Borneo Journal of Resource Science and Technology (2026), 16(1): 105-113
DOI: https:doi.org/10.33736/bjrst.10072.2026

Enhancing Germination Efficiency in *Dalbergia latifolia* Roxb. (Fabaceae) through Seed Pre-treatment

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Received: 29 June 2025 Accepted: 02 December 2025 Published: 30 June 2026

ABSTRACT

Dalbergia latifolia, commonly known as Indian rosewood, is found in the Terai and distributed up to 1000 m in the Chure region of Nepal. This slow-growing species is threatened by overexploitation and illegal logging for its timber. This research was carried out to examine different seed treatment methods for the germination of *D. latifolia*. The primary objective of our research was to investigate the effect of various treatments and determine the most effective seed treatment method for enhancing seed germination of this species. The fallen pods from mother trees at Thulo Garduwa, Sindhuli, were collected in March. From the sun-dried fruits, the seeds were manually extracted and treated with water, 300 ppm Gibberlic Acid (GA_3), Sodium Hydroxide (NaOH) and Potassium Hydroxide (KOH). The treated and untreated (control) seeds were sown in completely randomized designed (CRD) plots inside the greenhouse. The germination data was recorded and analyzed. The germination parameters, like germination percentage (GP), total length of seedlings, vigor index (VI), germination rate (GR) and mean germination time (MGT), were calculated from the collected data. The result indicated that the seed treated with 300 ppm GA_3 showed the best germination with higher GP (53.67), total length (18.16 cm), VI (976.46), GR (6.94) and shorter MGT (8.01 days). Similarly, the least germination was given by KOH with GP (31.33) and GR (3.11), however, minimum seedling length (12.74 cm) and VI (557.62) were observed for the control. Surprisingly, KOH showed the best performance after GA_3 for seedling length. In conclusion, *D. latifolia* seeds possess low to moderate physiological dormancy and treatment with 300 ppm GA_3 significantly improved both germination performance and seedling growth.

Keywords: *Dalbergia latifolia*, seed germination, gibberellic acid, seed treatment

INTRODUCTION

Dalbergia latifolia Roxb. (Fabaceae) which is commonly known as East Indian rosewood is one of the most valuable timber producing species in the Indian sub-continent. This species is naturally distributed in tropical region of Nepal, India, Indonesia and Malaysia (Praciak *et al.*, 2013). However, large scale plantations have also been established across Kenya, Myanmar, Nigeria, Philippines and Vietnam (Orwa *et al.*, 2009). It is well known for producing very hard and durable wood with a long straight bole (Kumar & Chavan, 2018). A significant source of lumber, trees typically reproduce by seed and sporadically by root suckers (Sukhadiya *et al.*, 2020). Wild subpopulations of *D. latifolia* are heavily overexploited due to the valuable timber they offer, with illegal felling adding considerable pressure on them (Groves & Rutherford, 2016). Ultimately, this species has been listed in Appendix-II of CITES and also included in the vulnerable category of the IUCN Red List of Threatened Species (Damaiyani & Prabowo, 2019).

The natural habitat of *D. latifolia* is rare on a global scale, yet it is renowned worldwide for its contributions to the guitar industry, as well as its diverse uses in furniture, plywood, veneer and carved wood products. It is regarded as an iconic material in the production of guitars because of its distinctive tonal qualities and lovely wood finishing (Arunkumar *et al.*, 2022). It is used in the traditional medical systems to cure a variety of illnesses, including diarrhea, leukoderma, dyspepsia and dysentery (Khare, 2008; Kazembe *et al.*, 2012).

Conventional propagation of *D. latifolia* is only through seeds; however, its success is limited due to poor seed viability (Swamy *et al.*, 1992). When mature, seeds are uniformly shaped, brown in color and weigh somewhere between 20,000 and 40,000 seeds per kilogram. Despite their conventional nature, seeds cannot be kept in storage for extended periods of time. Germination frequently declines from 50 – 75% to 30 – 40% after 9 – 12 months (Sukhadiya *et al.*, 2020). According to Kumar and Chavan (2018), seed soaking in tap water for 12 hours has a deep and significant impact on seed germination. According to Sukhadiya *et al.* (2020), seeds should be dried to a low moisture content of about 8% prior to storage in airtight containers. The species grows best in deep, moist, well-drained soils and is commonly found on fertile loam with a pH range of 5.5 to 7.5, representing moderately acidic to slightly alkaline conditions.

