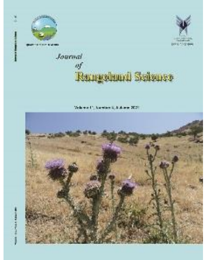

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Journal homepage: www.rangeland.ir

Research and Full Length Article:

Germplasm Collection and Germination Rate Determination of *D esmodium dichotomum* in Eastern Amhara, Ethiopia

Hunegnaw Abebe
Lecturer, Wollo University, Department of Animal Sciences, Dessie, Ethiopia, (corresponding author). Email: hunegnaw332@gmail.com

Received on: 31/08/2020
Accepted on: 11/03/2021

Abstract. The study was conducted to collect germplasm and identify appropriate seed treatment technique to enhance germination of *Bouffordia dichotoma* syn. *Desmodium dichotomum,* which is self-generating wild legume forage growing in eastern Amhara (North Wollo, South Wollo and Oromia Special Zones), Ethiopia. Twenty six *Desmodium dichotomum seed* populations (1000 seeds from each) were collected in 2018 from fields using Ethiopian biodiversity institute sample population collection format by considering agro ecological similarity and physical barrier to genetic mixing. The samples were collected from sorghum growing farm-lands. Breaking of dormancy in seeds of *Desmodium dichotomum* collections was investigated through laboratory experiment to elucidate the best method that can be used to enhance germination of the seed. To test the effectiveness of pre sowing treatments on germination of *Desmodium dichotomum* collected seed lots, the following treatments were imposed under a completely randomized design with 3 replications of 60 seeds each: mechanical scarification by sand paper; boiled water at 100^{o}C for 4 minutes; and untreated seeds as control. Germination percentage was highest ($p < 0.05$) for scarification (99.4%) followed by boiled water (79.15%) and untreated seeds (36.58%), respectively. Mechanical scarification greatly reduced germination time as most seeds (68.7%) sprouted in the first 5 days and germination was completed at day 9. Similarly, boiled water made sprout the seeds in the first 5 days (65%) and completed germination at day 10. However, non-treated seeds had started to germinate at day 12 (1%). Scarification by sand paper was quite effective in increasing germination of *Desmodium dichotomum* collected seeds. Further improvements in germination of *Desmodium dichotomum* could be expected in different boiling water temperature with different minutes and acid treatment methods.

Key words: Boiled water, Germination, Germplasm collection, Scarification

Introduction

From a recent study, *Bouffordia dichotoma* syn. *Desmodium dichotomum,* an herbaceous native legume is recognized in North Wollo, South Wollo and Oromia Special Zones of Ethiopia. The average yield of *Desmodium dichotomum* (locally called *chimero*) growing as a self-sown legume with sorghum (*Sorghum* spp.) was 4,400 kg DM/ha **(**Hunegnaw, 2020**)**. Mean chemical composition was 15.4% ash, 22% CP, 31% NDF, 26% ADF and 5.8% ADL, while IVDMD was 61%. Mineral concentrations were: 0.6% Ca, 0.23% P, 1.5% K, 0.78% Mg, 0.01% Na, 0.27% S, 0.16% Fe, 4.4 mg/kg Cu, 45 mg/kg Mn and 12.3 mg/kg Zn. Chimero appears useful as a supplement for feeding to ruminant animals **(**Hunegnaw, 2020**).**

Seed collecting is a well-defined scientific procedure, widely used for the ex-situ conservation of plant genetic resources (Smith *et al*., 2003). Studies about *Desmodium dichotomum* for preservation and further studies was not carried out yet. Hence, there is a need to collect germplasm (seed) and document its natural distribution and ecological status as to any threats of its habitat.

