academicJournals

Vol. 13(50), pp. 4557-4561, 10 December, 2014
DOI: 10.5897/AJB2014.14223
Article Number: 5A4153548964
ISSN 1684-5315
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Seed pre-treatment methods for improving germination of *Acacia tortilis*

So Hanaoka[1]*, Norio Nakawa[1], Norihisa Okubo[1], Stephen Fredrick Omondi[2] and Jason Kariuki[2]

[1]Forest Tree Breeding Center, Forestry and Forest Products Research Institute, 3809-1 Ishi, Hitachi City, Ibaraki, 319-1301, Japan.
[2]Kenya Forestry Research Institute, P.O. Box 20412 – 00200, Nairobi, Kenya.

Received 6 October, 2014; Accepted 2 December, 2014

***Acacia tortilis* (Forsk.) Hayne is considered as an important dryland tree species in Africa and Middle East, and establishing an effective and efficient seed-germination method is considered necessary for producing planting materials. In this study, new germination method was compared with some of the previously reported methods and evaluated in *A. tortilis*. In the new method, root setting was observed within 3 days and the final germination percentage was 94%; higher than 5 to 86% reported in the previous methods. New method is therefore considered to be suitable for geminating *A. tortilis* seeds.**

Key words: *Acacia tortilis*, seed germination, pre-treatment of seed germination.

INTRODUCTION

Acacia tortilis (Forsk.) Hayne (subfamily Mimosoideae, family Leguminosae) is one of approximately 135 African *acacia* species. The morphology of *A. tortilis* varies considerably, ranging from multi-stemmed shrubs (ssp. tortilis) to trees measuring up to 20 m in height with rounded (ssp. raddiana) or flat-topped (ssp. heteracantha and spirocarpa) crowns. The species is widely distributed throughout the Middle East and south into eastern and southern Africa, in habitats ranging from semi-desert to dry bushland and grassland (Allen, 2007; Hines and Eckman, 1993). In Africa, *A. tortilis* occurs in the drier parts of Senegal and Somalia and extends all the way down to South Africa. The species is highly drought resistant and grows in areas with annual rainfall ranging from as low as 40 mm to as much as 1200 mm, with dry seasons lasting 1-12 months. *A. tortilis* tree can be used for a variety of purposes. The pods, which are high in protein (15-20%), fall to the ground where they are eaten by livestock and wildlife. Leaves, new shoots, and seedlings are also eaten, with leaves being available throughout most of the dry season when other fodder sources are scarce. The gum is edible, and the bark can be used to produce strong fibre. *A. tortilis* starts producing firewood at 8-18 years, at a rate of 50 kg/tree/year. Its fast growth and good coppicing ability, coupled with the high calorific value of its wood of about

*Corresponding author. E-mail: sohana@affrc.go.jp. Tel: +81-294-39-7013. Fax: +81-294-39-7306.

Author(s) agree that this article remains permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

4400-4900 kcal/kg, make it well suited for use as firewood and charcoal (Orwa et al., 2009; National Academy of Sciences, 1980). Given the economic value of this species, efficient and stable seedling production is important. However, several challenges exist regarding artificial seed germination.

Acacia species generally have a water-impermeable seed coat, which means that physical and/or chemical pre-germination treatments are necessary in order to overcome seed dormancy and obtain rapid and synchronous germination. For example, soaking seeds in boiling water, sulphuric acid, or mechanical scarification treatments have been demonstrated to promote germination in *Acacia* species (Danthu et al., 1992; Teketay, 1998; Aref, 2000; Rodrigues et al., 2008). As with other *Acacia* species, the seeds of *A. tortilis* also have a hard seed coat and pre-treatment is essential to overcome seed dormancy. Loth et al. (2005) indicated that ingestion by herbivores or stimulation by fire may promote germination of *A. tortilis* seeds under natural conditions. In addition, the study also showed that a wet environment and optimal temperature conditions (from 15 to 25°C) is suitable for germination of the species. More recently, several studies have demonstrated varying levels of success in promoting *A. tortilis* seed germination. For example, Abari et al. (2012) reported a germination percentage of around 80% through scarification of the seed coat or combined use of sulphuric acid and hydrogen peroxide. However, the germination percentage after treatment with sulphuric acid alone was low in the study. On the other hand, Azazi et al. (2013) tested a number of chemical treatments and found that boiling in water or treatment with sulphuric acid for more than 20 min were both comparatively efficient, and the germination percentages obtained with these treatments was approximately 75%.

