut effect of seed coat removal on dry matter content of seedlings (Fig. 5), When seed coat is removed, the dry matter content of seedlings is increased in terms of percent of total seedling fresh weight. 100 ppm GA_3 treatment in both seed coat removed and seed coat intact seeds produced seedlings with highest dry matter content (31.07% and 24.72% respectively). Similarly seed priming with 500 ppm ethrel also resulted in seedlings with high dry matter content (30.84% and 18.88%, respectively). The dry matter content of untreated control of seeds with seed coat removed and seed coat intact was 24.47% and 15.63%, respectively.

Table 4
Summary of different priming treatment's effects on leaf length and leaf width.

Treatments	Leaf length (mm)		Leaf width (mm)	
	With seed coat	Without seed coat	With seed coat	Without seed coat
T1	34.26	37.31	8.85	6.81
T2	28.73	34.94	6.99	7.00
T3	33.49	20.94	5.57	2.25
T4	27.65	26.81	3.20	6.81
T5	26.19	25.06	6.61	5.50
T6	18.65	21.50	3.84	3.94
T7	36.17	37.75	8.16	6.94
T8	30.06	36.31	7.16	6.50
T9	32.30	32.31	7.92	6.25
T10	35.37	27.50	7.72	4.50
T11	26.07	29.44	5.17	6.38
SE(m)	2.47	0.66	0.43	0.21
C.D. for treatments (A)	7.75	2.43	1.38	0.68
C.D. for seed coat factors (B)	5.62		1.07	
C.D. for B at same level of A	5.84		1.13	
C.D. for A at same level of B	5.69		1.04	

3.8. Effect of removing seed coat

Root length in general was greater in seeds with seed coat intact (mean 63.99 mm) as compared to dehusked seeds (mean 43.73 mm). Similarly, higher shoot length (mean 65.20 mm) was recorded in seeds with seed coat intact in comparison to seed coat removed (mean 47.00 mm). As a result the total seedling length was also more in seeds with seed coat intact. Although, not much difference was observed in seedling leaf length when seed coat was intact but leaf width was greater when the seed coat was intact. The seedlings with seed coat intact retained higher moisture content (measured in percent) as against the seeds when seed coat was removed. The seedlings were found to accumulate more of dry matter when germinated (Table 5) after removing the seed coat (mean value 0.175 g/10 seedlings for dehusked seeds and 0.135 g/10 seedlings for seeds with intact seed coat). Finally and most importantly, the seeds when germinated after removing seed coat gave 100% germination in all treatments as well as untreated control. In certain cases, the factors inhibiting germination as in *Poaceae* species are located in the embryo, but may also be found in the grain-coat layers [8]. The results of this study also suggest presence of germination inhibiting principals in the seed coat in this case also. The author [9] has explained that the effect of chemical scarification on *Leymus chinensis* seed might be due to oxidation of germination inhibitors, a non-enzymatic oxidation reaction [25] or corrosion of the

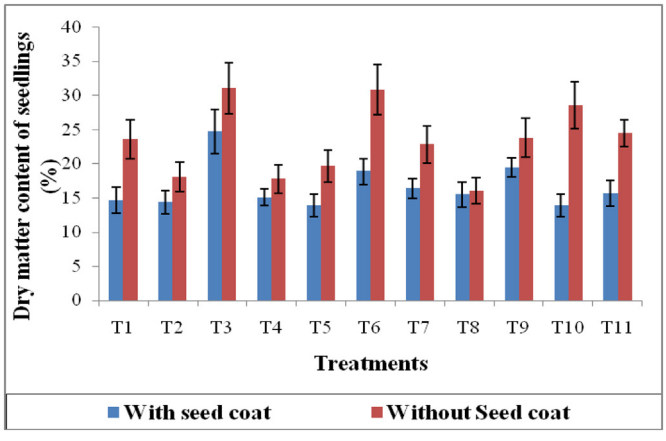

Fig. 5. Effect of different priming treatments on dry matter content of seedlings.