Moreover, conducting seed germination test of *Desmodium dichotomum* is essential to know if it needs further seed treatment once seeds were collected from fields of the seven districts. In seed testing germination has been defined as "the emergence and development of the seedling to a stage where the aspects of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favorable conditions in soil" (ISTA, 2015). Germination, emergence and establishment of legumes depend on the interaction of biological, environmental and management variables. In semi-arid and arid conditions, which prevail in parts of Ethiopia, seedling emergence and establishment are constrained mainly by the irregular distribution of rainfall within a season. Apart from this, seed size, weight, dormancy and integument thickness have significant effects on the emergence and establishment of seedlings from soil seed banks under natural conditions (Sy *et al*., 2001). Important factors controlling the variation in seed dormancy within species include the environment of the mother plant during the time of seed maturation and environmental conditions after the seeds have been released (Liebst and Schneller, 2008).Despite the great importance, establishment of most forage legumes is difficult. One of the major constraint in successful stand establishment of forage legumes is its high degree of hard seed, which can cause delayed or decreased their germination, seedling emergence and growth. Many efforts have been made to investigate seed germination and seedling emergence of different annual and perennial species (Chauhan and Johnson, 2008; Liebst and Schneller, 2008; Liza *et al*., 2010). However, yet a study has not been conducted on germination patterns of *Desmodium dichotomum.* As a result, this study is proposed to collect germplasm and identify appropriate pre-planting seed treatment on dormancy breaking and germination of *Bouffordia dichotoma* syn. *Desmodium dichotomum*.

Materials and Methods

Germplasm collections

The seed samples of *Desmodium dichotomum* were collected from South Wollo Zone (Ambasel, Tehuledere and Kalu districts), and from North Wollo Zone (Habru, Gubalafto and Kobo districts) and from Oromia Special Zone (Dawa-Chafa district) (Figure 1). After collection was completed, the seeds were air dried, cleaned and stored in brown paper bags at room temperature (17 °C) for one week an d then examined immediately. The Total pr ecipitation ranges from 500 mm/year to 15 57 mm/year. The average minimum and maximum annual temperature ranges from 13ºC to 25ºC (N MSAKS, 2019). The major soil types exten sively distributed in the districts are Lithic Leptosols, Eutric Cambisols and the Eutric Leptosols.

