



## Germination and Seedling Growth Response on *Lithocarpus elegans* (Fagaceae) Seeds to Pre-sowing Treatments and Fertilizer Application

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### Authors' contributions

This work was carried out in collaboration among all authors. Author RN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author MKH approved the design and final draft of the study and author SD managed the analyses of the study. All authors read and approved the final manuscript.

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### ABSTRACT

This study was conducted to investigate the effect of different pre-sowing treatments of seeds on germination and growth performance of native threatened tree species *Lithocarpus elegans* at the nursery of Chittagong University, Bangladesh. Seeds were placed to eight pre-sowing treatments e.g. control (PT<sub>0</sub>), treated with sand paper rubbing (PT<sub>1</sub>), nicking (PT<sub>2</sub>), seeds immersed in cold water for 24 hours (PT<sub>3</sub>), seeds immersed in cold water for 48 hours (PT<sub>4</sub>) and seeds immersed in cold water for 7 days (PT<sub>5</sub>), seeds sown at propagator house (PT<sub>6</sub>), seeds immersed in cold water for 7 days and then sown at propagator house (PT<sub>7</sub>). Germination percentage was found highest (100%) in seeds sown at propagator house (PT<sub>6</sub>) followed by seeds immersion in cold water for 24 hours (PT<sub>3</sub>) (91.11%). For fertilizer dose experiment to seedlings at the nursery level, treatment FT<sub>1</sub>: 100 kg/ha (0.33679 g urea/pot/seedling) comparing with other treatments FT<sub>0</sub>: 0 kg/ha (Control), FT<sub>2</sub>: 200 kg/ha (0.67358 g urea/pot/seedling) showed better performance in case of shoot length, collar diameter, number of leaves, shoot fresh weight, total fresh weight, total dry

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weight, volume index. Finally, it can be concluded that *Lithocarpus elegans* seedlings revealed better performances under the seed pre-treatment PT<sub>6</sub> (seeds sown at propagator house) and fertilizer dose FT<sub>1</sub> (100 kg N ha<sup>-1</sup>) in growth and biomass production.

**Keywords:** Germination value; quality index; volume index; vigor index.

## 1. INTRODUCTION

*Lithocarpus elegans* (Blume) Hatus. ex Soepadmo, is a tree in the beech family Fagaceae. Worldwide, there are more than 1,000 species belonging to the Fagaceae. All Fagaceae species are woody plants and are spread throughout the northern hemisphere, from the tropical to the boreal regions. The family comprises seven genera [1], and the number of species is extremely variable among genera: *Castanea* Mill. (12), *Castanopsis* (100 to 200), *Chrysolepis* (2), *Fagus* (11), *Lithocarpus* Blume (300), *Quercus* (450 to 600), *Trigonobalanus* (3). Oaks (*Quercus*), chestnuts (*Castanea*), and beeches (*Fagus*) are widely used in forestry for wood products over the three continents (Asia, Europe, and America) and are important economic species. Consequently, they have received more attention in forest genetic research than other genera. The specific epithet *elegans* is from the Latin meaning "elegant", referring to the acorns and cupules. It grows as a tree up to 30 m tall with a trunk diameter of up to 70 cm. The greyish brown bark is fissured or lenticellate. The coriaceous leaves measure up to 17 cm long. Its edible brown acorns are ovoid to roundish and measure up to 2.5 cm across. Its habitat is dipterocarp to lower montane forests up to 1,500 m altitude. The timber is used locally as firewood and for charcoal. The genus *Lithocarpus* is closely related to the oaks (*Quercus*) and occurs in South and South-East Asia, with a single species in North America. Seeds possessed very hard seed coat that produces poor natural germination and ultimately the population of the species is becoming very poor in natural forests. It is widely distributed, occurring from Bangladesh, Nepal to southern China and Malaysia. In Bangladesh, this tree is naturally grown in Teknaf Wildlife Sanctuary located in the southeastern corner of Bangladesh and in Lawachara National Park, Sylhet.