Table 5
Summary of different priming treatment's effects on seedling dry weight.

Treatments	Seedling dry weight (g)	
	With seed coat	Without seed coat
T1	0.175	0.105
T2	0.175	0.110
T3	0.240	0.160
T4	0.175	0.125
T5	0.185	0.120
T6	0.160	0.165
T7	0.155	0.170
T8	0.170	0.110
T9	0.175	0.145
T10	0.165	0.150
T11	0.160	0.115
SE(m)	0.010	0.005
C.D. for treatments (A)	0.025	0.015
C.D. for seed coat factors (B)	0.020	
C.D. for B at same level of A	0.020	
C.D. for A at same level of B	0.020	

distal margin of the lemma [15], which increased inhibitor diffusion, gas exchange or water.

3.9. Overall effect of treatments

In terms of germination, seed priming with 1.0% KNO_3 was found the best treatment (87.30% germination) followed by hydropriming for 16 h (83.80% germination). Bamboo (*D. strictus*) seeds give 100% germination even without any treatment when germinated in laboratory after removing seed coat (Fig. 1). Therefore, on small scale *i.e.*, for germinating a few seeds for any purpose simply removing the seed coat before keeping for germination can be recommended. But on commercial scale, where removing the seed coats is not possible and the seeds with seed coats intact and without any treatment give only 48.40% germination in laboratory conditions and only 9.50% germination in field conditions (Fig. 6).

4. Conclusion

Germination is the best and ultimate manifestation of seed quality. Therefore, it is a must to give some priming treatment when germinating seeds with seed coat intact. The KNO_3 1.0% solution, in addition to highest germination percent (87.30%) also gives higher seedling length (144.17 mm) next only to 100 ppm GA_3 solution (147.30 mm). GA_3 100 ppm solution although produces seedlings with highest seedling length, it gives a germination percent of 68.60% only. When seedlings germinated in laboratory were transferred to soil, all seedlings from all treatments established successfully without any mortality whatsoever. Hence, seed priming with 1.0% KNO_3 solution can be recommended for germination of bamboo (*D. strictus*) seeds on commercial scale.

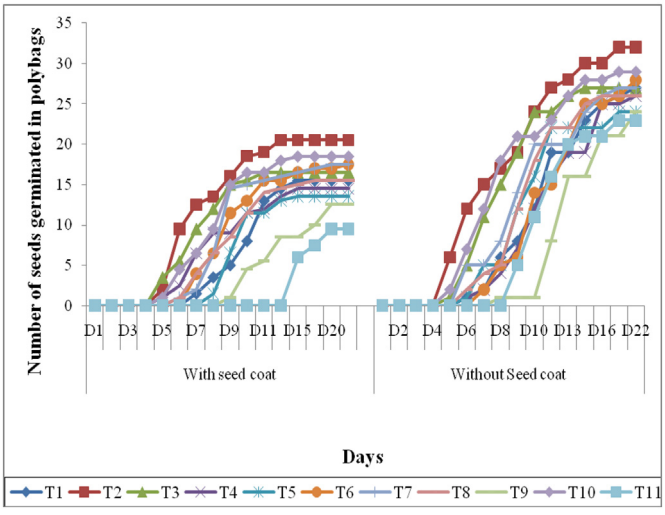

Fig. 6. Effect of different priming treatments on mean germination time of seeds germinated under field conditions.

Contribution of authors

P. K. Sarkar and B. P. Bhatt: Formulation of research work, planning and collection of seeds.
P. R. Kumar and A. K. Singh: Preparing seed priming solutions, treatment of seeds.
P. K. Sarkar and P. R. Kumar: Taking observations in field.
P. R. Kumar and A. K. Singh: Taking observations in laboratory.
P. K. Sarkar and B. P. Bhatt: Tabulation of data, and analysis of data.
All authors: Preparation of manuscript.

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