Comparative study of various pretreatment on seed germination of *Dalbergia cochinchinensis*

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**ABSTRACT**

*Dalbergia cochinchinensis* belongs to family Fabaceae, is a hardwood tree species listed as vulnerable on the IUCN Red List of Threatened Species. This species is a slow-growing and it is threatened by overexploitation for its timber and illegal logging. Because of the increased demand for the timber, the species is rapidly disappearing in many habitats and threatened to extinction in the near future. Prevention of illegal logging and conservation efforts are required to protect the species. Seed germination and seedling growth is a critical step for the abiding conservation of plant germplasm. An experiment was to find out appropriate pre-sowing treatment for maximizing the germination. Eighteen pre-sowing treatments were used for both the seeds sown on moist filter paper and soil. Data was collected daily for calculated germination percentage, mean germination time, mean germination rate and growth performance. Results revealed that hot water treatment was effective to increase the germination rate comparison to the control and other treatments such as cold treatment and scarification. Significantly higher germination percentage (34.4%) and mean germination rate (0.17) were recorded in seeds were soaked in hot water 70 °C for 1 min (T1). Beside, seedling growth such as number of leaves, leaf length and seedling height originated from hot water treatment performed significantly higher than others. The results indicated that seeds treated with hot water had profound and significant impact on seed germination and growth of *D. cochinchinensis*.

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needed to provide information for mass production of seedlings and avoidable of waste of seed consumption. Therefore, the purpose of this study was to find out the appropriate pretreatments that maximize total germination and effect on seedlings growth. The information from this study will facilitate nursery operations.

Material and method

Plant material

Seeds of *D. cochichinensis* were collected from 16 selected plus trees in the Forest Restoration and Development “Bonteay Srey” (1888 ha) that located in Trapeang Tmor village, Khun Ream Commune, Banteay Srey district, Siem Reap province, Cambodia in October 2018. The mature seeds were collected from the dark brown pod. After collection, the pods are dried in the open sun for about three days. Brownish seeds were extracted manually from the pods. Seeds were characterized on size and weight. Morphologically similar seeds were selected for the experiment. The seed weight was determined according to International Seed Testing Association (ISTA) method. The number of seeds per unit weight (gram) is determined on the pure-seed fraction. The seeds were counted by hand with 100 seeds in order not to lose one’s place, then weighed for eight replications separately. The mean of these weights was taken to get average weight of a 1000 seeds. The seeds size such as seed width and length were measured by manual measurement through using calibrate ruler. Ten seeds were selected randomly with four replications for measured. This present study was conducted over a period 4 weeks in laboratory of Department of Forest Resources, Kangwon National University. The observation of the germination period is 2 weeks and growth performance for 2 weeks.

Experimental design and pretreatment

The experiment was laid out in a randomized complete block design (RCBD) and was carried out in series. Seeds were subjected to an 18 sub-treatments of seven main treatments: (1) hot water treatment, (2) cold water treatment, (3) chemical application, (4) plant growth regulator treatment, (5) cold stratification, (6) scarification and (7) control. The detail of pre-sowing treatments are summary in Table 1. For each treatment, healthy seeds were selected randomly. Ten seeds was used in sub-treatments of each treatment with three replication. Germination was performed on moist filter paper in sterilized petri-plate (90 × 15 mm) except scarification treatment (sowed on soil). All cultures were maintained in culture room at 25 °C under white fluorescent tubes and 16/8 (light/dark) photoperiod for germination. The seven main treatments and procedure used in the experiment were:

1. Hot water treatment: Seeds were soak in the hot water 70 °C for 1, 3, 5, 10 minutes and 12 hours.
2. Cold water treatment: Seeds were soak in cold water 12 hours.
3. Chemical application (Sodium hypochlorite, NaOCl): Treatment was used various NaOCl concentration (2, 4, and 6%).
4. Plant growth regulator (Gibberellin Acid, GA3) application: Seeds were soaked in only GA3 or combined with the optimal of hot water treatment in previous experiment. GA3 solution 1 mM was used in experiment.
5. Cold stratification: Seeds were packaged in hermetic paper bag and kept in moist sand then frozen at 4 °C in ordinary freezer 30, 40 and 50 days.
6. Scarification: Seeds were scratched with sand paper for few second and sowed on the soil in the magenta box.
7. Control: The dry seeds were sown without any treatment.