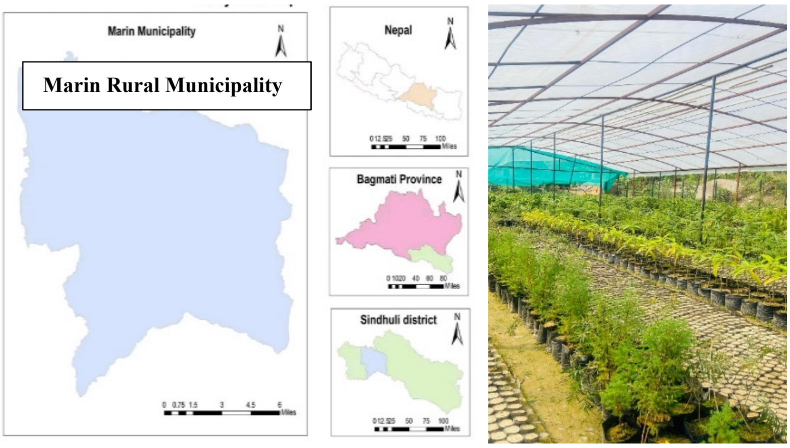
The tree species was previously widely dispersed, but due to excessive habitat exploitation, deforestation, forest conversion for agriculture, illegal logging and the invasion of alien plant species, its regeneration and establishment in natural environments have been drastically reduced (Kumar & Chavan, 2018; Mahatara *et al.*, 2021). For large scale plantation, seedlings of *D. latifolia* are raised in nurseries. Seedlings grown in nursery beds and polyethylene containers were found to be highly susceptible to various diseases (Sehgal, 1983), which resulted in significant mortality and negatively impacted their overall health and vigor (Harsh *et al.*, 1992). A new seedling die-back disease of *D. latifolia* was observed at Jabalpur in Central India (Verma *et al.*, 2016).

Although numerous tree planting techniques exist, species like *D. latifolia* face challenges due to their slow growth and difficulty in establishment. This highlights the need to standardize nursery practices to ensure the production of high-quality planting material. *D. latifolia*, is one of the threatened species in Nepal and is currently receiving limited conservation attention. Direct seeding is a common method of propagating many woody species; however, seed dormancy often reduces production efficiency. According to Baskin and Baskin (2004), seeds are categorized into five dormancy classes, among which physical and physiological dormancy are the most frequent causes of reduced germination success. These physiological and physical dormancy can be overcome through scarification, acid and alkali treatments, moist or warm scarification and the use of plant growth regulators (Baskin *et al.,* 2004; Ghosh & Maiti, 2014; Koutouan-Kontchoi *et al.,* 2020). Although acid scarification is commonly used in woody plants to break physical dormancy, there are limited studies of using strong alkaline treatments like KOH and NaOH. However, studies in non-woody species like *Stipa bungeana* (Hu *et al.,* 2014), *Carica papaya* (Rodríguez *et al.,* 2020) and *Kobresia* spp. (Wang *et. al.,* 2022) show that NaOH treatments can significantly promote germination. The findings indicate that alkaline scarification could serve as a potential alternative to acid treatments for woody seeds. Research on the effects of pre-sowing seed treatments is essential to improve germination parameters, which can enhance seedling production for plantation forestry. This not only supports the timber industry but also contributes to the conservation of species by promoting sustainable management and preserving its genetic diversity. Accordingly, this study investigated the effects of various pre-sowing treatments, including NaOH and KOH, on the germination of *D. latifolia* by assessing multiple germination parameters. In addition, we aimed to identify the most effective treatment among the tested methods.

MATERIALS AND METHODS

Study Area

The experiment was carried out in a nursery at the Division Forest Office, Marin, Sindhuli, Nepal, located at 27°10′32.16″N latitude and 85°56′30.84″E longitude (Figure 1). The site experiences a humid subtropical climate with dry winters, characterized by an annual mean temperature of 30.71 °C, which is 8.71% higher than the national average. On average, the area receives 147.1 mm of rainfall annually, spread over approximately 141.61 rainy days, accounting for about 38.8% of the year.



