

*Full Length Research Paper*

# Effect of seed size and pre-treatment methods of *Bauhinia thonningii* Schum. on germination and seedling growth

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*Bauhinia thonningii* Schum. a multipurpose tree species found in the savanna wood lands is a priority tree species for conservation in Malawi. The different plant parts are used in traditional medicine to treat different ailments. However, the seeds are dormant and the tree species remain undomesticated. A study was conducted to evaluate the effect of pre-sowing seed treatment and seed size on germination and seedling growth of *B. thonningii* in a nursery at Bunda College. Seeds grouped into two categories: small (1 to 5 mm) and large (>5 to 10 mm) were subjected to five main pre-sowing seed treatment methods namely; soaking in cold water for 12 h, soaking in hot water for 10 min, nicking, soaking in potassium nitrate (0.2%) for 10 min and soaking in concentrated hydrochloric acid (0.3 M) for 5 min, and a control where seeds were sown without any treatment. The results showed that the combination of nicking and large seeds produced the highest (100%) germination and highest height and diameter growth. Hot water treatment was also observed to be effective in both seed sizes producing 40 and 53.3% germination for small and large seeds, respectively. The increased seed germination and height for physically scarified seeds through nicking suggest that seed dormancy in *B. thonningii* is mainly due to relatively hard seed coat which renders the seed testa impermeable to water and gases required for germination process.

**Key words:** *Bauhinia thonningii*, dormancy, germination, pre-sowing treatment, seed size.

## INTRODUCTION

*Bauhinia (Piliostigma) thonningii* Schum. is a savanna deciduous leguminous tree belonging to the family Caesalpiniaceae. The tree has a wide distribution range in tropical Africa, extending from West Africa to the Sudan and south wards to east and central southern Africa, including countries such as Mozambique, Malawi, Zimbabwe, Zambia, Botswana, Tanzania and Namibia (Chidumayo, 2007; Chidumayo, 2008). *B. thonningii* trees typically grow to a height of 6 to 12 m and their branches spread 3 to 6 m outwards. The flowers are usually five-petaled and are 7 to 12 cm in diameter, generally in shades of red, pink, purple, orange or yellow colour. The species is widely used in sub-Saharan Africa for poles, firewood, charcoal and its pods are eaten by wild animals

and livestock. The seeds are a good source of anti-oxidant micronutrient and are rich in crude protein and carbohydrates (Jimoh and Oladiji, 2005). The inner bark of the tree is used to make rope.

The roots, bark and twigs of *B. thonningii* are used as medicine in many African countries to treat wounds, ulcers, gastric pain and as an antipyretic. In countries like Malawi, Tanzania and Zimbabwe, a cough remedy is prepared from the root bark. The root bark is also used to cure dysentery, diarrhoea, enhance delivery and ease menstrual pains (Gowela, 2003). Both roots and twigs have been used in the treatment of fever, respiratory ailments, hookworm and snake bites (Jimoh and Oladiji, 2005).

Despite its importance, *B. thonningii* is threatened by an increasing rate of exploitation for firewood, charcoal production, poles, medicine and many products. Continued availability of the medicinal plant is increasingly endangered by ongoing land-clearing, deforestation and

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in some cases, over-harvesting, wildfires, habitat change and climate change factors. The tree species has been prioritized as one of the species for conservation in Malawi to enhance its contribution to health and livelihood of communities. Traditional healers and farmers have failed to cultivate the plant species due to high seed dormancy. In view of its importance in the health sector, it is imperative to find methods of breaking seed dormancy for successful regeneration and promote cultivation of the species in medicinal plant gardens. Therefore, the objective of the study was to assess the effects of different seed pretreatment methods and seed size on germination and seedling growth of *B. thonningii*.