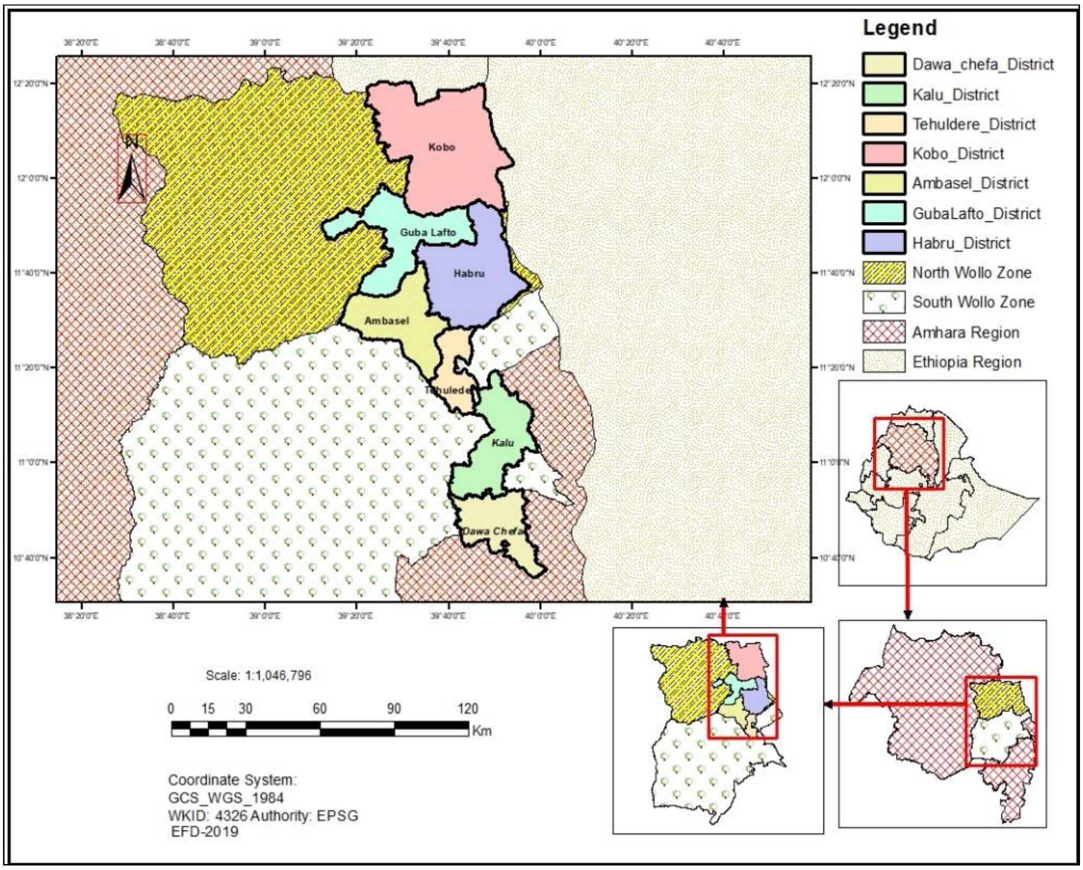

Fig. 1. Map of the study districts

Field data forms used for collection were Ethiopian biodiversity institute collection format for forage genetic resource conservation. The collecting equipment used was Geographical Positioning System (GPS), protective clothing and seed collecting bag. The collection sites of *Desmodium dichotomum* germplasm extend between latitudes 11° N in each collection site and between longitudes 39° E (Table 1). There seem to be ecological niches specific to *Desmodium dichotomum*. It is usually found under sorghum species. No studies have been indicating why usually *Desmodium dichotomum* have been more pronounced under sorghum species. The dominant species were herbaceous weedy legumes and no as such major threats were observed but in some areas invasive species like Parthenium hysterophorus observed as a threat**.**

Table 1. Locations (Altitude, Latitude and Longitude) of *Desmodium dichotomum* seed sample populations

Collections/entries	Altitude	Latitude	Longitude
*D. dichotomum*1979	1659	11º57’53.795’’N	39º41’35.582’’E
*D. dichotomum*1980	1539	11º33’47.256’’N	39º40’31.289’’E
*D. dichotomum*1981	1496	11º22’29.264’’N	39º39’23.684’’E
*D. dichotomum*1982	1495	11º50’13.447’’N	39º45’01.234’’E
*D. dichotomum*1983	1500	11º21’33.563’’N	39º34’36.254’’E
*D. dichotomum*1984	1874	11º48’56.996’’N	39º35’07.539’’E
*D. dichotomum*1985	1625	11º40’53.675’’N	39º39’34.114’’E
*D. dichotomum*1986	1652	11º41’26.135’’N	39º38’56.951’’E
*D. dichotomum*1987	1605	11º33’48.563’’N	39º39’36.682’’E
*D. dichotomum*1988	1888	11º44’40.400’’N	39º37’43.143’’E
*D. dichotomum*1989	1716	11º58’74.635’’N	39º42’37.635’’E
*D. dichotomum*1990	1496	11º26’04.621’’N	39º39’12.423’’E
*D. dichotomum*1991	1583	11º27’49.231’’N	39º37’10.779’’E
*D. dichotomum*1992	1680	11º45’67.358’’N	39º41’29.643’’E
*D. dichotomum*1993	1596	11º40’37.271’’N	39º37’35.692’’E
*D. dichotomum*1994	1694	11º44’08.245’’N	39º43’35.593’’E
*D. dichotomum*1995	1616	11º24’49.038’’N	39º37’01.232’’E
*D. dichotomum*1996	1642	11º22’54.842’’N	39º39’35.582’’E
*D. dichotomum*1997	1632	11º35’65.654’’N	39º38’36.524’’E
*D. dichotomum*1998	1597	11º25’06.685’’N	39º32’35.289’’E
*D. dichotomum*1999	1607	11º42’08.286’’N	39º31’31.659’’E
*D. dichotomum*2000	1478	10º26’09.036’’N	39º39’35.582’’E
*D. dichotomum*2001	1475	10º53’44.011’’N	39º48’23.066’’E
*D. dichotomum*2002	1473	10º53’50.452’’N	39º48’22.862’’E
*D. dichotomum*2003	1537	11º56’36.416’’N	39º46’41.610’’E
*D. dichotomum*2004	1506	11º48’56.996’’N	39º39’35.582’’E

Germination test

The collected *Desmodium dichotomum* seeds were transported and stored in Wollo University (at laboratory of animal sciences department), Ethiopia. A total of 26 seed populations of *Desmodium dichotomum* were used for germination test.

