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Enhancement of Seed Germination and Seedling Growth of Siratro (*Macroptiliumatropurpureum*)

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Abstract: ***The effect of cold water, hot water and sulfuric acid treatment on germination and seedling growth of Sirtaro (Macroptiliumatropurpureum) were studied. The results of various pre-treatments showed that soaking the seeds in cold water for 24 hours had the highest germination percentage and seedling growth, followed by immersing the seeds in sulfuric acid for 4 minutes and control. The lowest germination percentage and seedling vigor were found in the seeds soaked in hot water (80^{o}C) for 4 minutes. Thus, while the seeds of Siratro could be germinated without pre-treatment, to attain higher germination and better seedling growth, soaking the seeds in cold water at room temperature for 24 hour is recommended.***

Keywords: cold water, hot water, sulfuric acid, Siratro, germination, seedling growth

1. Introduction

Siratro (*Macroptiliumatropurpureum*) is a perennial herb with deep, swollen tap root and trailing, climbing and twining stems. Siratro originates from Central and South America and the Caribbean islands and now it has been widely cultivated and naturalized throughout the tropics and subtropics (Cook *et al*., 2005). Siratrois mainly used for permanent and short-term pastures. While best suited to grazing, Siratro also suited to cut and carry system or conserved as hay, although its twining habit makes harvesting is difficult. It is also used for soil conservation, as cover crop, and fallow crop. Siratro can be made to hay with difficulty because of the heavy loss by leaf drop, leaving stemmy material and young shoot. It can also be made to silage but ensilage of Siratrowithout adding molasses is never successful (Anon., 2016). Siratrois one of the tropical legumes released for commercial use in the 1990s (Jones, 2014).

Dry matter weight of Siratro is estimated to be 2.4 – 5.5 ton/ha and accumulated about 62 – 178 kg N/ha (Sato, 2016). The yield of Siratro decreased when intercropped with Nandi Setaria.but total annual yield increased up to 6.6 ton/ha/year. Siratro yield increased with increasing cutting intervaland lenient grazing should be utilized (Jones, 1974). N fixed by Siratro is approximately 291 kg/yr (Nutman, 1976).

Like most legumes, nutritive composition of Siratro is quite high. The crude protein content varied from 16.76 to 24.55%, cellulose and lignin contents varied from 14.42 to 26.82 and 5.82 to 9.17%, respectively (Singh *et al*., 1999). Its nutritive composition is affected by age at harvest;at 8 weeks of age, crude protein of Siratro was 17.9% and NDF and ADF were 35.1 and 32.5%, respectively. With increasing of age of harvest to 14 weeks, crude protein content decreased to 23.8%, while NDF and ADF content increased to 53.4 and 47.2%, respectively (Mupangwa*et al*., 2003).

The success and failure of pasture species is initially determined during the phase of germination and seedling establishment. The seed and seedling behavior characteristics help to determine establishment, spread, and resistance to aggression of competing plants (Cook and Lowe, 1977). Siratro is established by seeds. Hard seed levels are often high and seed should be scarified before planting (Anon., 2016a). Siratro when not scarified showed high percentages of hard seeds (de Almeida *et al*., 1979). As a successful establishment of pasture depends initially on high germination time over short period of time, it was decided to evaluate the effect of cold water, hot water, and acid scarification on germination and seedling growth of Siratro.

2. Materials and Method

Seed collection

Mature seeds of Siratro were obtained from cultivated plants in Sumba island, East Nusa Tenggara Province, Indonesia. The seeds were selected bysorting out the healthy and uniform seeds. Malformed and unhealthy seeds weeds were not used for the study. Uniform seeds were selected to reduce non-treatment variation as germination percentage and seedling vigor is correlated with seed size. Before the study started, the seeds were tested their viability by floating them in distilled water. The seeds that floated were assumed not to be viable and discarded and only the sunken seeds were used for the study.

Experimental design and treatment

The experiment was assigned in completely randomized design with treatments and each treatment consisted of three replications. The treatments were as follows:

1) Control (no seed treatment)
2) Cold water scarifications: seeds soaked in cold water at room temperatures for 24 hours and 48 hours.
3) Hot water: seeds immersed in hot water (80^{0} C) for 2 minutes and 4 minutes.
4) Acid scarification: seeds immersed in concentrated sulfuric acid (96%) for 4, 8, 12, 16 and 20 minutes.

