Enhancing Germination in Seeds of *Centrosema pubescens*

Muhammad Rusdy

Department of Forage Science and Grassland Management, Faculty of Animal Science Hasanuddin University, Indonesia

Abstract- Breaking of dormancy in seeds of *Centrosema pubescens* was investigated through laboratory experiment to elucidate the best method that can be used to enhance germination of the seed. The treatment were: 1) control, 2) scarification with sand paper, 3) immersion in hot water (80^{o}C) for 2, 4, 6, 8 and 10 minutes and 4) immersion in sulfuric acid for 3, 6, 9, 12, 15, 18, 21 and 24 minutes. The results showed that regardless of immersion time, sulfuric acid scarification had the highest germination percentage, followed by scarification with sand paper and immersion in hot water. The lowest germination percentage was obtained in control. Scarifications with sulfuric acid for 18 minutes was the best method for breaking dormancy of *Centrosema pubescens* which resulted in an increased total germination to 100% , the highest germination speed and the lowest germination time.

Index Terms- *Centrosema pubescens*, sulfuric acid, sandpapering, hot water, germination

I. INTRODUCTION

Centrosema pubescens, common name centro , is a perennial twining, trailing and climbing legume that native to sub-humid and humid regions of central and South America and now it has been naturalized in tropical Asia and Africa. It has a high potential adaptation to diverse habitat such as dry and high altitude of the tropics, poorly drained and/or seasonally flooded conditions and acids, low fertility soils (Schultze-Kraft et al., 1990). Centro is widely used as forage and source of protein, calcium and phosphorus to livestock. It can be intercropped with grasses and increasing crude protein content of associated grasses. Centro is also used as green manure crop in rubber, coconut and oil palm plantation (Lascano *et al.*, 1990). It can be grown for cover crops because it naturally suppressed weeds. Like other N-fixing legumes, centro is soil improver. Its association with grasses is beneficial to grass yield making N fertilization is unnecessary (Castillo *et al*., 2003). The amount of nitrogen fixed by centro is average of 259 kg/ha with ranged from 126 – 395 kg/ha (Adegboola and Fayemi, 1972).

Centro is suitable to be used as animal feed. Annual yields of green matter are around 5 – 14 tons/ha, but 40 tons/ha has been recorded (Ecocrop, 2009). It contains 18.29 crude protein, 57.35% NDF, 27.40% ADF, 8.14% ADL and 8.81% ash (Ajayi and Babayemi, 2008). Centro is a high quality forage and is recommended for pasture improvement in Indonesia.

Despite the great importance and characteristics, establishment of centro is difficult. One of the major constraint in its successful establishment is due to high proportion of hard seed (Verhoeven, 1958). Field observations have indicated that without seed treatment, germination of centro is low (Serpa and Achicar, 1970). High hard seed content in a seed lot can lead to delayed or decreased seedling emergence. As a result, plant stand becomes thin, sporadic and less competitive with weeds or undesirable species. Therefore, reduction of hard seed content in seed lot of centro is very important. As a successful establishment of plant depends initially on high germination rate over a short period of time, therefore, this study was conducted to find suitable treatment that would increase germination as well as to improve seedling quality of *Centrosema pubescens*.

II. MATERIALS AND METHOD

Seed collection

Mature seeds of *Centrosema pubescens* were collected from plant growing naturally in Hasanuddin University campus, Makassar, Indonesia (latitude 5^{o}10'S, longitude 119^{o}20'E) and 7 m above sea level, from August to September 2015. Seeds were selected by sorting out the healthy and uniform seeds. Malformed and unhealthy seeds were discarded. Uniform seeds were used to reduce non-treatment variation since germination percentage and seedling vigor is positively correlated with seed size. Before the experiment was done, the seeds were tested for viability by floatation method in distilled water. The seeds that floated were discarded and assumed not to be viable and only the seed that sunk were used for study.

Experimental design and treatment

The experiment was completely randomized design with 15 treatments and each treatment containing four replicates.
The seed treatments were as follows:

- Control (no seed treatment) (T0)
- Mechanical scarification: seeds scarified by rubbing between sand paper for 15 seconds (T1)
- Hot water scarification: seeds immersed in hot water (80^{o} C) for 2, 4, 6, 8 and 10 minutes (T2, T3, T4, T5 and T6).
- Acid scarification: seeds immersed in concentrated sulfuric acid (96%) for 3, 6, 9, 12 , 15, 18, 21 and 24 minutes (T7, T8, T9, T10, T11, T12, T13 and T15).