A factorial combination of these seed populations and three seed pretreatments (untreated or control, mechanical scarification and boiling water at 100ºc for 4 minutes) were used in a completely randomised design with three replications. Two grams of seeds from each collection were subjected to either mechanical scarification (rubbing the seeds between sand papers) or boiling water treatment (placing seed in boiling water and leaving until the water cooled). After the seed treatments, 60 seeds from each treatment were placed in petri dishes fitted with moist filter paper for the test of germination. These were placed in a growth cabinet set to 12 hours light/12 hours’ dark. Seeds were adequately watered throughout the experimental period with distilled water.

Germination counts were made every three days for 15 days. Seeds were considered germinated when the radicle was emerged through the integument and the germinated seeds were removed after each count. At the end of the test, seeds that are not germinated were categorised into hard and dead components by touching and piercing with a needle. While dead seeds could be pierced with the needle, hard seeds could not.

Statistical Analysis

The percentages of germinated, hard and dead seeds were subjected, after arcsine transformation, to analysis of variance using Proc GLM of SAS (2002). When Fisher’s F values were significant at $p < 0.05$, the analysis were continued by comparing the means using Tukey’s test at $p < 0.05$.

Results

Germination test

There was a significant ($p < 0.05$) difference between effect of the seed treatments, but there was no a significant ($p > 0.05$) difference between *Desmodium dichotomum* seeds.

Hard seed breakdown

In all collected seed populations, the percentage of hard seed remaining at the end of the germination test was significantly higher ($p < 0.05$) in the control seeds than in those either scarified or treated with boiled water. Scarification broke hard seed dormancy to a significantly ($p < 0.05$) greater extent than boiling water treatment in all accessions (Table 2). Scarification would fracture the seed testa in many places and allow rapid imbibition of water, while the boiling water treatment would rupture the seed coat by ejecting the strophiolar plug and cracking the testa. In the case of the boiling water treatment, water imbibition would occur over a relatively longer period of time than with the fractured seed testa from scarification.

Table 2. Percentage of hard seeds after incubation

Collections/entry	Untreated (%)	Seed treatment (%)	
		Scarification[1]	Boiled water[2]
*D. dichotomum*1979	65[a]	2[c]	19[b]
*D. dichotomum*1980	70[a]	1[c]	19[b]
*D. dichotomum*1981	73[a]	0[c]	24[b]
*D. dichotomum*1982	55[a]	0[c]	17[b]
*D. dichotomum*1983	68[a]	0[c]	17[b]
*D. dichotomum*1984	71[a]	0[c]	16[b]
*D. dichotomum*1985	56[a]	1[c]	20[b]
*D. dichotomum*1986	60[a]	1[c]	22[b]
*D. dichotomum*1987	74[a]	2[c]	22[b]
*D. dichotomum*1988	70[a]	0[c]	22[b]
*D. dichotomum*1989	67[a]	0[c]	21[b]
*D. dichotomum*1990	59[a]	0[c]	17[b]
*D. dichotomum*1991	60[a]	0[c]	17[b]
*D. dichotomum*1992	62[a]	2[c]	15[b]
*D. dichotomum*1993	72[a]	1[c]	20[b]
*D. dichotomum*1994	58[a]	2[c]	21[b]
*D. dichotomum*1995	65[a]	0[c]	20[b]
*D. dichotomum*1996	55[a]	0[c]	17[b]
*D. dichotomum*1997	60[a]	0[c]	21[b]
*D. dichotomum*1998	62[a]	2[c]	19[b]
*D. dichotomum*1999	63[a]	0[c]	23[b]
*D. dichotomum*2000	59[a]	1[c]	24[b]
*D. dichotomum*2001	62[a]	0[c]	18[b]
*D. dichotomum*2002	66[a]	0[c]	19[b]
*D. dichotomum*2003	60[a]	0[c]	23[b]
*D. dichotomum*2004	57[a]	0[c]	20[b]

[1]Seed rubbed with sandpaper.