## MATERIALS AND METHODS

### Seed collection and preparation

Dry mature fruits of *B. thonningii* were collected in October, 2009 from Dzalanyama Forest Reserve near Chiwulongwe River (13° 59' S, 33° 30' E and 1124 m above sea level). After seed extraction and cleaning, the moisture content was determined by oven drying at 103°C for 17 h (ISTA, 2003). The intact plump seeds were separated into small and large seed categories. The seeds were then taken to Bunda College Forestry nursery (33° 45' S, 14° E, 1118 m above sea level) during 2009/2010 crop growing season. Bunda receives 875 to 1000 mm rainfall per annum and is situated south east of Lilongwe. Soils were collected from *miombo* wood land and then mixed in 1:2 ratio. The soils were analyzed for phosphorus, organic matter content, texture and nitrogen.

### Experimental design and treatments

The treatment combination consisted of two factors namely, seed size and pretreatment methods. The first factor where seeds have seed length of 1 to 5 mm were denoted as small seeds and >5 to 10 mm were denoted as large seeds. The second factor was five pretreatment methods namely: soaking in cold water for 6 h, soaking in hot water for 10 min, nicking, immersing in potassium nitrate (KNO<sub>3</sub>) and concentrated hydrochloric acid (0.3 M HCl) and a control which consisted of seeds that were left intact. The treatments were arranged in a factorial randomized complete block design with three replications.

### Cold and hot water treatment

Fifty (50) seeds of each small and large seeds were put in similar beaker sizes where cold water at room temperature was poured and the seeds were soaked for 6 h. Water was then removed and the seeds were planted on the same day. For the hot water treatment, water was boiled to approximately 100°C and was then poured into both beakers containing 50 seeds and was left to stand for 10 min after which the seeds were planted.

### Nicking

Fifty (50) seeds from each size category were mechanically nicked on one side away from the micropyle using secateurs and then planted immediately. The control experiment consisted of 50 seeds of each size category which were left intact.

### Immersion in concentrated hydrochloric acid and potassium nitrate

Small and large seeds were put into separate beakers and concentrated 0.3 M hydrochloric acid (HCl) was then added to the beakers each containing 50 seeds and were left to soak for 5 min. After immersion, the solution was drained off and seeds were repeatedly rinsed in running tap water until considered safe to handle. Then the seeds were planted. On the other hand, 0.2% potassium nitrate (KNO<sub>3</sub>) was added on another set of large and small seeds and left to soak for 10 min before rinsing and planting. In total, there were 12 treatment combinations and were denoted as follows:

- T1: S1P1, Small seeds soaked in hot H<sub>2</sub>O at 100°C for 10 min;
- T2: S1P2, small seeds soaked in cold H<sub>2</sub>O at room temperature for 6 h;
- T3: S1P3, small seeds with nicking;
- T4: S1P4, small seeds soaked in 0.2% potassium nitrate (KNO<sub>3</sub>);
- T5: S1P5, small seeds immersed in 0.3 M hydrochloric acid (HCl);
- T6: S1P6, small seed planted without pretreatment;
- T7: S2P1, large seeds soaked in hot H<sub>2</sub>O at 100°C for 10 min;
- T8: S2P2, large seeds soaked in cold H<sub>2</sub>O at room temperature for 6 h;
- T9: S2P3, large seeds with nicking;
- T10: S2P4, large seeds soaked in 0.2% potassium nitrate (KNO<sub>3</sub>);
- T11: S2P5, large seeds immersed in 0.3 M hydrochloric acid (HCl); and
- T12: S2P6, large seed size planted without pretreatment.

Treated and non treated seed were sown directly in 10 cm plastic pots and one seed was planted per pot. Watering was done accordingly to keep the beds with adequate moisture.