(A) (B)

Figure 1. Map of study area (A), plastic house where experiment was done (B)

Seed Collection of *D. latifolia*

Seeds of *D. latifolia* are orthodox in nature and viability normally reduces after six months of storage. The pods are brown in color and up to 15 cm long, 1.5 – 2.5 cm wide with pointed ends. The pods are indehiscent in nature and each pod contain 1– 4 seeds. Seeds are 6 – 7 mm long and 4 – 5 mm wide, smooth, shiny brown to black and flat. Seed weight is variable with an average of 20,000-40,000 seeds per kg. Mature pods that had naturally fallen from mother trees at Thulo Garduwa, Sindhuli, were collected in March 2023. The pods were sun-dried for several days and once adequately dried, were manually cracked open to extract clean seeds for treatment.

Seed Treatment

The seeds were subjected to pre-sowing treatments using different methods. The major treatments applied in this study were as follows: T1 = Control (no pre-sowing treatment); T2 = Tap water treatment (soaking seeds in tap water for 12 hours); T3 = Gibberellic acid treatment (soaking seeds in 300 ppm GA_3 solution for 12 hours); T4 = Soaking in 0.5 M KOH for 1 hour; and T5 = Soaking in 0.5 M NaOH for 1 hour.

Preparation of Soil Beds/Experimental Plots

Separate experimental plots were established for each treatment, with each plot filled with a 2:1:1 mixture of soil, sand and farmyard manure (FYM). A total of 100 seeds were sown per plot and each treatment, including the control, was replicated three times—amounting to 300 seeds per treatment. In total, 15 plots were prepared. Plot placement within the nursery beds followed a Completely Randomized Design (CRD) (Table 1). All treatments were scheduled to ensure uniform sowing time across the experiment.

Table 1. CRD layout with equal replication

T1	T5	T3
T5	T3	T5
T4	T1	T2
T2	T4	T1
T3	T2	T4

Data Collection

All plots were uniformly managed, receiving equal amounts of manure, irrigation and weeding. Data collection began on the 5th day after sowing and continued until the final seedling count was recorded for each treatment. Germination was monitored daily up to the 21st day, as repeated seedling emergence was observed beyond the 18th day. Seedling lengths were measured from samples collected from each treatment and documented for further analysis.

The collected data regarding different germination parameters such as rate of germination, germination percentage, length of seedling, seedling vigor index and mean germination time with different treatment and with the control was compared.

Data Analysis

The data collected during an experiment was recorded in an excel and is analyzed using IBM SPSS (Statistical Package for Social Science) for an ANOVA test at 5% level of significance for each germination parameters and multiple comparison using Least Significant Difference (LSD). Given formula were used to calculate the value of germination parameter;

1. Germination percentage (GP): the number of germinated seeds as a percentage of the total number of the tested seeds (Tanaka-Oda *et al*., 2009).

$$GP = \frac{\text{Germinated seed}}{\text{Tested seeds}} \times 100 \quad \text{Eq. (1)}$$

2. Vigor index (VI) in terms of length were determined as per formulae given by Abdul-Baki and Anderson (1973).

$$VI = \text{Seedling length} \times \text{Gemination} \quad \text{Eq. (2)}$$

3. Mean germination time (MGT) given by (Ellis & Roberts, 1981)

$$MGT = \sum \frac{(n \times d)}{N} \quad \text{Eq. (3)}$$

Where, n = No. of seeds germinated on each day; d = No. of days from the beginning of the test; N = Total no. of seeds germinated at the termination of the experiment.

4. Germination Rate (GR): Average number of seed germinated over given interval of time given by (Awasthi, 2016).

$$GR = \sum \frac{Gt}{Dt} \quad \text{Eq. (4).}$$

Where, Gt = The number of the germinated seed on day t; Dt = The time corresponding germination period.

RESULTS

Germination Percentage of *D. latifolia* with Different Treatments

The percentage of germination was significantly affected by the applied treatments ($p \leq 0.01$). Among the five treatments applied to the *D. latifolia* seeds, gibberellic acid showed the highest germination percentage (53.67%) followed by the water (46.33%) and control (40.33%). The lowest percentage of germination was observed in the seeds treated with NaOH (37.33%) and KOH (31.33%) (Figure 2A).