Moreover, forest resources in hill forests and sal forests of Bangladesh show poor natural regeneration because of the depletion of resources due to anthropogenic interference, inappropriate methods of silviculture and a preference for exotic species over native for

afforestation [2]. At the same time, plant genetic diversity is being disrupted in an alarming way [3,4]. It is observed in some cases that if somehow seeds germinate, but survival is poor [5]. If this trend continues, species under threat will become extinct in the near future. So to restore the forest with native species, regeneration through nursery and plantation programs as well as development of seed orchards are essential. However, poor germination, delayed nursery establishment and slow growth limit the cultivation of threatened species in both forestry and homestead plantation programs. Good planning and appropriate nursery techniques could speed up germination rates and assure maximum quality seedlings from minimum seed sources.

*L. elegans* was once abundant in hill forests but are becoming very rare because of habitat destruction. Considering the importance of native and rare species to the ecological and environmental balance of the forest ecosystems, this research has been designed to assess the effects on seed germination by different pre-sowing treatments and the initial seedling growth attributes of *Lithocarpus elegans*. Good planning and appropriate nursery techniques could speed up germination rates and assure maximum quality seedlings from minimum seed sources. There is, however, little information on germination ecology of native threatened species [6] especially those of the hill forests. Although much research has been conducted on common plantation species, germination behavior and initial seedling growth of threatened native species has been largely ignored. This research investigates the germination rate and growth performance under different pre-sowing treatments at the nursery level and as well as biomass growth under different doses of urea fertilizers at the nursery level for better plantation level growth.

## 2. MATERIALS AND METHODS

The experiment was conducted in a propagator house and nursery located at the Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU) and at the Seed

Research Laboratory of IFESCU, Bangladesh. Fruits of *Lithocarpus elegans* were collected from the Ukhia-Teknaf reserve forest in August, 2017. After collection, the brownish seeds were dried for four days in open sun in order to reduce the moisture (Fig.1).

Only healthy seeds were sown in polybags (15 × 10 cm) and propagator house. The media of the polybags was mixture of forest topsoil collected from forest floor and cow dung in a ratio of 3:1. The media used in propagator house was fine Sylhet sand. The experiment consists of eight pre-sowing treatments with three replications (15 seeds per replication) in a randomized design. For each treatment, 45 healthy seeds were selected randomly and then provided with the treatments as follows:

- PT<sub>0</sub>:Seeds with no treatment and sown in polybag only (Control),
- PT<sub>1</sub>:Seeds treated with sand paper rubbing at distal end of the seed,
- PT<sub>2</sub>:Seeds treated with nicking/nail clipping at distal end of the seed,
- PT<sub>3</sub>:Seeds immersed in cold water for 24 hours,
- PT<sub>4</sub>:Seeds immersed in cold water for 48 hours,
- PT<sub>5</sub>:Seeds immersed in cold water for 7 days,
- PT<sub>6</sub>:Seeds sown at propagator house,
- PT<sub>7</sub>:Seeds immersed in cold water for 7 days and then sown at propagator house,

The effects of pre-sowing treatments were assessed periodically by counting germinated seeds. The germination was recorded daily from the date of sowing and continued till the germination ceased. The imbibition period (number of days from sowing to commencement of germination) was recorded. In addition, germination value (GV) was also calculated using the formula of [7] below:

$$GV = (DDGs/N) \times GP/10$$

where DG is daily germination speed obtained by dividing the cumulative germination percentage by the number of days since sowing.

DDGs is total germination obtained by adding DGs value obtained from the daily counts.

N is the total number of daily counts, starting from the date of first germination.

GP is Germination percentage at the end of the test and 10 as a constant.

Volume index and vigor index was calculated using the following formula:

$$\text{Volume index (VI)} = [\text{root collar diameter (mm}^2) \times \text{shoot height (cm)}] \text{ [8]}$$

$$\text{Seedling Vigor index (SVI)} = [\text{seedling length (cm)} \times \text{germination percentage}] \text{ [9].}$$



Fig. 1. *Lithocarpus elegans* seeds

The quality index (QI) [10] to quantify seedling morphological quality was calculated.