### Data collection and analysis

Data on germination were recorded on daily basis for a period of 70 days from the date of sowing. Germination was defined as the emergence of radicle from the seed coat. In addition, plant height and root collar diameter were also measured for six weeks to determine the response of different seed sizes. Daily germination percentages were summed up to obtain cumulative germination for each treatment. Height and diameter were summed up to obtain the cumulative heights and diameters for each treatment after six weeks. The results of the germination studies were subjected to an analysis of variance (ANOVA) using GenStat third edition following arcsine transformation of all percent germination data. Differences between treatment means were separated using Fischer's least significant difference (LSD) at the 0.05 level. Arcsine-transformed data were back transformed for graphic presentation.

## RESULTS

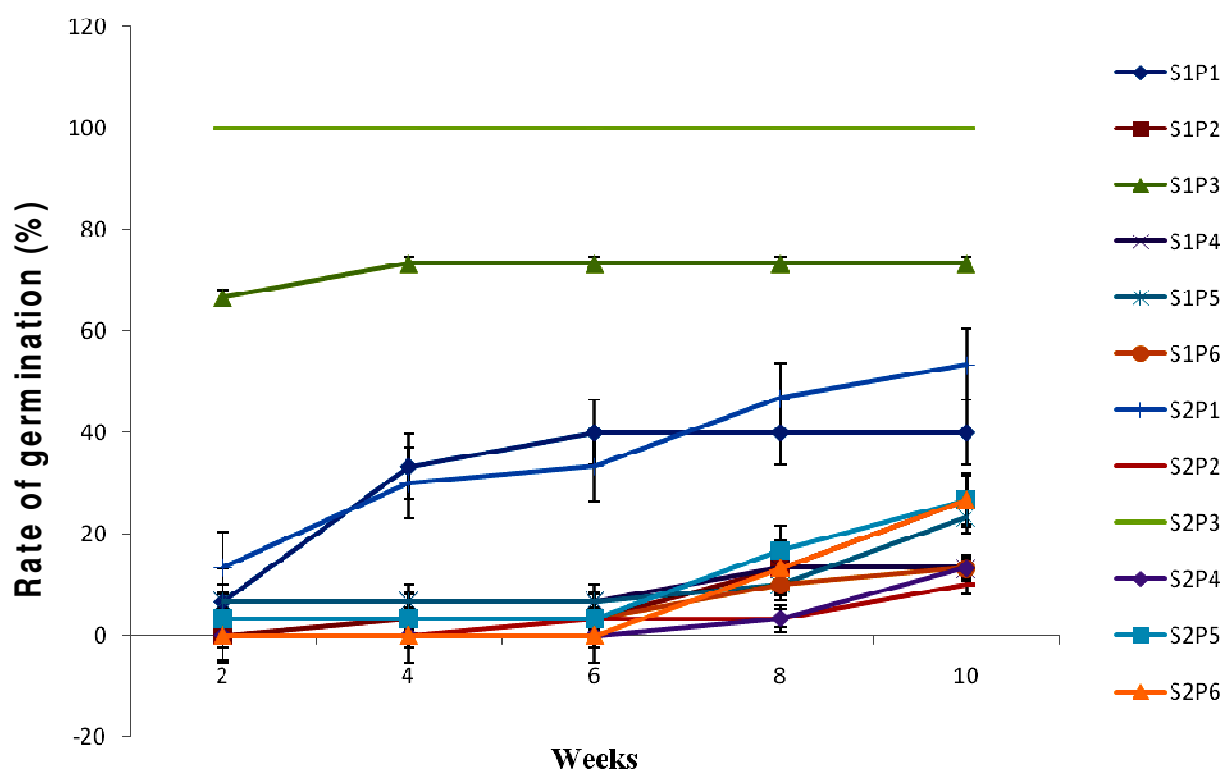
### Seed size and pre-sowing treatment

There were no significant differences in germination ( $p > 0.05$ ) between seed sizes, although larger seeds had a higher germination (38.3%) percentage than the small seeds (31.7%). However, there were significant differences ( $p < 0.05$ ) in germination among pre-sowing treatments where nicking gave the highest germination (86.7%) followed by immersion in water (46.7%) and then immersion in 0.3 M HCl acid with 25.0% germination

