Improvement of Seed Germination and Early Seedling Growth of *Leucaena leucocephala* by Cold Water, Mechanical and Acid Scarification Pretreatment

Muhammad Rusdy
Department of Forage Science and Grassland Management, Faculty of Animal Science Hasanuddin University, Indonesia
Email id: muhrusdy79@yahoo.co.id

**Abstract** Seed dormancy is the limiting factor in the propagation of *Leucaena leucocephala*, a leguminous tree with wide important utilization. With the aim of obtaining the best treatment for breaking of seed dormancy in this plant, the present research was conducted. For this reason, three treatments: 1 control, 2 soaking the seeds in cold water for 24, 48 and 72 minutes, 3 mechanical scarification: clipping of seeds around micropyle, clipping of seeds at distal end and sandpapering, and 4 soaking the seeds in sulfuric acid for 4, 8, 12, 16, 20 and 24 minutes were used. Results of the study revealed that regardless of soaking time, soaking the seeds in sulfuric acid resulted in the highest germination and seedling growth, followed by mechanical scarification and control, respectively. The lowest germination and early seedling growth was recorded when the seed soaked in cold water.

**Introduction**

Leucaena (*Leucaena leucocephala* Lam. de Wit), is a fast growing leguminous tree native to southern Mexico and northern Central America and now, it has been naturalized throughout the tropics and subtropics. During 1970s and 1980s, Leucaena was promoted as miracle tree for its multiple uses. Leucaena can provide nutritious forage, firewood, timber, human food, green manure, shade, and can aid in erosion control and improving soil fertility.

As forage, Leucaena provides palatable, digestible and nutritious forage for ruminants, but causes weight loss and health problems in non-ruminants when it is fed at high levels. Leucaena foliage contains both nutrients and roughage and makes an almost complete ruminant feed, comparable to alfalfa (D’Mello and Thomas, 1977), and its amino acid pattern is comparable to soya bean and fish meal (TerMeulen* et al.*, 1979). Leucaena contains organic matter 89%, crude protein 27.6%, NDF 42.5%, ADF 30.9%, ADL 9.3%, ash 11%, calcium 2.4% and phosphorus 0.2% (Yadete, 2014). Since the protein content is so high, in Malawi, Thailand and Philippines, the leaflets of Leucaena are sun-dried for export to Japan and Singapore (TerMeulen* et al.*, 1979).

In spite of excellent source of nutrients, a Leucaenacounts toxic constituent who may severely limits its utilization to non-adapted animals. The foliage and pods of Leucaena contain toxic amino acid mimosine. Although quite toxic to non-ruminant animals, mimosine can be broken down by microbes in the rumen into non-toxic compounds. The microbes are naturally present in ruminants in Indonesia and Hawaii and probably other countries of South East Asia and Pacific (Shelton and Brewbaker, 1998).

Leucaena is also a good soil fertility improver because it can fix large amounts of atmospheric nitrogen. Sanginga* et al.* (1985) using difference method to evaluate nitrogen fixation by this plant give figures of nitrogen fixed in the range of 100 –
Because of its high nitrogen fixing ability and deep root systems, Ray et al. (1995) recommended to use Leucaena for developing sustainable grazing, increase productivity and protect biodiversity of the rangelands.

Despite its great importance and wide assortment of uses, establishment of Leucaena is difficult. One of the major constraints to its successful establishment is its high degree of hard seed due to an impermeable waxy coat which must be broken to increase its germination. Hard seeds that persist under unfavorable conditions are important in the regeneration of many species, but too high proportion of hard seed at the initial plant sowing can markedly reduce establishment. Various pretreatments have been proposed to overcoming hard seediness such as hot water, cold water, mechanical and chemical scarifications. As the successful establishment of plants depends initially on high germination percentage over the shortest period of time, the present study was carried out to determine the effects cold water, mechanical, and sulfuric acid scarifications on germination and early seedling growth of Leucaena.

Materials and Method

Seed collection

Mature seeds of Leucaena were harvested from the stand growing naturally in Hasanuddin University campus, Makassar, Indonesia (5º10’ S, 119º20’ E) with elevation of 7 m above sea level. Seeds were selected by sorting out the healthy and uniform seeds. Unhealthy and malformed seeds were discarded. Before the experiment was conducted, the viability of seeds was tested by floating the seeds in a container filled with tap water. The sunken seeds were selected for study while the floated seeds were discarded.

Experimental design and treatments

The experiment was conducted using Completely Randomized Design (CRD) with thirteen treatments and four replications. The treatments were as follows:

1. Freshly harvested seeds (control) (T1)
2. Soaking of seeds in cold water for 24, 48 and 72 hours (T5, T6 and T7)
3. Clipping of seed coat 1 – 2 mm from micropyle (T2)
4. Clipping of seed coat 1 – 2 mm from distal end (T3)
5. Sandpapering around the seed circumference for 10 minutes (T4)
6. Soaking of seeds in concentrated sulfuric acid (96%) for 4, 8, 12, 16, 20, and 24 minutes (T8, T9, T10, T11, T12 and T13).