Sturdiness of seedling was calculated using the following formula:

$$\text{Sturdiness} = \text{Shoot height (cm)} / \text{Collar diameter (cm) of the seedling}$$

## 2.1 Experimental Design for Fertilizer Application

Second experiment under this study was set to investigate the effect of organic fertilizer (Urea) on the initial growth of *Lithocarpus elegans* at the nursery for selecting the best fertilizer dose for field level plantation of this species. In second experiment, 90 best seedlings were chosen before fertilizer application from the first experiment which showed shoot length range within 30-39 cm and collar dia within 3-5 mm. So a range for shoot length and collar dia was maintained for the fertilizer experiment to show reliability of evaluation of result.

A randomized complete block design with three treatments (3 replications each) and ten (10) seedlings were used in each replicates. Before, fertilizer application, data on shoot height, collar diameter from the best ten (10) seedlings of each treatment among 90 seedlings was collected. Then different doses of fertilizers as different treatments were applied in April, 2018 to the selected seedlings. Watering was carried regularly by fine shower, which could not disturb the seedlings physically. Removal of weeds, grasses etc. were done as far as possible.

At the end of four months, again data on shoot height (cm) and collar diameter (mm) from the same seedlings/treatment were collected and then the seedlings were uprooted and separated into leaves, shoot and root components and were dried in electric oven at 70°C until the constant weight was obtained for studying biomass productions in different doses of fertilizer treatments. The following treatments were used as different doses of Urea fertilizer at the nursery:

$$\begin{aligned} \text{FT}_0 &: 0 \text{ kg/ha (Control),} \\ \text{FT}_1 &: 100 \text{ kg/ha}=0.33679 \text{ g urea/pot/seedling,} \\ \text{FT}_2 &: 200 \text{ kg/ha}=0.67358 \text{ g urea/pot/seedling} \end{aligned}$$

Height of seedlings was taken from the ground level to the tip of the seedlings by using meter

scales. Diameter at collar region of seedlings was measured at the ground level using slide calipers. After 120 days (4 months) of fertilizer application, seedlings were harvested and separated into root, shoot and leaf components. Finally, data on fresh weight (g) and dry weight (g) of each part of the seedling under three treatments were taken at the IFESCU laboratory by using weight machine for measuring fresh weight and then using oven to make the fresh sample dry and then took dry weight again by weight machine. Data were statistically analyzed by using SPSS© and data were subjected to analysis of variance and Duncan's Multiple Range Test (DMRT).

## 3. RESULTS

### 3.1 Germination Period and Germination Percentage

Germination behavior of *Lithocarpus elegans* seeds was affected by different pre-sowing treatments under this experiment. Germination percentage was found highest (100%) in seeds sown at propagator house (PT<sub>6</sub>) followed by seeds immersion in cold water for 24 hours (PT<sub>3</sub>) (91.11%) (Table 1). Moreover, germination was started earlier in all treatments except in PT<sub>5</sub> in seeds immersed with cold water for 7 days where as it took 74 days for initiation of germination (Table 1).

Moreover, germination was started just after 34 days in seeds soaked in cold water for 24 hours and 48 hours respectively. Germination of seeds continued up to 205 and 208 days in those two treatments. In addition, it is found that seeds sown in propagator house (PT<sub>6</sub>) and seeds immersed in cold water for 7 days plus sown in propagator house (PT<sub>7</sub>) took 38 days to start germination. Therefore, it is evident from the study that all the treatments except PT<sub>5</sub> reduced the seed dormancy period (Table 1).

The highest germination percentage (100%) was observed in PT<sub>6</sub> (Seeds sown at propagator house) followed by 91.11% in PT<sub>3</sub> (seeds immersed with cold water for 24 hours), 88.88% in PT<sub>4</sub> (seeds immersed in cold water for 48 hours) and 82.22% in PT<sub>1</sub> (sand paper rubbing), 73.33% in PT<sub>2</sub> (sand paper nicking) (Table 1). Germination percentage was least (8.88%) and started later (74 days) in PT<sub>5</sub> (seeds immersed with cold water for 7 days).