Data recording and germination assessment

The effects of treatment were assessed by daily counting of number of germinated seeds. Germinated seeds were counted and removed from the date of sowing until there was no more germination. A seed was considered to have germinated when the tip of radicle emerged free of the seed coat (Wiese and Binning 1987;
Auld et al. 1988). The parameter of seed germination measurements are germination percentage, time spent to germinated or emerge (mean germination time), and mean germination rate using the formula of (Ranal et al. 2009). Germination percentage (GP) was calculated using the equation: GP = number of germinated seeds/ total number of seeds × 100. MGT (mean germination time) expression by:

$$ t = \frac{\sum_{i=1}^{n} t_i}{\sum_{i=1}^{n} n_i} $$

Where $t_i$: time from the start of the experiment; $n_i$: number of seeds germinated.

Mean germination rate (MGR) is calculated as the reciprocal of the mean germination time:

$$ MGR = \frac{1}{MGT} $$

**Growth determination**

Germinated seedlings were transplanted in pots each, watered once in two days and seedling growth was measured after 2 weeks. Twelve seedlings of each treatment were selected for growth measurement. The growth parameters of seedling in terms of number of leaves and seedling height were recorded.

**Data analysis**

Germination assessment, Germination percentage (GP), and Mean Germination Time (MGT), Mean germination rate (MGR) and growth parameters of seedlings were subjected to analysis the variance (ANOVA) to statistically verify the significant difference of the treatments and the results were classified with Duncan’s multiple range test (DMRT) ($p < 0.05$) by using MS Excel and International Rice Research Institute (IRRI, 2014) program.

**Results**

**Morphology of the seeds of D. cochinchinensis**

Seed size is about 4 mm by 6 mm in dimension, flat in shape and its color is gray or brown (Figure 1). *Dalbergia cochinchinensis* seed is seems impervious to water. The weight of 1000 seeds was found as 24 g.

**Seed germination**

Data presented in Table 2 regarding germination percentage (GP), mean germination time (MGT) and mean germination rate (MGR) showed that there are significant differences between effective treatments on germination characteristics and the different treatments resulted in significant differences among germination properties.

The results of this research showed that seeds were soak in hot water 70°C for 1 min (T1) 34.44% follow by seeds soak in hot water 70°C for 5 (T3) and 10 min (T4) 32.22% as compared to other treatments. The minimum germination percentage was recorded in seeds were scratch and soak in hot water 50°C for 10 min (T16) 3.33% followed by scratch and sow dry seed (T15) 6.67% and cold stratification 50 days (T14) 8.33%. Seed treated by NaOCl and GA3 had neither negative nor positive effect on seed germination of *D. cochinchinensis*.

Among the various treatments scarification treatment (T15 and T16) was decreased day spend to emerge (2 days) after sowing, due to radicles appearance. Other treatments, the average day spend to germinated is 6 to 10 days, and the highest mean germination time was observed in control treatment (12 days).

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**Table 2. Summary of different pretreatments methods effects on germination percentage, mean germination time and mean germination rate in parenthesis of *D. cochinchinensis***

| Treatment | GP (%) | MGT (day) | MGR |
|-----------|--------|-----------|-----|
| T1: HW (1 min) | 34.44<sup>a</sup> | 6.19<sup>cd</sup> | 0.17<sup>a</sup> |
| T2: HW (3 min) | 31.11<sup>ab</sup> | 6.48<sup>bcd</sup> | 0.16<sup>ab</sup> |
| T3: HW (5 min) | 32.22<sup>ab</sup> | 6.19<sup>cd</sup> | 0.16<sup>ab</sup> |
| T4: HW (10 min) | 32.22<sup>ab</sup> | 6.13<sup>cd</sup> | 0.16<sup>ab</sup> |
| T5: HW (12 h) | 30.56<sup>bc</sup> | 7.06<sup>bc</sup> | 0.14<sup>b</sup> |
| T6: CW (12 h) | 25.00<sup>bcd</sup> | 9.76<sup>b</sup> | 0.11<sup>b</sup> |
| T7: NaOCl 2% | 27.50<sup>bcd</sup> | 8.78<sup>b</sup> | 0.12<sup>b</sup> |
| T8: NaOCl 4% | 23.33<sup>bcd</sup> | 8.48<sup>b</sup> | 0.13<sup>bcd</sup> |
| T9: NaOCl 6% | 20.83<sup>bcd</sup> | 9.37<sup>b</sup> | 0.11<sup>bcd</sup> |
| T10: GA3 (12 h) | 13.33<sup>cde</sup> | 7.83<sup>b</sup> | 0.12<sup>bcd</sup> |
| T11: HW 70°C (1 min) + GA3 (12 h) | 16.67<sup>bcd</sup> | 11.13<sup>b</sup> | 0.09<sup>bcd</sup> |
| T12: CS 30 d | 10.00<sup>de</sup> | 10.67<sup>b</sup> | 0.09<sup>bcd</sup> |
| T13: CS 40 d | 10.00<sup>de</sup> | 10.67<sup>b</sup> | 0.10<sup>bcd</sup> |
| T14: CS 50 d | 8.33<sup>de</sup> | 10.00<sup>b</sup> | 0.10<sup>bcd</sup> |
| T15: SD | 6.67<sup>de</sup> | 2.00<sup>d</sup> | 0.05<sup>d</sup> |
| T16: S + HW 50°C (10 min) | 3.33<sup>e</sup> | 2.00<sup>d</sup> | 0.05<sup>d</sup> |
| T17: S + H2O (10 min) | 16.67<sup>bcd</sup> | 7.17<sup)b</sup> | 0.15<sup>bc</sup> |
| T18: Control | 13.33<sup>cde</sup> | 12.00<sup>a</sup> | 0.08<sup>d</sup> |