[2]Seed immersed in boiled water and left until the water cooled down.

[abc]Means in the same row with significantly different letters are different at $p < 0.05$.

Germination and dead seeds

The percentages of germinated seed popula tions and dead seed populations from the tr eatments are presented in Tables 3 and 4, r espectively. In all the seed collections, whi le scarification significantly ($p<0.05$) incre ased the total germination percentage comp ared with the control (Tables 3), it simultan eously and significantly ($p< 0.05$) increase d the level of seed mortality collections relative to the control (Tables 4). In the present study, most seeds germinated within the first 5 days of count under the sand paper scarification treatment. This was closely followed by the boiled water treatment where the majority of germination was observed at the day 5 count.

Table 3. Percentage of germinated seeds after incubation

Collections/entry	Untreated (%)	Seed treatment (%)	
		Scarification[1]	Boiled water[2]
*D. dichotomum*1979	35^{c}	98^{a}	78^{b}
*D. dichotomum*1980	30^{c}	99^{a}	80^{b}
*D. dichotomum*1981	27^{c}	100^{a}	76^{b}
*D. dichotomum*1982	45^{c}	100^{a}	81^{b}
*D. dichotomum*1983	32^{c}	100^{a}	80^{b}
*D. dichotomum*1984	29^{c}	100^{a}	80^{b}
*D. dichotomum*1985	44^{c}	99^{a}	79^{b}
*D. dichotomum*1986	40^{c}	99^{a}	78^{b}
*D. dichotomum*1987	26^{c}	98^{a}	78^{b}
*D. dichotomum*1988	30^{c}	100^{a}	78^{b}
*D. dichotomum*1989	33^{c}	100^{a}	79^{b}
*D. dichotomum*1990	41^{c}	100^{a}	81^{b}
*D. dichotomum*1991	40^{c}	100^{a}	82^{b}
*D. dichotomum*1992	38^{c}	98^{a}	82^{b}
*D. dichotomum*1993	28^{c}	99^{a}	78^{b}
*D. dichotomum*1994	42^{c}	98^{a}	79^{b}
*D. dichotomum*1995	35^{c}	100^{a}	80^{b}
*D. dichotomum*1996	45^{c}	100^{a}	82^{b}
*D. dichotomum*1997	40^{c}	100^{a}	79^{b}
*D. dichotomum*1998	38^{c}	98^{a}	79^{b}
*D. dichotomum*1999	37^{c}	100^{a}	77^{b}
*D. dichotomum*2000	41^{c}	99^{a}	76^{b}
*D. dichotomum*2001	38^{c}	100^{a}	80^{b}
*D. dichotomum*2002	34^{c}	100^{a}	80^{b}
*D. dichotomum*2003	40^{c}	100^{a}	76^{b}
*D. dichotomum*2004	43^{c}	100^{a}	80^{b}

[1]Seed rubbed with sandpaper.
[2]Seed immersed in boiled water and left until the water cooled down.
[abc]Means in the same row with significantly different letters are different at $p<0.05$.