The seeds immersed in sulfuric acid were gently stirred periodically and after treatment duration, the seeds were washed repeatedly withrunning tap water until they were considered safe to handle. Twenty seeds of Siratro were kept in sterile Petri dish (9 cm diameter) lined with one layer of

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filter paper. The Petri dishes were kept in laboratory at the temperature of 26 – 34^0 C and covered with lid to prevent the loss of moisture by evaporation. The germinated seeds were recorded daily until germination ceased. Germination was regarded to have occurred when the radicle was observed.After seven days incubation, the final germination and seedling indices i.e.rootlength (the longest root), shoot length and seedling vigor index were recorded/calculated. The length of root and shoot were measured by ruler by taking five seedling per Petri dish at random. When the number of germinated seeds were less than five, all seedlings were used as sample.

Measurement

Germination indices measured were: 1 germination percentage (GP): number of germinated seeds/total number of seeds in Petri dish x 100, 2 mean daily germination (MDG): germination percentage/total number of days of germination period, 3 germination period (GP): 4 germination speed (GS) was calculated from the following formula (Czabator, 1962) as follows: n1/d1 + n2/d2 + n3/d3 + ……., where: n – number of germinated seeds, and d – number of days, and 5 mean germination time (MGT), was calculated as follows (Ellis and Roberts, 1981):

$$MGT = \frac{\sum TiNi}{\sum Ni}$$

Where: Ti is the number of days from the beginning of experiment and Ni in number seeds germinated per day. Seedling vigor index (SGI) was determined as formula given by Abdul-Baki and Anderson (1973) as seedling length x germination percentage/100.

Data analysis

The data on germination and seedling indices were subjected to analysis of variance using SPSS software version 16. The means of treatment were compared using Least Significant Difference (LSD) test at 5% probability level.

3. Results and Discussion

Various parameters related to germination and seedling growth were significantly ($P < 0.05$) affected by treatments. In general, compared to control, the treatments had positive and negative effects on seed germination and seedling growth.The stimulatory effects were found in seeds soaked in cold water and sulfuric acid with short duration and the negative effect was found in seeds soaked in hot water (Table 1)

Table 1: Effect of cold water, hot water and sulfuric acid scarification onparameters related to germination and seedling growth of Siratro

Treatment	*GP (%)*	*MDG (%/day)*	*GS (seed/ day)*	*MGT (days)*	*RL (cm)*	*SL (cm)*	*SVI*
Control	43.0	10.8	10.1	2.07	5.25	4.67	4.67
Cold water for 24 h	66.7	13.4	8.75	2.03	6.33	7.91	7.91
Cold water for 48 h	45.0	9.0	8.75	2.02	4.07	4.80	4.80
Hot water for 2 min.	36.0	9.0	8.85	1.89	3.40	2.59	2.59
Hot water for 4 min.	16.6	4.15	6.58	1.89	2.80	1.06	1.08
H_2SO_4 for 4 min.	63.3	21.1	13.3	1.35	5.03	6.46	6.46
H_2SO_4 for 8 min.	55.0	18.3	11.2	1.13	3.20	3.92	3.92
H_2SO_4 for 12 min.	50.0	16.6	10.6	1.20	3.03	3.45	3.43
H_2SO_4 for 16 min.	20.0	6.67	4.33	1.14	1.89	0.86	1.08
H_2SO_4 for 20 min.	0	0	0	0	0	0	0
LSD 5%	22.3	3.24	2.40	0.72	2.24	3.24	3.35

Effect of soaking the seeds in cold water

Soaking the seeds in cold water for 24 hproduced the highest GP, RL, SL value, resulting in the highest SVIvalue (Table 1). The highest germination percentageas resulted from soaking the seeds in cold water for 24 hwas also was reported byOwonubi*et al* (2005) in *Azadirachtaindica* and by Haider*et al*. (2014) in*Acacia catechu*. This may be attributed to softening the seed coats, removal of inhibitor and reducing the time for germination (Hartman *et al*., 2007). Although soaking in the cold water for 24 h increased seed germination and seedling growth, however, soaking the seeds in coldwater for 48 h resulted in the lower GP, RL, SL and SVI compared to seeds soaked in cold water for 24 h (Table 1). This indicates that Siratro seeds are sensitive to prolonged soaking in cold water. This might have been due to possible leaching out of soluble food and auxins and the action of bacteria after soaking for a long time (Wheeler, 1965), and occurrence of oxygen deficiency of seeds with prolonged soaking (Roberston and Small, 1977)..