In each column, mean with same letter(s) are not significantly different at $p < 0.05$, Duncan’s Multiple Range Test (DMRT). GP: germination percentage; MGT: Mean germination time; MGR: mean germination rate.
The seed germination rates of *D. cochinchinensis* increased significantly in seeds were treated by soak in hot water 70°C for 1 min (T1) 0.17 compared to control and other treatments. However, the germination rate was decreased when seeds were treated by scratch and sow dry seed and scratch and soak in how water 50°C for 10 min (T15 and T16) 0.05 followed by control treatment (T18) 0.08 and seed were soaked in hot water 70°C then immersed in GA3, 1 mM overnight (T11) and pre-chilling 30 days (T12) 0.09.

Seed germination process spent about 10–12 days for germinated in all treatment (Figure 3). Day 6 initiated growth of radicle, at day 8 radicle grown 0.8–1 cm, at day 10 radicle grown 1.5–2 cm and at day 12 cotyledon already released from the husk.

**Growth performance of *D. cochinchinensis* seedlings**

After transplanted to the pot, mean of seedlings growth were recorded. Analysis of variance showed significance difference of number of leaves and height of seedlings (p < 0.05) originated from seeds among the pre-sowing treatments. At the end of 2 weeks, number of leaves and height of seedlings originated from seeds with hot treatment significantly higher than other treatments (Table 3).

**Discussion**

Seed dormancy affect the use of dormant species in nurseries for the production of seedlings, it is known that seed dormancy vary from species to species, so the type of pretreatments should be given in accordance with the forest tree species (Amen 1968; Rees 1996). Several authors (Khasa 1992; Rees 1996; Yadav 1992; Azad et al. 2006a, 2006b, 2010a, 2010b, 2011) have discussed different methods of pre-sowing treatments for seed germination in order to break dormancy and enhance the rate of germination and speed up the germination process. The finding of this present study shows that seed germination of *D. cochinchinensis* under pretreatment methods significantly affected various germination and growth parameters. Among the pretreatments, seeds pretreated with hot water had optimum germination, number of leaves and seedlings height than those pretreated with chemical, cold stratification, scarification and control. Similar studies (Sharma et al. 2008) have been done on conducted a germination trial by using different seed pretreatment to increase germination percentage and they observed that hot water pre-sowing treatment in seeds of *Albizia lebbeck* and *P. pterocarpum* for one minute can enhance germination up to 94–97%, respectively. Azad et al. (2006b) carried out an experiment in different pre-sowing treatment effect on seed germination on *Xylia carrii* in Bangladesh. They found the similar result in hot water treatment (87%). Azad et al. (2010a) found 69% germination success in hot water (80°C for 10 min) treatment on *Melia azedarach*. Ali et al. (1997) carried out an experiment on hot water treatment (50°C and boiling for 3 min) on *A. procera* and found 43% seed germination. (Alamgir and Hossain 2005b) showed that hot water (immersion in boiled water 1 min, and then flowed cold water treatment for 24 h). Soaking seeds in hot water significantly improved seed germination percentage in *Bohgannia*
malagascarionsis (Thokozani et al. 2011). Growth performances of the seedlings derived from the pretreated seeds were significantly greater than untreated control (Doody and O’Reilly 2008; Amoakoh et al. 2017). Azad et al. (2011) mentioned that germination percentage, seedlings growth including root, shoot and total length of Acacia auriculiformis increased significantly with pre-sowing treatment especially by hot water treatment. It may be due to the difference of seed coat thickness. The difference of germination percentages may be due to the difference of temperature and the boiling time. The influence of different physical parameters have been explained by different models. Several dormancy types can be associated with the seed-coat, e.g. mechanical resistance, physical barrier to moisture absorption or gaseous exchange, temperature or chemical inhibition, and light sensitivity (Bewley and Black 1982, 1994; Ellis et al. 1985; Schmidt 2000). Hot-water treatment has been reported to enhance germination by affecting various factors, viz. seed coat permeability for water to maximize seed hydration (Longer and Degago 1996), for gaseous exchange and release of inhibitors (Mohamed-Yasseen et al. 1994) like phenolic etc. Soaking D. cochinchinensis seeds in hot water may help in softening the seed coats and removal of inhibitors, thus increased germination. This finding also reported in Hartman et al. (2007), hot water reduces required time for germination and enhances germination percentage. In addition, seeds soak in the hot water may had advantage makes the seed coats permeable to water the seeds imibe and swell as the water cools. This treatment is known to break physical dormancy of seeds which enhance the water uptake and gaseous exchange. Otherwise, enhancing of seedlings growth by hot water treatment may due to an advantage of absorbing much water and started the photosynthesis process much faster than others.