Germination Rate

The germination rate was positively influenced by the applied treatments ($p \leq 0.01$) except KOH and NaOH. The result showed that the germination rate was higher for GA_3 treated seeds (6.94) followed by tap water-soaked seeds (5.54) and control (4.79). Whereas the lowest germination rate was found for KOH treated seeds (3.11) followed by NaOH treated seeds (4.50) (Figure 2B).

Mean Germination Time

The mean germination time was also positively affected by different germination parameters ($p \leq 0.01$) (Table 2). The seeds germinated in controlled environment showed higher time for germination (11.06 days) whereas the seeds treated with GA_3 germinated quickly in 8.01 days followed by the seeds soaked in tap water (8.86 days) (Figure 2C).

Seedling Length

Seed treatment had a significant effect on seedling growth, as evidenced by the differences in mean seedling length among treatments ($p \leq 0.01$) (Table 2). The greatest seedling length was recorded in GA_3-treated seeds (18.16 cm), followed by KOH treatment (17.83 cm) and tap water soaking (17.14 cm). In contrast, the shortest seedlings were observed in the control (12.74 cm), with NaOH treatment yielding slightly longer seedlings (16.28 cm) (Figure 2D).

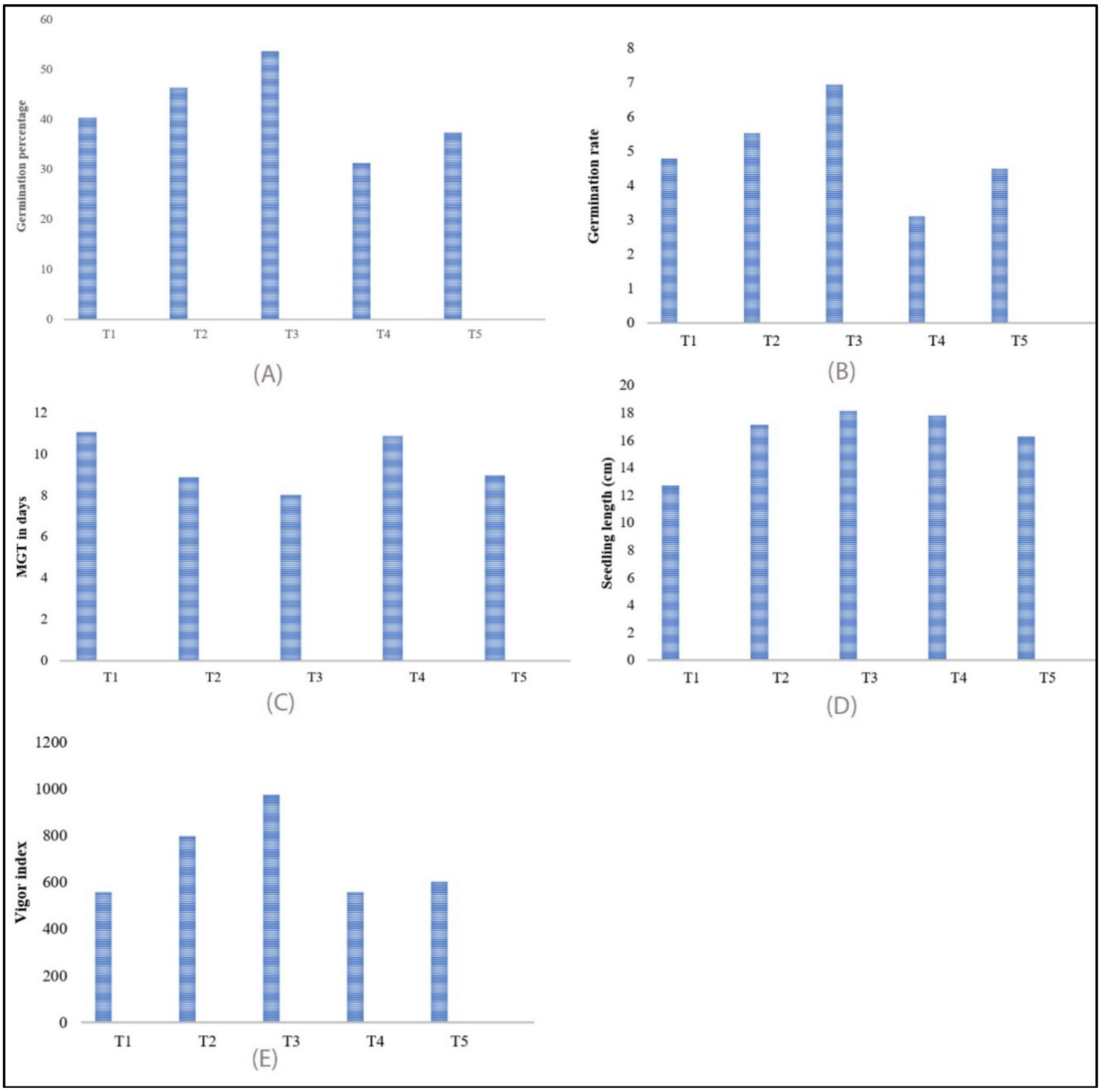


Figure 2. Germination parameters against different treatments on *D. latifolia* seeds. (T1 = control; T2 = tap water; T3 = gibberellic acid; T4 = KOH; and T5 = NaOH)

Table 2. ANOVA of the effect of different treatments on various germination parameters

Germination parameter	F–value	*p*–Value
Germination percentage	6.593	0.007**
Mean germination time	10.089	0.002**
Seedling length	11.102	0.001**
Vigor index	9.245	0.002**
Germination rate	8.651	0.003**

** $p<0.01$

Seedling Vigor Index

The higher vigor index was obtained with GA_3 treated seeds (976.46) followed by tap water-soaked seeds (794.88) and NaOH treated seeds (606.11), whereas the lowest vigor index was obtained with control (557.62) followed by KOH treated seeds (557.69) (Figure 2E). The overall vigor index was positively affected by the applied treatment parameters ($p \leq 0.01$).

Table 3. Multiple comparison of different treatments for germination parameters

Treatment (I)	Treatment (J)	*p* value				
		GP	MGT	GR	Length	VI
T1	T2	0.232	0.906	0.292	0.001*	0.019*
	T3	0.018*	0.084	0.01*	.000*	0.001**
	T4	0.085	0.002**	0.032*	.000*	0.999
	T5	0.539	0.925	0.678	0.009*	0.583
T2	T3	0.151	0.102	0.066	0.232	0.063
	T4	0.01*	0.002**	0.005**	0.372	0.019*