In addition, a germination percent-tage of 52% was obtained after mechanical scarification. Although both studies concluded that mechanical scarification, immersion in boiling water or sulphuric acid were all effective, the obtained germination percentages were relatively variable. For the sulphuric acid treatment, differences in immersion duration may have accounted for the observed differences in germination percentages in both studies, with immersion for a minimum of 20 min associated with optimal results. However, in addition to being potentially dangerous and unsafe for local farmers, chemical treatments are typically difficult to achieve in rural areas. Regarding treatments involving boiling water or mechanical scarification, it is considered likely that slight differences in experimental conditions might affect germination percentages. If such condition-dependent differences do exist, then it may be difficult for local farmers to repeat experimental conditions and obtain the same the results. In this paper, we report and discuss simpler, safer and more efficient method for improving germination percentages in *A. tortilis* seeds.

MATERIALS AND METHODS

Seed materials and pre-treatments

Seeds of *A. tortilis* were collected from several mother trees in Kibwezi, Kenya. The seeds were processed, bulked, and sent to the Forest Tree Breeding Centre (FTBC) in Japan where they were stored at room temperature for two months until the following experiments were initiated: Treatment 1(*Cut-Hilum*: The seeds of *A. tortilis* are flattened and ovoid in shape, with a white hilum on the seed coat (Figure 1a). We defined the surface with hilum as the “upper side”, which we removed by nail scissors (Figure 1b); Treatment 2 (*Cut-Side*): The seed coat of the “lower side” was removed by nail-scissors. *Treatment 3* (*Cut-Bot*): Seed coat of the “side” (that is, the seed coat between the upper and lower sides) was removed using nail scissors; Treatment 4 (*Scr-Bo*): The seed coat was first scratched using sandpaper and then placed in boiling water at 98°C. The boiling water was allowed to cool naturally to room temperature following Abari et al (2012); Treatment 5 (*Bo*): Without removing or scratching the seed coat, seeds were placed in boiling water at 98°C. The boiling water was allowed to cool naturally to room temperature; Treatment 6 (*Scr-Sul*): The seed coat was scratched using sandpaper, before being immersed in concentrated sulphuric acid solution for 20 min and washed in distilled water; Treatment 7 (*Sul*): Seeds were immersed in concentrated sulphuric acid solution for 20 min and washed in distilled water; Treatment 8 (*Scr*): Seed coat was scratched using sandpaper; Treatment 9 (*Cont*): No treatment (control).

Mechanical scarification of *A. tortilis* seeds using sandpaper is complicated by small size of the seeds and the hardness of the coat. We therefore only scratched the surface of the seed coat and did not produce deep scars on the coat. Treatments 1 to 3 are unique to this study while treatments 4 to 8 were based on previous studies (Abari et al., 2012; Azazi et al., 2013).

Comparison of the time to root production among treatments

Fifty seeds were used in each of the nine treatments described above. After completing each treatment, the seeds were placed in water-filled Petri dishes and root setting (produced a root of > 2 mm) was observed every morning at 8:30 am for 14 days. We also identified which seeds absorbed water within the first 3 days. However, the presence of mould on some seeds at day 15, meant that the experiments had to be stopped. Expected cumulative rate of root setting over time and their 95% confidence interval were calculated by the Kaplan Meier method (Measurement of the effect of intervention over a period of time, sometimes called “time-to-event analysis”). In addition, the differences in rate of root setting among the treatments were tested by Log rank test. Seeds with roots were planted in soil containing compost and their rates of cotyledon establishment were confirmed and noted. The experiments were performed in a greenhouse at a mean temperature of 27.9±6.9°C.

Germination percentage of seeds in each treatment

In the above experiments, observations were stopped on the 15th day. To examine germination percentages over an extended period of time, 100 seeds were planted in sand at a depth of 10 mm after each of the above treatments. Because we understood that treatment 1 was more efficient than treatment 2 and 3 in above experiments, we did not try treatment 2 and 3 in this experiment. Watering was performed once every two to three days to prevent seeds from drying. The number of seedlings was counted on the 28th day and germination percentage was calculated as: (numbers of germinated seed / numbers of total seeds examined). The

Figure 1. Seeds of *Acacia tortilis*; a, untreated seeds; b, seeds with hilum removed. Hilum is circled.

experiments were performed in a greenhouse at a mean temperature of 23.9±4.5°C.