**Table 1. Germination parameters of talbatna (*Lithocarpus elegans*) seeds under different pre-sowing treatments**

| Treatments  | Germination start (day) | Germination end (day) | Germination percentage (%) | Germination value |
|---|-------------------------|-----------------------|----------------------------|-------------------|
| PT <sub>0</sub> (Control)   | 33                      | 150                   | 88.88                      | 4.57              |
| PT <sub>1</sub> (Sand paper)  | 30                      | 105                   | 82.22                      | 12.60             |
| PT <sub>2</sub> (Nicking)   | 30                      | 106                   | 73.33                      | 9.29              |
| PT <sub>3</sub> (Cold water, 24 hrs.)                                 | 34                      | 205                   | 91.11                      | 10.82             |
| PT <sub>4</sub> (Cold water, 48 hrs.)                                 | 34                      | 208                   | 88.88                      | 6.04              |
| PT <sub>5</sub> (Cold water, 7 days)                                  | 74                      | 189                   | 8.88                       | 0.057             |
| PT <sub>6</sub> (Seeds sown at propagator house)                      | 38                      | 188                   | 100                        | 9.15              |
| PT <sub>7</sub> (Cold water, 7 days + seeds sown at propagator house) | 38                      | 125                   | 66.66                      | 6.19              |

### 3.2 Growth Performance of the Seedlings under Different Doses of Fertilizer Application

In case of the total length of seedlings, the value was highest (121.00 cm) in FT<sub>1</sub> (100 kg/ha=0.33679 g urea/pot/seedling) and lowest in FT<sub>2</sub> (90.50 cm). There was significant variation ( $p < 0.05$ ) among FT<sub>0</sub>, FT<sub>1</sub>, and FT<sub>2</sub> (Table 2). Collar diameter (mm) of seedlings was maximum (7.96 mm) in FT<sub>1</sub> (100 kg/ha=0.33679 g urea/pot/seedling) and lowest in FT<sub>2</sub> (200 kg/ha=0.67358 g urea/pot/seedling). There was significant difference between FT<sub>1</sub> and FT<sub>2</sub> treatments and slightly significance difference between FT<sub>0</sub> and FT<sub>1</sub>. Maximum leaf number was recorded in FT<sub>1</sub> (32.40) and minimum was in FT<sub>2</sub> (21.80). Highest shoot fresh weight (50.10 gm) and highest total fresh weight (67.90 gm) was found in FT<sub>1</sub> treatment which showed significant variation from FT<sub>0</sub> and FT<sub>2</sub> (Table 2).

Total dry weight (g) was found maximum in treatment FT<sub>1</sub> (37.10 g). The highest quality index was recorded in FT<sub>2</sub> (2.69) and there is no significance difference among FT<sub>0</sub>, FT<sub>1</sub>, and FT<sub>2</sub> (Table 3). Besides, highest volume index (5861.49) was recorded in treatment FT<sub>1</sub> and there was significant difference between FT<sub>1</sub> and other treatments. Sturdiness was found maximum (116.61) in FT<sub>1</sub> and minimum (93.87) in FT<sub>2</sub>. Greatest root-shoot ratio was found in FT<sub>2</sub> (0.5565) (Table 3).

The results of the present study demonstrate that *Lithocarpus elegans* seeds, when seeds sown at propagator house showed better in germination and at the same time, with the fertilizer application dose of 100 kg/ha (0.33679 g urea/pot/seedling), this species shows better biomass production. But it is also evident that with the increased dose of fertilizers, growth parameters of this seedling were reduced.

**Table 2. Effect of fertilizer treatment on shoot length, root length, total length, collar diameter, number of leaves, fresh weight of *Lithocarpus elegans* seedlings at the nursery**