**Table 1.** Effect of pre-treatment methods and size of *B. thonningii* Schum. seeds on germination at 10 weeks after sowing.

| Seed size      | Pre-sowing treatment method |                   |                   |                   |                   |                   | Mean |
|----------------|-----------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|------|
|                | Cold water                  | Hot water         | HCl               | KNO <sub>3</sub>  | Nicking           | Control           |      |
| Small          | 26.7                        | 40.0              | 23.3              | 13.3              | 73.3              | 13.3              | 31.7 |
| Large          | 10.0                        | 53.3              | 26.7              | 13.3              | 100.0             | 26.7              | 38.3 |
| Mean           | 18.4 <sup>b</sup>           | 46.7 <sup>b</sup> | 25.0 <sup>b</sup> | 13.3 <sup>b</sup> | 86.7 <sup>a</sup> | 20.0 <sup>b</sup> |      |
| SE             | 0.235                       |                   |                   |                   |                   |                   |      |
| LSD (p < 0.05) | 28.5                        |                   |                   |                   |                   |                   |      |
| CV %           | 56.2                        |                   |                   |                   |                   |                   |      |

Means in the same row followed by the same letter do not significantly differ at p < 0.05.

**Figure 1.** Effect of pre-treatment methods and size of *B. thonningii* Schum. seeds on maximum germination.

(Table 1). A similar trend in germination percentage was also observed for the small seed categories.

### Maximum germination ( $G_{max}$ )

When the pre-sown seed were observed over time, germination increased rapidly for hot water treatment for small seeds (S1P1) between the second to sixth week after which it remained constant, while in the large seeds (S2P1), germination increased rapidly between the second to sixth week. However, nicking for both small (S1P3) and large seeds (S2P3) had the highest rate of  $G_{max}$  in the first two weeks, then the rate of germination became constant (Figure 1).

### Effect of pre-sowing treatment on seedlings growth

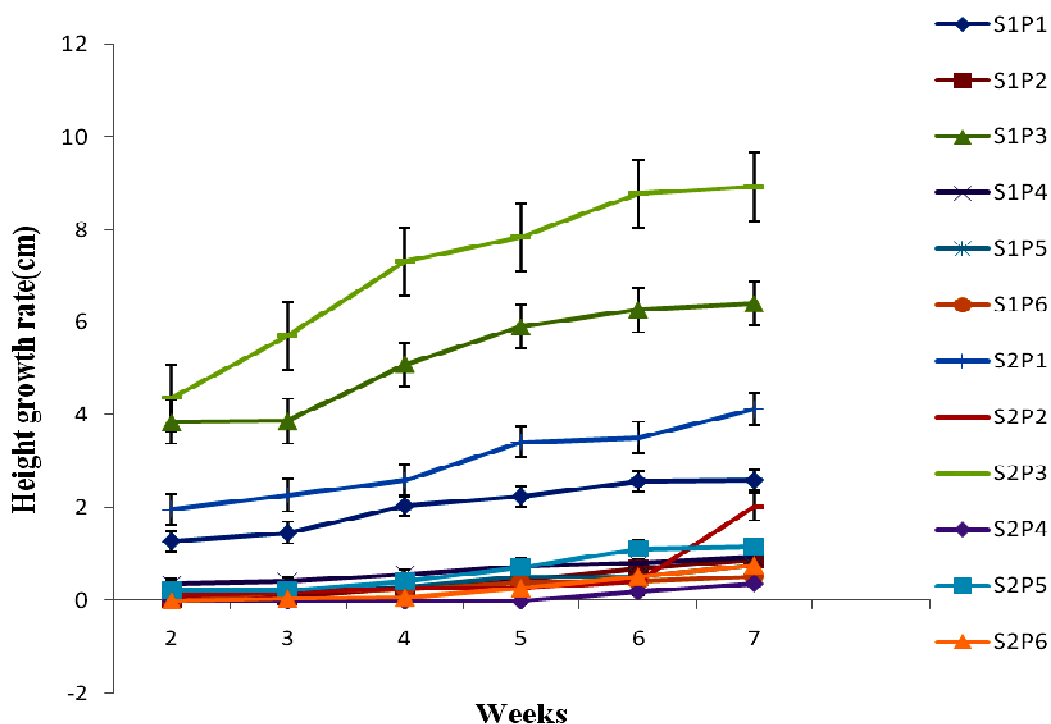
There were no significant differences in the seedling growth due to seed size, though larger seed sizes showed higher seedlings height (2.87 cm) than seeds with smaller sizes (1.97 cm). Height growth was significantly different (p < 0.05) among the different pre-sowing seed treatment methods. Nicking gave the highest seedlings height (7.52 cm) followed by soaking in hot water (3.36 cm). The use of KNO<sub>3</sub> as a pre-sowing treatment and planting of seed without any treatment had the least height growth (Table 2).