RESULTS AND DISCUSSION

Root setting

The percentage of seeds that absorbed water within the first 3 days was 100, 100, 100, 82, 8, 82, 70, 8 and 10% for Treatments 1 to 9, respectively. The expected rates of root setting for time in each treatment are shown in Figure 2. Rate of root setting was highest in treatment 1 (*Cut-Hilum*), with most seeds producing roots (that is, produced a root of > 2 mm) within 3 days, and all seeds rooted within 5 days in our experiment. Rate of root setting was also comparatively high in treatment 2 (*Cut-Side*) and 4 (*Scr-Bo*), with a cumulative rate of root setting peaking at approximately 9 days. In treatments 3 (*Cut-Bot*), 6 (*Scr-Sul*) and 7 (*Sul*), cumulative rate of root setting gradually increased, while root setting was very limited in the seeds subjected to treatments 5 (*Bo*), 8 (*Scr*) and 9 (*Cont*). Significant differences in germination percentages among the treatments were detected in Log-rank test ($p < 0.01$).

Abari et al. (2012) and Azazi et al. (2013), reported a slightly different results; treatments 5 (*Bo*) and 8 (*Scr*) were associated with high germination percentages (around 75%) in their studies. One possible reason for this disparity is because the volume of boiling water used in treatments 4 (*Scr-Bo*) and 5 (*Bo*) in this study was small as the treatments were conducted in Petri dishes. It is thus possible that the boiling water may have cooled very quickly, affecting germination efficiency. To comprehensively test the various effects of boiling water on seed germination, conditions such as soaking time and different water temperatures should be examined (Rehman et al., 1999). However, complicated pretreatment conditions are not ideal for local practitioners, such as farmers. In treatment 8 (*Scr*), it is possible that the low rate of water absorption may have been because we only lightly scratched the seed coat. Success rate of treatment 8 may depend on the technique and/or device used by the practitioner. Given the difficulties and time consuming nature of using sandpaper, Abari et al. (2012) concluded that this method is not recommend for promoting germination in *Acacia* seeds. On the other hand, the results of treatment 4 (*Scr-Bo*) indicate that combined use of mechanical scarification and treatment with boiling water may promote germination.

In this study, we found that roots always grow from the point where the seed coat was removed in treatment 1 (that is, the hilum). The seed coat is known to retain elasticity, even if the seed absorbs water, and our results suggest that root setting may sometimes be prevented and/or take time to break through the seed coat (treatment 1 (*Cut-Hilum*) vs. 2 (*Cut-Side*), 3 (*Cut-Bot*)). Indeed, this ability to penetrate the seed coat is considered to be the main cause of variation in rate and duration of germination. Consequently, by identifying the point of root protrusion in treatment 1, the findings of this study will be useful in maximizing germination of *A. tortilis* seed. All rooted seeds were planted in composted soil and we confirmed that cotyledons opened within 3 days after sowing. Therefore, the efficiency of root setting was directly linked to seedling establishment (Table 1).