| Treatment       | Shoot length (cm)  | Root length (cm)   | Total length (cm)   | Collar diameter (mm) | No of leaves       | Root fresh wt      | Shoot fresh wt     | Total fresh wt     |
|-----------------|--------------------|--------------------|---------------------|----------------------|--------------------|--------------------|--------------------|--------------------|
| FT <sub>0</sub> | 71.40 <sup>b</sup> | 35.10 <sup>a</sup> | 106.50 <sup>b</sup> | 7.29 <sup>ab</sup>   | 26.00 <sup>a</sup> | 12.30 <sup>a</sup> | 30.70 <sup>b</sup> | 43.00 <sup>b</sup> |
| FT <sub>1</sub> | 92.40 <sup>a</sup> | 28.60 <sup>a</sup> | 121.00 <sup>a</sup> | 7.96 <sup>a</sup>    | 32.40 <sup>a</sup> | 17.80 <sup>a</sup> | 50.10 <sup>a</sup> | 67.90 <sup>a</sup> |
| FT <sub>2</sub> | 64.20 <sup>b</sup> | 26.30 <sup>a</sup> | 90.50 <sup>c</sup>  | 6.90 <sup>b</sup>    | 21.80 <sup>a</sup> | 20.50 <sup>a</sup> | 29.70 <sup>b</sup> | 50.20 <sup>b</sup> |

<sup>a, b, c</sup> - Figures followed by the same letter(s) are not statistically significant at  $p < 0.05$ , DMRT test

**Table 3. Effect of fertilizer treatment on dry weight, quality index, volume index, root-shoot ratio and sturdiness of *Lithocarpus elegans* seedlings in the nursery**

| Treatment       | Root dry wt (g)    | Shoot dry wt (g)   | Total dry wt (g)   | Quality index       | Volume index         | Root-shoot ratio    | Sturdiness          |
|-----------------|--------------------|--------------------|--------------------|---------------------|----------------------|---------------------|---------------------|
| FT <sub>0</sub> | 8.00 <sup>a</sup>  | 16.30 <sup>b</sup> | 24.30 <sup>b</sup> | 2.1082 <sup>a</sup> | 3780.58 <sup>b</sup> | 0.4783 <sup>a</sup> | 99.44 <sup>a</sup>  |
| FT <sub>1</sub> | 11.50 <sup>a</sup> | 25.60 <sup>a</sup> | 37.10 <sup>a</sup> | 2.2268 <sup>a</sup> | 5861.49 <sup>a</sup> | 0.4474 <sup>a</sup> | 116.61 <sup>a</sup> |
| FT <sub>2</sub> | 8.40 <sup>a</sup>  | 15.80 <sup>b</sup> | 24.20 <sup>b</sup> | 2.6953 <sup>a</sup> | 3098.27 <sup>b</sup> | 0.5565 <sup>a</sup> | 93.87 <sup>a</sup>  |

<sup>a, b, c</sup> - Figures followed by the same letter(s) are not statistically significant at  $p < 0.05$ , DMRT test

#### 4. DISCUSSION

Findings from the study reveals that *Lithocarpus elegans* seedlings, when treated with FT<sub>1</sub> (100 kg N ha<sup>-1</sup>) shows better performances in growth and biomass production. These findings relate with the findings of [11] where it was observed that there was significant increase in height of seedlings of *Michelia champaca* L., after N and P fertilization. Moreover, another study from [12] demonstrates that *Khaya ivorensis* A. Chev., showed better growth parameter on application of organic and inorganic fertilizers and it was found that growth attributes were increased with the application of 75 mg urea that is added to the seedlings. This study is also related with the present study. In addition, another study on the effect of NPK fertilizer on growth of *Hopea odorata* Roxb. (Telsur) seedlings found that N has significantly increased the seedling height, leaf and shoot dry matter production [13]. Moreover, further study on the response of four species of tropical timber seedlings to Urea and Folivert fertilizers in nursery revealed that plants treated with 3 g of urea produced the highest number of leaves in *Albizia zygia* (DC.) J.F. Macbr., seedlings [14].

Moreover, there are several studies on improvement of growth performances of seedlings through application of organic fertilizers which is reported by [15,16,17,18] earlier. However, it is observed from the study that with the increased doses of fertilizers, seedlings growth parameters e.g. collar diameter (mm), total shoot length (cm), total dry weight (gm), volume index, sturdiness was reduced. It means, FT<sub>2</sub> (200 kg/ha) treatment reduced growth performance of *Lithocarpus elegans* seedlings.

#### 5. CONCLUSION

It can be concluded from this study that 100 kg urea/ha is recommended for better growth performance of *Lithocarpus elegans* seedlings. Moreover, seeds sown at propagator house can be suggested for best germination of this species.

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#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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