Height growth performance for *B. thonningii* seedlings showed that nicking for both small (S1P3) and large seeds (S2P3) produced the highest seedlings height, for

**Table 2.** Height (cm) of *B. thonningii* pre-treated seeds 7 weeks after sowing

| Seed size      | Pre-sowing treatment method |                  |                   |                   |                   |                   | Mean |
|----------------|-----------------------------|------------------|-------------------|-------------------|-------------------|-------------------|------|
|                | Cold water                  | Hot water        | HCl               | KNO <sub>3</sub>  | Nicking           | Control           |      |
| Small          | 0.7                         | 2.6              | 0.77              | 0.93              | 6.27              | 0.58              | 1.97 |
| Large          | 2.0                         | 4.1              | 1.17              | 0.36              | 8.77              | 0.76              | 2.87 |
| Mean           | 1.4 <sup>c</sup>            | 3.4 <sup>b</sup> | 0.97 <sup>c</sup> | 0.65 <sup>c</sup> | 7.57 <sup>a</sup> | 0.65 <sup>c</sup> |      |
| SE             | 1.34                        |                  |                   |                   |                   |                   |      |
| LSD (p < 0.05) | 1.61                        |                  |                   |                   |                   |                   |      |
| CV %           | 55.4                        |                  |                   |                   |                   |                   |      |

Means in the same row followed by the same letter do not significantly differ (p < 0.05).

**Figure 2.** Seedlings growth performance of small and large seeds of *B. thonningii*.

instance nicked seeds gave the highest seedlings height which increased rapidly between week 2 and 4, then increased at a decreasing rate between week 4 and 6, and then remained stable in weeks 6 and 7. Treatment of seed with hot water (S2P1) showed the second height growth with an increased rate of growth between weeks 2 and 4 and then increased at an increasing rate in weeks 4 to 5, and remained stable between weeks 5 to 6 (Figure 2).

## DISCUSSION

The results obtained in this study show the important role of pre-sowing seed treatment in the germination and

domestication of *B. thonningii*. Germination and height growth varied among the different seed pre-treatment methods. Just like many savanna woodland species, *B. thonningii* has a hard seed coat which has evolved to withstand unfavourable conditions such as heat, strong teeth of dispersing animals, severe drought and other mechanical damage (Baskin and Baskin, 1998). High seed germination and height seedlings growth for seeds that were nicked suggests that this is the best method to be applied before planting seeds of *B. thonningii*. Nicking is known to break physical dormancy of seeds with hard coats which inhibit water uptake and gaseous exchange. The seed coat is the restricting force to water imbibitions, a process necessary for seed germination. Earlier germination and fast height growth of nicked seeds is as a

result of cracks made on the seed for entry of water and exchange of gases resulting in enzymatic hydrolysis and thus transforming the embryo into a seedling. Permeability of seed to water and oxygen is improved by nicking the seed coat and this is in agreement with the findings of Ayisire et al. (2009). Nicking has been found to be extremely effective for most species but it requires care, for instance, it has to be done on one side away from the micropyle to prevent destruction of the embryo. The results are also supported by the findings of Likoswe et al. (2008) who reported nicking and soaking in hot water for 12 h to have enhanced the highest germination and growth in *Terminalia sericea* Burch. ex DC. However, nicking is laborious and slow such that its applicability is only limited to small seed samples. There is need to design seed scarifying drums with sandpaper lining that could physically scarify large quantities of seed at a time than nicking individual seeds.