The seeds under hot water and sulfuric acids scarification were gently stirred periodically and after the treatment duration, seeds were washed thoroughly. Seeds that had been immersed in sulfuric acid, were repeatedly washed in running tap water until they were considered safe to handle.

Twenty five seeds of centro were kept in sterile Petri dish (9 cm diameter) lined with one layer of filter paper. The filter papers were kept saturated with addition of distilled water throughout the experimental period. The petri dishes were kept on laboratory bench at the temperature of 27 – 36° C and covered to prevent the loss of moisture by evaporation. The germinated seeds were recorded daily till the germination ceased. After 10 days of incubation, the final germination and length of seedling were recorded. Germination was regarded to have occurred when the radicle was observed.

Measurement

Germination indices measured were: 1) total germination (TG): number of germinated seeds/total number of seeds in petri dish x 100, 2) mean daily germination (MDG): total number of germinated seeds/total number of days of germination period, 3) germination period (GP): the time in the days between the first and the last germination events occurred, 5) germination speed (GS) was calculated following the formula given by Czabator (1962) as follows: n1/d1 + n2/ d2 + n3/d3 + ……, where: n – number of germinated seeds and d – number of days, 6) mean germination time (MGT), was calculated as formula given by Ellis and Roberts (1981) as follow:

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

Where Ti is the number of days from the beginning of experiment and Ni is number of seeds germinated per day.

Seedling vigor index (SGI) was determined according to the formula given by Abdul-Baki and Anderson (1973) as seedling length (cm) x germination percentage/100.

Data collection and analysis

The data on germination indices and seedling growth index were subjected to statistical analysis of variance. The means were compared using Least Significant Difference (LSD) test at 5% probability level.

III. RESULTS AND DISCUSSION

The results of the germination test for different methods of breaking dormancy are shown in Table 1. There was significant difference ($P < 0.05$) in parameters measured between treatments. Generally, it was observed that regardless of immersion time, sulfuric acid scarification was the method that had the highest TG, MDG, GS, SGI values and the lowest GP and MGT values, followed by seeds treated with sand paper and seeds immersed in hot water. The lowest TG, MDG, GS, SGI and the highest GP and MGT values were recorded in control (T0).

Table 1. Effect of different pre-treatment on germination indices and seedling growth of

Centrosem pubescens.

Treatments	TG (%)	MDG (%/day)	GP	GS (seed/day)	MGT	SGI
			(days)		(days)	
T0	11.00	1.10	10.20	0.38	5.17	1.04
T1	82.00	10.25	8.10	8.54	2.81	7.87
T2	66.00	8.25	10.40	3.85	5.62	5.48
T3	76.00	9.50	9.60	5.20	4.93	6.31
T4	58.00	7.25	8.70	4.72	3.15	4.79
T5	53.00	5.90	8.20	3.62	3.52	4.68
T6	47.00	5.22	7.60	2.94	4.52	3.90
T7	65.00	6.50	10.20	4.68	3.86	5.91
T8	86.00	9.55	9.80	7.93	3.40	7.40
T9	95.00	10.55	9.20	10.61	2.53	8.20
T10	100.00	11.11	8.30	11.71	2.10	8.50
T11	100.00	25.00	5.40	12.11	2.04	8.20
T12	100.00	33.33	2.10	12.50	2.01	7.90
T13	96.00	19.20	5.40	11.77	2.09	7.60
T14	95.00	19.00	5.40	11.60	2.19	7.50
LSD at 5%	14.25	5.40	4.21	4.65	1.21	1.32

Sulfuric acid scarification is well known to be effective to improving germination of species with hard seed coat (Youssef, 2008). The positive effect of sulfuric acid scarification on seeds of centro indicates that the low germination of centro is probably due to physical dormancy which is agreement with previous research reported in legumes (Missanjo *et al*., 2013; Olatunji *et al*., 2013)). This also is in agreement with Wine Pe *et al.* (1974) who reported that percentage of germination of centro increased from 30 to 80% when the seeds were treated with concentrated sulfuric acid. The superiority of sulfuric acid scarification over other breaking dormancy methods had also been reported in *Flemingia macrophylla* by Asare and Otsyina (1980), in *Parkia biglobosa* by Aliero (2004), and in *Vitellaria paradoxa* by Iroko *et al*. (2013)