	T5	0.085	0.832	0.155	0.214	0.049*
T3	T4	0.001**	0.000***	0.000***	0.742	0.001**
	T5	0.006**	0.071	0.005**	0.026**	0.001**
T4	T5	0.232	0.002**	0.067	0.047*	0.584

* $p<0.05$; ** $p<0.01$; *** $p<0.001$.

DISCUSSION

This study investigated the effects of various pre-sowing treatments on the seed germination of *Dalbergia latifolia*, an ecologically and economically important species of Nepal's Terai region. Five treatment methods were tested, each using 100 seeds with three replications, totaling 300 seeds per treatment. The results revealed that different pre-sowing treatments had a significant impact on the germination parameters of *D. latifolia*, with GA_3 proving to be the most effective.

Among the treatments, GA_3 resulted in the most favorable outcomes, including the highest germination percentage (53.67%), longest mean seedling length (18.16 cm), highest vigor index (976.46) and greatest germination rate (6.94), as well as the shortest mean germination time (8.01 days). A study conducted on the same species in India reported that seeds soaked in tap water for 12 hours exhibited a higher germination percentage (70.00%), germination rate (5.11), seedling length (19.66 cm) and vigor index (1376), followed by treatment with 300 ppm GA_3, which yielded a germination percentage of 59.17%, germination rate of 4.24, seedling length of 17.42 cm and vigor index of 1032 (Kumar & Chavan, 2018). In our study, tap water was the second most effective treatment, following GA_3, demonstrating variations in seed response to different pre-sowing treatments. This may be due to because simple imbibition initiates metabolic activity, leaches inhibitory substances and promotes embryo growth. A study on *Prunus mahaleb* by Al-Absi (2010) found that GA_3-treated seeds exhibited the highest germination rate and percentage. Similarly, research on *Zanthoxylum armatum* in Jordan showed that GA_3 effectively enhanced germination rate and percentage, but only after an extended stratification period (Datt *et al.,* 2017). These contrasting results across different or even the same species may be attributed to variations in seed collection locations and inherent seed dormancy characteristics of species. The enhanced germination observed in GA_3-treated seeds could be due to its growth-stimulating effects, potentially linked to the depression of GAI/RGA proteins, which regulate growth and its role in improving photosynthetic efficiency through the accumulation of photosynthates (Richards *et al.,* 2001).