Table 4. Percentage of dead seeds after incubation

Collections/entry	Untreated (%)	Seed treatment (%)	
		Scarification[1]	Boiled water[2]
*D. dichotomum*1979	0^{a}	0^{a}	3^{a}
*D. dichotomum*1980	0^{a}	0^{a}	1^{a}
*D. dichotomum*1981	0^{a}	0^{a}	0^{a}
*D. dichotomum*1982	0^{a}	0^{a}	2^{a}
*D. dichotomum*1983	0^{a}	0^{a}	3^{a}
*D. dichotomum*1984	0^{a}	0^{a}	4^{a}
*D. dichotomum*1985	0^{a}	0^{a}	1^{a}
*D. dichotomum*1986	0^{a}	0^{a}	0^{a}
*D. dichotomum*1987	0^{a}	0^{a}	0^{a}
*D. dichotomum*1988	0^{a}	0^{a}	0^{a}
*D. dichotomum*1989	0^{a}	0^{a}	0^{a}
*D. dichotomum*1990	0^{a}	0^{a}	2^{a}
*D. dichotomum*1991	0^{a}	0^{a}	1^{a}
*D. dichotomum*1992	0^{a}	0^{a}	3^{a}
*D. dichotomum*1993	0^{a}	0^{a}	2^{a}
*D. dichotomum*1994	0^{a}	0^{a}	0^{a}
*D. dichotomum*1995	0^{a}	0^{a}	0^{a}
*D. dichotomum*1996	0^{a}	0^{a}	1^{a}
*D. dichotomum*1997	0^{a}	0^{a}	0^{a}
*D. dichotomum*1998	0^{a}	0^{a}	2^{a}
*D. dichotomum*1999	0^{a}	0^{a}	0^{a}
*D. dichotomum*2000	0^{a}	0^{a}	0^{a}
*D. dichotomum*2001	0^{a}	0^{a}	2^{a}
*D. dichotomum*2002	0^{a}	0^{a}	1^{a}
*D. dichotomum*2003	0^{a}	0^{a}	1^{a}
*D. dichotomum*2004	0^{a}	0^{a}	0^{a}

[1]Seed rubbed with sandpaper.
[2]Seed immersed in boiled water and left until the water cooled down.
[a]Means in the same row with significantly different letters are different at $p < 0.05$.

Discussion

In essence, there was no problem in collecting within a population until an obvious barrier (the physical bridge that prevents genetic mixing such as valley, mountain) to genetic exchange. It was kept then samples either side of these barriers separate. The nature of these barriers will depend on the pollen and seed dispersal method of the species. Occasionally, some pollen or seed may travel extreme distances but where this happens the genetic effects within the recipient population are assumed to be swamped by locally-produced pollen and seeds. Therefore, isolation will rarely be absolute; a low probability of exchange will exist. Most dispersal will usually be local. For instance, most seeds disperse less than 100m (Cain *et al*., 2000). Wind-blown pollen and animal carried pollen can travel much greater distances. *Desmodium dichotomum* is wind-blown cross pollinating plant and animal and human is the seed dispersal method.

In an ideal world with infinite resources, it would be advisable to collect every population within a taxon’s distribution to ensure complete sampling of its genetic variation. In reality, this will not be possible except for the most restricted species where all populations are known (Brown and Marshall, 1995). In some cases, details such as breeding system, ecological specialization and detailed distribution may be known and deductions can

be made about likely gene flow and the nu mber of populations that should be sample d (Hamrick *et al*., 1991). It would then be a dvisable to keep samples either side of barr ier separate. There was no as such differen ces in environmental conditions at different geographic locations are likely to impose different selection pressures on the target taxon’s populations and thereby promote genetic differentiation between them. Thus, the territory was dividing under consideration into areas using available eco-geographic data, and assuming that the more distant and environmentally diverse two populations were the more genetically diverse. Maxted *et al*., (1995) described the use of eco-geographical surveys in the selection of collecting sites while the use of gene ecological zonation is outlined by Dulloo *et al*., (2008). Extensive information can also be found in Bacchetta *et al*., (2008).

The effect of environment on plant species is a factor that should currently be considered in collecting strategies using Geographic Information Systems GIS (Draper *et al*., 2004). GIS is also an important tool to characterise environmental features of the provenance of the samples improving success when the material will be used. The main benefit is the increment in efficiency of collections, cost-reduction of collecting missions, and the increase in the genetic diversity of species sampled. Considering the agro ecological similarity and physical barrier to genetic mixing 26 *Desmodium dichotomum seed* populations (1000 seeds from each) were collected from Kobo, Gubalafto, Tehuledere, Habru, Ambasel, Kalu and Dawa Chafa districts. The collected samples were pre cleaned in the field to evaluate their quantity and to prepare them for transporting and were air dried, cleaned and stored in brown paper bags at room temperature of (17 °C).