Time of exposure of seeds to sulfuric acid is very critical. In the present study, as increasing soaking time, TG, MDG and GS values were increased and peaked at 18 minutes, but with the longer time of exposure, the values of the three germination indices were decreased. This is agree with *Missanjo* et al. (2013) that insufficient soaking may be not effective enough as it just makes the seed coat glossy, conversely, exposure of seeds to the chemical in the long time may damage the embryo.

Treatment with sulfuric acid for a period of 18 minutes gave the lowest MDG value. Compared to control, it reduced the amount of MGT value by 60%. This indicates that immersion of seeds in sulfuric acid for 18 minutes was quickly ruptured the seed coats, thereby resulted in high TG, MDG and GS values and less germination time. Therefore, immersion of seeds in sulfuric acid for 18 minutes is the best option to obtain uniform and rapid germination in seeds of centro. This is in line with Agbodogi *et al* (2007) that the soaking of *Dacryodes edulis* seeds in concentrated sulfuric acid reduced the GP value considerably and concluded that it was the best method although it dangerous to handling. The harmful effect of immersing sulfuric acid for 21 and 24 minutes indicates that prolonged immersion of seeds was injurious to seeds of centro as the acid may damage the vital parts of the embryo or may hinder the embryo metabolism. In nature, germination of centro may extend over weeks, months or even years and in order to propagate centro efficiently, it therefore is necessary to treat the it seeds with concentrated sulfuric acid before sowing to ensure not only a high final germination percentage but also a rapid and an uniform germination.

Sulfuric acid scarification affected SGI. SGI value continued to increase from 3 to 15 minutes of immersing of seeds in sulfuric acid, but at 18, 21 and 24 minutes of immersing, SGI value decreased (Table 1). This decrease in SGI value of seeds immersed for 21 and 21 minutes was attributed to both the lower TG value and length of seedling. However, in seeds immersed for 18 minutes, the decrease of SGI was purely attributed to lower seedling length, as TG value was 100%. This might be attributed to the abnormality of seed metabolism under prolonged of seed immersion.

In the present study scarification with sand paper was the second best pre-sowing treatment method. Mechanical scarification with sand paper significantly increased seed germination compared to control and hot water scarification. This result agrees with Duguma *et al*. (1998) and Aduradola *et al*. (2005) that mechanical scarification was the most effective way of improving seed coat permeability in seeds of *Leucaena leucocephala* and *Chrysophyllum abidum*, respectively. The damaging of lignified palisade cells after sandpapering that permitting water and oxygen entering the cells may be the causative factor for positive effect of mechanical scarification (Yildiztugay *et al*., 2012).

Hot water scarification had a positive effect on breaking dormancy. It was recorded that TG, MDG, GP, GS and SGI values increased as increasing soaking time up to 4 minutes, then they were decreased, conversely, MGT value increased up to 4 minutes of soaking and after that, it decreased (Table 1). The highest of some germination indices of seeds immersed in hot water for 4 minutes might be attributed to the increased penetration of water and oxygen into the seeds. Deleterious effects of immersing the seeds for 6, 8 and 10 minutes might be due to the death of embryo as caused by long time of exposure to hot water. Rincon et al. (2003) reported that soaking the seed in hot water induced seed germination, however, increasing the contact time of the seeds with hot water decreased seed germination.

IV. CONCLUSION

This study showed that scarification of seeds with sulfuric acid, sand paper, and hot water significantly induced germination in seeds of *Centrosema pubescens*. The best germination value was recorded from acid sulfuric scarification, followed by sand paper scarification and immersion in hot water. Although sulfuric acid had the highest positive effect in breaking seed dormancy, because its application by most farmers is not easy, therefore, sand papering and hot water treatments could be considered for substitution.

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AUTHORS

First Author – Dr.Muhammad Rusdy, Department of Forage Science and Pasture Management, Faculty of Animal Science Hasanuddin University, South Sulawesi, Indonesia
Email address: muhrusdy79@yahoo.co.id