Effect of immersing the seeds in hot water

The effect of hot water on germination and seedling growth of Siratro is presented in Table 1. The results showed that immersing the seeds in hot water (80^o C) for 2 and 4minutes resulted in reducing all germination and seedling growth indices compared to control. Deleterious effect of immersing of seeds in hot water also reported in *Afzeliaafricana*by Amusa (2011) and in *Sesbaniarostrata* by Nath and Prasad (2000). This indicates that hot water pretreatment at 80^o C for 2 and 4 minutesare not suitable to be used to improve germination and seedling growth of Siratro. Hot water treatment perhaps lead to seed embryo being killed because of prolonged contact with hot water. Results of this study is contrary with the reports ofRusdy (2015) that soaking the seeds of *Centrosemapubescens* in hot water (80^o C) for 2 and 4 minutes increased seed germination by 500 and 590%, respectively. Truong and Hans (2007) reported that high temperature may affect either initial process of water uptake by seeds or biochemical processes that result in cell division. The vulnerability of seeds of Siratro to hot water treatment might be due to the long time of exposure of seeds to hot water. Rincon *et al*. (2003) reported that soaking the seed in

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hot water can induce seed germination, however, increasing the contact time of the seeds with hot water decreased seed germination.

Seedling growth performance followed similar pattern as that of germination. Hot water treatment reduced germination percentage, root and shoot length, resulting in decreased seedling vigor index. It is suggested that hot water treatment besides impaired germination, it also reduced cell elongation of root and shoot of Siratro.

Sulfuric acid scarification

Sulfuric acid scarification is well known to be effective to improving germination of species with hard seed coat. In the present study, the highest increase in seed germination and seedling growth of Siratro with acid scarification was observed in seeds immersed in sulfuric acid for 4 minutes, thereafter, germination percentage decreased with increasing of exposure time to the acid, and no germination was observed in Siratro seeds immersed in sulfuric acid for 20 minutes (Table 1). Moreover, although positive effect on germination over control observed until 12 minutes of exposure time to acid, the longer time of exposure decreased SVI, as resulted from decreasing RL and SL value.

Enhancing of germination of seeds immersed in seeds in sulfuric acid for 4 minutes from this study was below that reported by Rusdy (2015) that immersion of *Centrosemapubescens* in sulfuric acid for 3 minutes increased germination by 490%. This indicates that different species have varying responses when they are subjected to immersion of sulfuric acid. Degree of seed dormancy in Siratro seeds probably was lower than Centrosema seeds and prolonged immersion the seeds of Siratroto the acid to more than 4 minutesmight increase the killing of embryo.

The seeds subjected to sulfuric acid exhibited the highest MDG and GS value and the lowest MGT value (Table 1). This indicates that sulfuric acid scarification yielded highest germination within the shortest time. The widespread cause of seed dormancy is the presence of hard seed coat which prevents entrance of water, exchange of gases and/or mechanically constrained the embryo. Sulfuric acid is thought to promote the early ruptureof seed coat and expose the lumens of macrosclereids cells to imbibition of water which triggers early and fastgermination (Nikoleave, 1977).However, although acid scarification for 4 minutes resulted in high germination and seedling vigor index, this treatment is inferior compared to soaking the seeds in cold water, because its cost is higher and the application is not easy.

4. Conclusion

It can be concluded that the best method for breaking dormancy ofSiratro which resulted in the highest germination and seedling growth is soaking the seeds in cold water for 24 h. Therefore, this study recommends to farmers to adopt use of soaking the seeds in cold water, since it is safer and effective.

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