In all the collected seed populations, the percentage of hard seed remaining at the end of the germination test was significantly higher ($p < 0.05$) in the control seeds than in those either scarified or treated with boiled water (Table 2). The integument of the seed of many leguminous species is resistant to the penetration of water (Skerman, 1982), e.g. Cassia obtusifolia (Sy *et al*., 2001). This results in poor germination caused by hard seed coat dormancy, which can be overcome in most by treating seed to reduce the impermeability of the integuments. Hard seediness is an important trait that enhances survival of a species to the next generation by ensuring sequential germination of seeds from the soil seed bank in semiarid and arid areas, which are often characterized by extreme and high climatic variability (Abubeker, 2004). Scarification broke hard seed dormancy to a significantly ($p < 0.05$) greater extent than boiled water treatment in all collected seeds. This is probably due to scarification fracturing the seed test and allowing rapid imbibition of water, while the effect of boiled water treatment is mainly through rupturing of the seed coat by ejecting the strophiolar plug and cracking the testa (Argel and Paton, 1999). Hot-water treatment has been reported to enhance germination by affecting various factors, viz. seed coat permeability for water and gaseous exchange and release of inhibitors (Sharma *et al*., 2008). The collections known to have low germination rates showed 75 – 95 % hard seed after the two-week germination test in untreated controls.

This high proportion of hard seed is similar to that reported in an earlier pilot study (Abubeker, 2004). High proportion of hard seed has been observed in the control.

Germination of seeds of *Desmodium dichotomum* was influenced by the different treatment techniques applied (Table 3). Scarification significantly ($p < 0.05$) increased the total germination percentage compared with boiled water and the control. Final germination percentage was highest for scarification (99.4%) and the hot water (79.15%) treatments. While untreated seeds (control) attained only 36.58% germination counts. Mechanical scarification by hand with sandpaper was quite effective in increasing germination of *Desmodium dichotomum* seed lots. This agrees with the results of Abubeker (2004), who reported scarification significantly ($p < 0.05$) increased the total germination percentage of *Indigofera accessions* compared with the hot water and control. Even if sulfuric acid treatment is best in studies such as Mohammed (2015), who reported immersion of *Centrosemapubescens* seeds in sulfuric acid for 18 minutes is the best option to obtain uniform and rapid germination and soaking of *Dacryodesedulis* seeds in concentrated sulfuric acid was the best method, it dangerous to handling (Agbodogi *et al*., 2007). This indicates that sulfuric acid treatment is not safe for the farmers though have short germination responses in many species. Sandpaper scarification of the seed testa greatly reduced germination time as most seeds (68.7%) sprouted in the first 5 days and germination was complete by day 9 followed by the boiled water start in germinate in the first 5 (65%) by day 10 germination was completed. The non-treated collections had started to germinate at day 12 (1%). Lignified palisade cell layer in the seeds could be damaged after sandpapering and germination occurs with water penetration (Yildiztugay *et al*., 2012). This response supports evidence that the seed coat of the plant is the main inhibitor of germination. In the other study by Tadros *et al*., (2011), sandpaper scarification treatment of *L. leucocephala* was not enough to overcome the physical barrier to allow germination and thus failed to facilitate water imbibition or permeability of the seed coat to water and oxygen. This indicates that the germination response of different forage species is quite different in different seed treatments. In the present study, most seeds germinated within the first 5 days of count under the sandpaper scarification, while none went beyond 9 days. This was closely followed by the boiled water treatment where the majority of germination was observed at the day 5 count.

Conclusion

An effective treatment method should significantly improve germination rate of the seed lots without causing a significant increase in the mortality of potentially viable seeds. This study showed that scarification of seeds by sand paper and boiled water significantly induced germination in *Desmodium dichotomum* *seed lots*. The best germination value was recorded from sand paper scarification. Further improvements in germination of *Desmodium dichotomum* could be expected in different boiling water temperature with different minutes and acid treatment methods.

Acknowledgements

I thank Wollo University for financial support, Mr. Muluken Getachew and Mr. Mekasha Seid (Wollo University Animal Sciences Department, laboratory assistants) for their help in all activities of collection and germination test.

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