The seeds under sulfuric acid scarification were gently stirred periodically and after treatment duration, the seeds were washed repeatedly in running tap water until they were considered safe to handle (5 to 10 minutes). After that, the seeds were spread in a thin layer for shade drying.

Germination and bioassay studies

Fifteen seeds of Leucaena were placed in each Petri dish (8 cm diameter) lined with one layer of filter paper. The filter papers were moistened with addition of distilled water throughout the experimental period. The Petri dishes were kept on laboratory bench at the temperature of 28 – 34º C and covered with lid to prevent moisture loss by evaporation. The germinated seeds were recorded daily until germination ended. After 9 days of incubation, germination indices, radicle length, hypocotyl length, and seedling vigor index were recorded/counted. Germination was regarded to have occurred when the radicle was observed. The length of radicle and hypocotyl were measured with ruler by taking five seedlings per Petri dish at random. When germination percentage was less than 30%, all seedlings were used as sample.

Germination indices measured were: 1 germination percentage (GP): total number of germinated seeds/total seeds x 100, 2 mean daily germination (MDG): total number of germinated seeds/total number of days of germination period, 3 germination speed (GS): was calculated following the formula given by Czabator (1962) as follows: \( n_1/d_1 + n_2/d_2 + n_3/d_3 + \ldots + n_n/d_n \), where \( n \) – number of germinated seeds and \( d \) – number of days, 4 mean germination time (MGT).
Volume 01//Issue 01//June 2016

was calculated following the formula given by Ellis and Roberts (1981):

\[ \text{MGT} = \frac{\sum nT_i}{\sum n} \]

where \( T_i \) is the number of days from the beginning of experiment and \( n \) is the number of newly germinated seeds on day \( T_i \), and 5 seedling vigor index (SVI) was determined according to formula given by Abdul-Baki and Anderson (1973) as: seedlings length (cm) x germination percentage/100.

**Statistical Analysis**

Data which obtained were subjected to analysis of variance using SPSS software version 16 and means of treatment were compared using least significant difference (LSD) at 5% probability level.

**Results and Discussion**

Different parameter related to seed germination and seedling growth were significantly affected by applied treatments. In general, compared to control, the treatments had stimulatory and inhibitory effects. The stimulatory effect was recorded in seeds soaked in sulfuric acid and mechanical scarification, with sulfuric acid had the highest stimulatory effect. The inhibitory effect was recorded in seeds soaked in cold water (Table 1).

**Table 1. Effects of treatments on various parameters related to germination and seedling growth of Leucaena.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GP (%)</th>
<th>MDG (%/day)</th>
<th>GS (seed/day)</th>
<th>MGT (days)</th>
<th>RL (cm)</th>
<th>HL (cm)</th>
<th>SVI</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>10.0</td>
<td>2.50</td>
<td>0.39</td>
<td>3.20</td>
<td>6.05</td>
<td>5.60</td>
<td>1.17</td>
</tr>
<tr>
<td>T2</td>
<td>6.00</td>
<td>2.00</td>
<td>0.21</td>
<td>3.25</td>
<td>4.83</td>
<td>4.90</td>
<td>0.58</td>
</tr>
<tr>
<td>T3</td>
<td>5.00</td>
<td>1.67</td>
<td>0.27</td>
<td>3.52</td>
<td>4.89</td>
<td>5.00</td>
<td>0.59</td>
</tr>
<tr>
<td>T4</td>
<td>6.00</td>
<td>1.50</td>
<td>0.31</td>
<td>3.64</td>
<td>4.81</td>
<td>4.13</td>
<td>0.54</td>
</tr>
<tr>
<td>T5</td>
<td>83.3</td>
<td>27.8</td>
<td>5.73</td>
<td>2.20</td>
<td>4.89</td>
<td>4.52</td>
<td>7.83</td>
</tr>
<tr>
<td>T6</td>
<td>83.0</td>
<td>27.7</td>
<td>5.89</td>
<td>2.16</td>
<td>4.50</td>
<td>4.47</td>
<td>7.45</td>
</tr>
<tr>
<td>T7</td>
<td>61.7</td>
<td>20.6</td>
<td>4.36</td>
<td>2.26</td>
<td>4.47</td>
<td>4.71</td>
<td>5.66</td>
</tr>
<tr>
<td>T8</td>
<td>55.0</td>
<td>9.17</td>
<td>1.91</td>
<td>3.34</td>
<td>4.43</td>
<td>4.70</td>
<td>5.02</td>
</tr>
<tr>
<td>T9</td>
<td>90.4</td>
<td>22.6</td>
<td>4.88</td>
<td>2.60</td>
<td>4.50</td>
<td>4.60</td>
<td>8.23</td>
</tr>
<tr>
<td>T10</td>
<td>92.6</td>
<td>23.2</td>
<td>6.25</td>
<td>2.28</td>
<td>4.30</td>
<td>4.50</td>
<td>8.15</td>
</tr>
<tr>
<td>T11</td>
<td>93.7</td>
<td>31.2</td>
<td>6.64</td>
<td>2.21</td>
<td>4.40</td>
<td>4.60</td>
<td>8.43</td>
</tr>
<tr>
<td>T12</td>
<td>96.7</td>
<td>48.4</td>
<td>6.83</td>
<td>2.09</td>
<td>4.30</td>
<td>4.47</td>
<td>8.48</td>
</tr>
<tr>
<td>T13</td>
<td>91.7</td>
<td>45.9</td>
<td>6.66</td>
<td>2.15</td>
<td>3.67</td>
<td>3.85</td>
<td>6.90</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>6.4</td>
<td>15.3</td>
<td>2.3</td>
<td>1.60</td>
<td>0.90</td>
<td>1.10</td>
<td>3.21</td>
</tr>
</tbody>
</table>