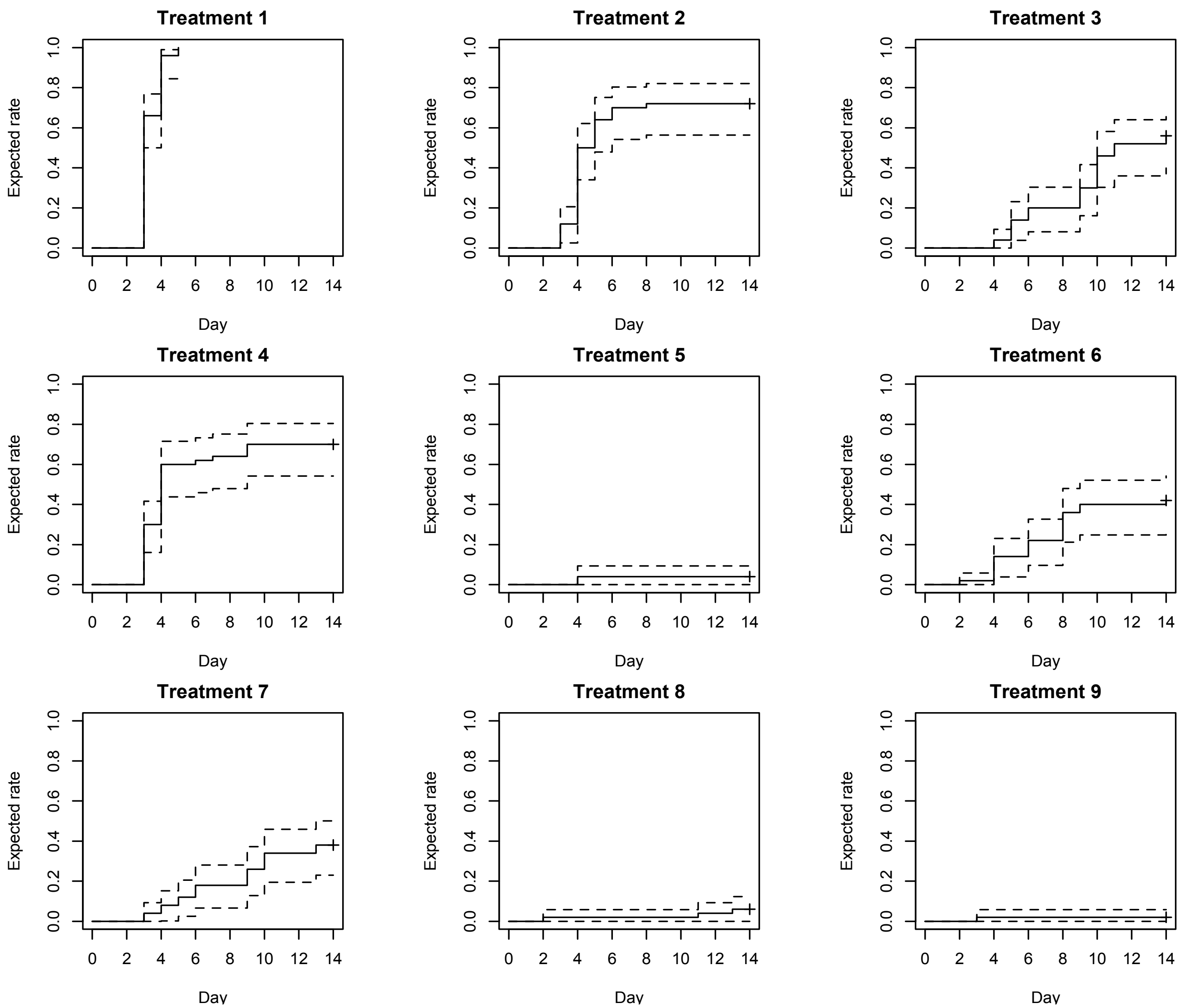

Figure 2. Kaplan-Meier plot for each treatment. Expected cumulative rate of root setting over time (Day) are shown in solid line, and dashed lines indicate the 95% confidence intervals.

Germination percentages

In this study, 100 seeds from each of the seven treatments were sown in sand. Of these, 94, 32, 5, 86, 85, 21 and 6% germinated within 28 days in treatments 1 and 4 to 9, respectively. Significant differences in germination percentage were observed between treatments 1 and 4, 5, 8, 9; 4 and 5; 6 and 4, 5, 8, 9; 7 and 4, 5, 8, 9; 8 and 5, 9 (Ryan's method, $p < 0.01$). Although the germination percentages for treatments 4, 5 and 8 were lower than those of the previous studies (Abari et al., 2012; Azazi et al., 2013), this may be attributed to differences in the way mechanical scarification was performed and how boiling water was applied. On the other hand, compared to the previous studies, germination percentages in treatments 6 (*Scr-Sul*) and 7 (*Sul*) were similar. Because treatments 6 and 7 in our experiments showed similar germination percentages, mechanical scarification is not considered to be useful in combination with sulphuric acid treatment. In above root setting experiments, 54 and 50% of the seeds in treatments 6 and 7 produced roots within 14 days, respectively. However, 86 and 85% of the seeds in treatments 6 and 7 in this experiment germinated. These results suggest that seeds that have been exposed to chemical treatment take longer to germinate, but that final germination levels in such seeds are not necessarily always low. Nevertheless, germination levels of the seeds in treatment 1 (*Cut-Hilum*) were higher than the levels of seeds in treatments 6 and 7. Thus, while it may be time consuming to remove seed coat (approximately five seconds/seed), the levels of germination that can be obtained using our method (that is, treatment 1; *Cut-Hilum*)

Table 1. The summary results of root setting experiments.