Role of hormones to relive dormancy of forest tree seeds are well established (Ahmadloo et al. 2015; Daneshvar et al. 2016). Exogenous applications of GA3 have been widely used to break physiological dormancy of hard seed coat species (Yao et al. 2015). In case of D. cochinchinensis the GA3 treatment was not effective to increase germination. Confounding to this by Raji and Siril (2018) the case of E. serratus the GA3 treatment was ineffective to improve germination or reduce MTG. Scarification treatment reduced MGT, but not improved the germination of D. cochinchinensis. Similarly, Raji and Siril (2018) reported that the mechanical scarification was superior in reduction of MTG. Amoakoh et al. (2017) found that mechanical scarification improves germination of P. campachiana while soaking with cold water has a negative influence on seed germination.

Figure 4. Growth performance of D. cochinchinensis at 28 days under different pretreatment.

Table 3. Summary of different pretreatments methods effects on growth of D. cochinchinensis.

| Treatment | Number of leaves | Seedling height (cm) |
|-----------|-----------------|---------------------|
| T1: HW (1 min) | 2.17a | 7.09a |
| T2: HW (3 min) | 2.00ab | 5.60bcd |
| T3: HW (5 min) | 1.25bc | 6.08bc |
| T4: HW (10 min) | 2.08a | 6.48ab |
| T5: HW (12 h) | 2.00ab | 5.12bde |
| T6: H2O (12) | 1.25bc | 4.53bcde |
| T7: NaOCl 2% | 1.00 | 6.27b |
| T8: NaOCl 4% | 1.17 | 5.22bde |
| T9: NaOCl 6% | 1.25bc | 5.62bde |
| T10: GA3 (12h) | 1.00 | 5.92bc |
| T11: HW 70 °C (1 min) + GA3 (12 h) | 0.92c | 3.27e |
| T12: CS 30 d | 0.83a | 3.07a |
| T13: CS 40 d | 1.25bc | 3.82bcde |
| T14: CS 50 d | 1.00a | 3.12a |
| T15: SD | 1.08 | 3.24b |
| T16: S + HW 50 °C (10 min) | 0.75a | 3.49be |
| T17: S + H2O (10 min) | 1.17 | 4.70bde |
| T18: Control | 1.08 | 5.05bde |

In each column, mean with same letter(s) are not significantly different at $p < 0.05$, Duncan’s Multiple Range Test (DMRT).
Conclusion

Dalbergia cochinchinensis is an important tree species for social forestry and agroforestry program due to its capability of nitrogen fixation. For establishing nursery of a particular species for producing maximum number of quality seedlings with minimum cost, time and labor, the seed pretreatments are required. The present study of pre-sowing treatments of seeds would prove itself potential in the practical fields. From the results obtained, there were significant differences between five main treatments. Among the treatments applied in the experiment for D. cochinchinensis seeds soaking in hot water 70 °C for 1 min then pour out and soak in cold water overnight (T1) was found more effective in respect to higher germination percentage and germination rate comparison to control and other treatments. Chemical application had neither negative nor positive effect on germination percentage, mean germination rate comparison to control and other treatments. Chemical application had neither negative nor positive effect on germination percentage, mean germination time, and mean germination rate of seed germination of this species. Seeds were scratched with sand paper then soak in hot water 50 °C for 10 min to modify the seed coat, remove inhibitors and soften the seed for easy germination. The improved germination method evolved through the study can be applied to large scale seedlings production and conservation of D. cochinchinensis. Hence, soaking of seeds in hot water 70 °C for 1 min could be recommended for getting higher germination of D. cochinchinensis.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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