**Soaking of seeds in cold water**

Soaking the seeds in cold water produced the lowest GP, MDG, GS and SVI values compared to other treatments, including control (Table 1). The lower germination in seeds soaked in cold water compared to control was also reported by Amusa (2011) in Afzelia Africana and Azad et al. (2010) in Melia azedarach. This might be attributed to leaching out soluble food and auxin and the action of bacteria after soaking for a long time (Wheeler, 1965) and reduced concentration and availability of dissolved oxygen compared to the air (Amusa, 2010). However, the results of this study were opposite to the report of Owanubi et al., (2005) that soaking of Azadirachta indica seeds for 1, 12 and 24 hours resulted in increasing germination rate. This indicates that different
species have varying effect when they are subjected to soaking in cold water.

Mechanical scarification

The effect of mechanical scarification on germination in seeds of Leucaena is presented in Table 1. The results showed that scarification significantly enhanced germination of seeds of Leucaena compared to control. Highest GP, MDG, GS and lowest MGT values were recorded when seeds were clipped around micropyle and at distal end. Lowest GP, MDG, GS values were recorded when the seeds were sandpapered around circumferences. The enhancing results obtained by mechanical scarification in this study are in agreement with Duguma et al. (1998) that mechanical scarification is an efficient way of improving seed coat permeability in Leucaena. The high germination percentage as resulted from clipping at the micropyle or distal end sides of the seed results also reported by Oyebamiji et al. (2014) in *Spondias mombin* Linn, Saharan et al. (2001) in *Evolvulus alsinoides* and by Yogeesha et al. (2005) in *Bixa orellana*. Zubairu (2014) stated that the opening into the cotyledon of Leucaena seeds through micropyle or distal end allowed ready imbibition of water, which enhanced early germination. Besides, the enhanced rate of respiration may promote germination in mechanically scarified seeds.

Sulfuric acid scarification

All sulfuric acid treatments increased germination and improved early seedling growth of Leucaena. However, the highest GP, MDG, GS and SVI values was recorded when seeds soaked in sulfuric acid for 20 minutes, followed by soaking in sulfuric acid for 16, 12, 24, 8 and 4 minutes, respectively. Effect of duration of soaking was significantly different at 5% level of probability level (Table1). This result is in agreement to findings of Duguma et al. (1998) that acid scarification is the most effective way of improving coat permeability in seeds of Leucaena. The highest stimulatory effect of sulfuric acid scarification on seed germination and seedling growth was also reported in other plants such as *Tamarindus indic* (Muhammad and Amusa, 2003), in *Atriplex canescens* (Nosrati et al. (2008) and *Centrosema pubescens* (Rusdy, 2015). The observed significant differences indicated that acid scarification of seeds stimulated prompt and uniform germination. This finding is similar to the report of Dachung and Verinumbe (2006) that acid treatment of seeds removes the waxy layer of the seed coat by chemical decomposition of the seed coat components as that similar to breakdown process occurring during microbial attack. Sulfuric acid is thought to disrupt the seed coat and expose the lumens of the macroslerids cells, permitting imbibition of water which triggers the release of simple sugar that could be readily used for protein synthesis, thereby encouraging germination (Jackson, 1994).

The stimulatory effect of sulfuric acid scarification on seedling growth in the present study was different with the reports of Rusdy (2015) that scarification of pre-chilled seeds of Leucaena with sulfuric acid was ineffective to improve germination of Leucena compared to control; even it harmed the length of radicle and hypocotyl, resulting in the lower value of SVI. The reason for reduced seedling length of pre-chilled seeds and SVI value in sulfuric acid treatment could be the reduction of division and elongation of meristem cell in radicle and hypocotyl (Soomarin et al., 2010).

Conclusion

The results obtained in this study entail the important role of pre-treating Leucaena seeds prior to sowing for enhanced germination. Germination and early seedling growth of Leucaena varied among different pre-treatment methods. Soaking in sulfuric acid for 20 minutes is the best to be applied before sowing Leucaena seeds, followed by mechanical scarification and control. Soaking in cold water should not be applied to the seeds, because it’s harmful effect on germination and seedling growth. From the above results, it can be inferred that dormancy of seeds of Leucaena is associated with the seed coat, since
the treatments that induce germination were those that can effect disruption of the seed coat.

References