Hot water treatment was the second best pre-sowing seed treatment method, producing higher germination percentage of 53.3% for large seeds of *B. thonningii*. Soaking seed in hot water may soften hard seed coats and leach out chemical inhibitors. Soaking in boiled water makes the seed coats permeable to water and the seeds imbibe and swell as the water cools. Hot water treatment has yielded beneficial results with a number of leguminous seeds. It has been shown that the initial water temperature has a larger effect on germination rate than the periods of soaking and cooling of the seed (Willan, 1985). This treatment is recommended for species that have little resistance to germination. Prescription of hot water must be applied carefully without killing the seeds, though with excessive heating (Phartyal et al., 2005).

Potassium nitrate in both seed size categories produced poor results in seed germination and height growth. Chemical stimulators like  $KNO_3$  enhance seed germination by creating a balance between hormonal ratios in the seed and reducing the growth preventable substances like abscisic acid. Use of  $KNO_3$  may not have resulted in improved seed germination and growth due to low sensitivity to  $KNO_3$  because of the hard seed coat. This is in agreement with the findings of Ali et al. (2010) who reported high germination in *Descurainia sophia*, a soft seed coated species than in *Plantago ovata* which is a medicinal plant species with a hard seed coat. Use of potassium nitrate has been an important seed treatment in seed-testing laboratories for many years without a good explanation for its action (Hartmann et al., 1997). Potassium nitrate was found to be effective in breaking dormancy of many species (Agrawal and Dadlani, 1995). Yet, Stidham et al. (1980) reported that the use of  $KNO_3$  in combination with other pretreatment methods had beneficial effects on seed germination of 18 shrub species. The use of hydrochloric acid in seed pretreatment was not the worst in percent germination. McDonald and Omoruyi (2003) reported higher seed germination of

species with hard impermeable seed coat when treated with HCl. However, concentration of the acid and time of exposure are very critical and need to be quantified for each species since seeds exposed for a long time gets damaged easily (Schmidt, 2000). In our study, only one concentration and time of exposure to the HCl was used.

Even though the differences were not significant, larger seeds produced a higher germination and height growth in *B. thonningii* than the smaller seeds. Emerging seedling depends on the mobilizable reserves and nutrients to provide an advantage for initial growth until it becomes autotrophic. Furthermore, larger seed produced robust seedlings due to greater amount of carbohydrates in their endosperm or cotyledons than smaller seeds, ensuring early development to produce faster growing plants (Hewitt, 1998). The findings are in line with the studies of Khurana and Singh (2004) who reported that large seeds have greater germination and survival value under water stress.

## Conclusion

Seed pretreatment methods and seed size affected the germination and growth of *B. thonningii*. The bigger seeds resulted in higher germination percent and high seedlings height since larger seeds contain more food reserves to support germination and growth. Nicking has shown to be the overall best pre-sowing seed treatment method in *B. thonningii* followed by hot water treatment. However, nicking of seeds is slow but safe and effective. This process is best suited for larger seeds. On most hard-coated species, sand paper may be used to reduce seed coat thickness by abrasion. A combination of different pretreatment methods, especially nicking and soaking in hot water can be studied to determine the best method to improve seed germination and growth of *B. thonningii*. While different methods may break seed coat allowing water to infiltrate and enhance seed germination, there is need to recommend to farmers the adoption of methods that are simple, with low cost, effective and quick in action.

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