Treatment	Percentage of absorbed water within 3 days (%)	Percentage of root setting within 14 days (%)
Treatment 1 (*Cut-Hilum*)	100	100
Treatment 2 (*Cut-side*)	100	72
Treatment 3 (*Cut-Bot*)	100	56
Treatment 4 (*Cut-Bo*)	82	70
Treatment 5 (*Cut-Bo*)	8	4
Treatment 6 (*Cut-Sul*)	82	54
Treatment 7 (*Cut- Sul*)	70	50
Treatment 8 (*Scr*)	8	6
Treatment 9 (*Con)*	10	2

Table 2. The results of germination in soil

Treatment	Germination percentage (%)
Treatment 1 (*Cut-Hilum*)	94
Treatment 4 (Scr-*Bo*)	32
Treatment 5 (*Bo*)	5
Treatment 6 (Scr-*Sul*)	86
Treatment 7 (*Sul*)	85
Treatment 8 (*Scr*)	21
Treatment 9 (*Con)*	6

are higher than those that can be achieved with sand-paper. Our new method should therefore be considered for seed pre-treatment in *A. tortilis* cultivation (Table 2).

Conclusion

We consider that the most efficient strategy for promoting germination in *A. tortilis* seeds is as follows: 1) Remove the upper seed coat, 2) soak the seed in water until root protrusion, and 3) transfer the rooted seed to a pot or planter. Using this method, it may be possible to minimise the number of pots required and increase the probability of successfully producing seedlings by more than 90%. In addition, the amount of time required to obtain seedlings for transplanting to pots will be approximately one week.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

We wish to thank Dr K. Kato and Mr S. Yamaguchi for their kind support during the experiments. This work was carried out as part of KEFRI/JICA project (Development of Drought Tolerant Trees for Adaptation to Climate Change in Drylands of Kenya).

REFERENCES

Abari AK, Nasr MH, Hodjati M, Bayat D, Radmehr M (2012). Maximizing seed germination in two *Acacia* species. J. For. Res. 23:241-244.
Allen DJ (2007) A traveller's guide to the wildflowers and common trees of east Africa. Camerapix Publishers International, Singapore. ISBN: 1-904722-28-8
Aref IM (2000) Effects of pre-germination treatments and sowing depths upon germination potential of some *Acacia* species. Res. Bull. 95:5-17.
Azazi EI, Sayed EI, Sourour MM, Belal AH, Khalifa EA (2013). Improving *Acacia tortilis* seed germination by breaking dormancy treatment. Int. J. Adv. Biol. Res. 3:103-109
Danthu A, Roussel J, Saar JA. (1992) Effect of different pretreatments on the germination of *Acacia senegal* seeds. Seed Sci. Technol. 20(1): 111−117.
Hines DA, Eckman K (1993) Indigenous multipurpose trees of Tanzania: uses and economic benefits for people. FAO
Loth PE, Boer WF, Heitköning MA, Prins HHT (2005) Germination strategy of East African savanna tree *Acacia toritlis*. J. Trop. Ecol. 21:509-517.
National Academy of Sciences (1980) Firewood Crops – Shrub and tree species for Energy Production. NAS, Washington DC
Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). Agroforestree database: a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya. Downloaded from (http://www.worldagroforestry.org/treedb/AFTPDFS/Acacia_tortilis.pdf
Rehman S, Loescher RNJ, Harris PJS (2000) Dormancy breaking and germination of *Acacia salicina* Lindl seeds. Seed Sci. Technol. 27(2): 553-558.
Rodrigues DC, Kohl MC, Pedrinho DR, Arias ERA, Favero S (2008) Treatments to overcome dormancy of *Acacia mangium* seeds. Acta. Sci. Agron. (UEM). 30:279-283.
Teketay D (1998). Germination of *Acacia origena*, *A. pilispina* and *Pterolobium stellatum* in response to different presowing seed treatments, temperature and light. J. Arid Environ. 38(4):551-560.