The lowest germination rate, germination percentage and vigor index were recorded in seeds treated with NaOH and KOH. Similar findings were reported by Nagaveni *et al.* (1988) and Karam & Salem (2001), who observed reduced germination rates in *Santalum album* (sandalwood) and *Arbutus andrachne* seeds, respectively, when treated with 0.5% NaOH and KOH. These results are consistent with our study, further highlighting the inhibitory effects of these treatments on seed germination. However, a study on *Syzygium cumini* showed contrasting results, where germination percentage and shoot length increased with NaOH and KOH soaking times of up to 10 and 15 minutes, respectively, before declining (Gaikwad & Borkar*,* 2017). Seeds treated with KOH exhibited greater seedling length, which may be attributed to increased water uptake compared to untreated seeds. The alkaline solution partially softened the seed coat, improving water uptake and leaching inhibitory compounds. However, the lower germination percentage observed in KOH-treated seeds may be attributed to its toxic effects (Gaikwad & Borkar, 2017), as strong bases can penetrate excessively and disrupt physiological processes during seedling development. In contrast, a study on *Meizotropis buteiformis* by Gaisamudre & Dhabe (2011) found that KOH and NaOH treatments resulted in the highest germination percentages, with KOH achieving 85.2% and NaOH 72.2%. These findings differ from our study, where KOH and NaOH treatments led to lower germination percentages. This variation could be attributed to the duration of seed exposure to chemical treatments. Additionally, the type and concentration of chemical treatments play a crucial role in seed germination. For example, *Tectona grandis* seeds exposed to a combination of sun drying for five days followed by soaking in 2% KOH for twelve hours achieved a higher germination rate of 39% (Masilamani *et al.,* 2012). However, germination percentage declined as KOH concentration increased, with complete inhibition observed at 8% of KOH. A similar trend was noted in NaOH-treated teak drupes, where 2% NaOH resulted in only a 17% germination rate, with no significant effect on germination beyond this concentration (Masilamani *et al.,* 2012).

The findings of this study emphasize the role of chemical pre-treatments, as both KOH and NaOH have been shown to influence germination by breaking seed dormancy and promoting seedling growth which was also demonstrated by Spyroglou & Radoglou (2017) in *Phillyrea latifolia*. However, excessively high concentrations can have inhibitory or toxic effects, a pattern observed in both our study and previous research. While GA_3 treatment combined with warm stratification enhanced germination in *P. latifolia* (Spyroglou & Radoglou, 2017), our study demonstrated a more significant impact of GA_3 on seed germination, indicating that its effectiveness varies by species and depends on optimal concentration levels. These variations emphasize the importance of selecting appropriate pre-treatment methods based on the specific dormancy mechanisms of different plant species.

CONCLUSION

In summary, *D. latifolia* seeds demonstrate low to moderate physiological and physical dormancy and 300 ppm GA_3 treatment serves as the most effective method for improving germination and subsequent seedling vigor. In contrast, KOH treatment was less effective in terms of germination percentage and germination rate but performed better in seedling length, vigor index and mean germination time than the control. Notably, KOH ranked second only to GA_3 in promoting seedling length. Alongside selecting the optimal seed treatment for improved seed germination, it is essential to monitor and evaluate the seedlings produced from treated seeds until they are fully established. Finally, further experiments involving different treatments, varying concentrations and seed soaking durations could be conducted to identify the most effective treatment for enhanced performance for this species.

ACKNOWLEDGEMENT

The authors would like to thank nursery co-worker Ms. Ganga Kuikel and Mr. Prakash Bajagai for their valuable support and help during seed bed preparation.

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