Research Note

BREAKING SEED COAT DORMANCY WITH PHYSICAL AND CHEMICAL METHODS IN TAMARIND (*TAMARINDUS INDICA* L.) SEEDS[1,2]

Tomas Ayala-Silva[3], Hamide Gubbuk[4] and Garry Gordon[3]*

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Tamarind, *Tamarindus indica* L. (Fabaceae), has a wide geographical distribution in the subtropics and semiarid tropics, and is cultivated in numerous regions. Various geographical areas have been proposed for the origin of tamarind: India or the Far East (Morton, 1987), and Africa (Coates-Palgrave, 1988), the latter being the consensus (Williams, 2006). Troup (1921) placed it in Ethiopia, but others considered it indigenous to the drier savannahs of tropical Africa, from Sudan, Ethiopia, Kenya and Tanzania, westward through sub-Sahelian Africa to Senegal (Brandis, 1921; Ridley, 1922; National Research Council, 2008).

Tamarind is a multipurpose tropical tree especially promising for restoring deforested and damaged lands to health and productivity. It is already used in anti-desertification programs because it grows in arid and other challenging sites, and it resists savannah ground fires (National Research Council, 2008).

Tamarind fruits are eaten fresh, processed into a paste, made into a beverage, and used for tanning hides (Morton, 1987; Gunasena and Hughes, 2000; Barwick, 2004; and Janick and Paull, 2008). The seeds also have medicinal properties and, when pulverized, are used to treat chronic diarrhea, jaundice, dysentery, and as a gentle laxative. Seeds are also ground with salt and ingested to prevent throat infections, cough, fever, intestinal worms and liver ailments (Rama, 1975; Jayaweera, 1981). The wood is used for making furniture, canoes, boats, and many other products (Gunasena and Hughes, 2000). It is also used as fuel, as the wood has a very high calorific value (4850 k cal/kg), making it excellent for brick making and for use as charcoal (www.icuc-iwmi.org/files/tamarind manual, The International Centre for Underutilised Crops, South Hampton, UK).

The fruits are brittle, indehiscent pods, oblong, curved or straight, with rounded ends, and somewhat compressed. Each pod contains one to 12 seeds which are flattened, glossy, orbicular to rhomboid. Seeds are hard, red to purple brown, and the size is very variable and there are 320 to 1000 seeds per kilo (Von Carlowitz, 1986; Hong et al., 1996; El-Siddig et al., 2000). Pods ripen about six months after flowering and can remain on the tree until the next flowering period, unless harvested (Rama, 1975; Chaturvedi, 1985).

The potential of the tamarind tree within rural farming communities has been well recognized, although unimproved wild trees are continuously being exploited to meet

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[3]National Germplasm Repository System, Subtropical Horticulture Research Station, 13601 Old Cutler Road, Miami, FL 33158. *To whom reprint requests should be addressed. Email address: tomas.ayala-silva@ars.usda.gov.

[4]Department of Horticulture, Faculty of Agriculture, Akdeniz University, Antalya 07058, Turkey.

growing domestic and international demand (Parrotta, 1990; Smith et al., 2002). In many areas of the world the growth and management of tamarind is handled by small nurseries and local farmers.

Nursery phases are an important part of the operation in the cultivation of many tropical/subtropical tree crops (Landis, 2008). Keeping seedlings growing in a nursery until they are big enough for grafting makes them stronger and more vigorous and avoids wasting seeds, space and water, and reduces the risk of damage to or loss of the plant (Landis, 2008). In most regions of the world (e.g., India, Africa) tamarind is propagated from seeds. Orchards and tree stands are steadily disappearing (ex. harvesting of trees for wood and charcoal, and raising livestock) without replacements and seeds do not germinate readily, perhaps due to failure to break seed dormancy.

The family Fabaceae (Leguminaceae) has many species exhibiting seed dormancy (Morrison et al., 1998). Legume seed is generally characterized by a thin impermeable cuticle preventing water imbibition (Baskin and Baskin, 1998; Ortega Baes et al., 2002). To enhance the propagation of tamarind trees and seedlings there is a need to understand its germination requirements. Knowledge of seed germination is important for successful propagation (Karrfalt, 2008).

Chemical scarification with acids is broadly used, but should be applied with some care, since long periods of exposure cause damage to the seed and subsequent reduction in germination (Egley, 1972). Scarification with acids was used effectively to overcome the dormancy of seeds of *Ceratonia siliqua* and *Pistacia lentiscus* (Tsakaldimi and Ganatsas, 2001); Okra (Velempini et al., 2003); and Vigna (Wang et al., 2007). Germination rate can be increased by adopting suitable pre-sowing treatments such as hot water immersion, hormone treatment (gibberellic acid) (Alamgir and Hossain, 2005a and 2005b; Azad et al., 2006a and 2006b, Wang et al., 2007; Azad et al., 2010a and 2010b). The purpose of this study was to determine the effect of scarification methods on germination of tamarind seeds, and subsequent root and shoot development.

Seeds of two tamarind accessions (MIA 25443 and MIA 25811) were obtained from the USDA-ARS Subtropical Horticulture Research Station, Miami, FL, in March/April 2012. Immediately after collection they were cleaned from the pulp, placed in plastic bags until June 2012. The seeds were subjected to nine treatments and then thoroughly washed in distilled water and dried on paper towels.

Scarification treatments consisted of: 1) Control; 2) mechanical bottom cutting (BC); 3) both side cutting (BSC); 4) bottom cutting + both side cutting (BC+BSC), and; 5-9) soaking in 98% sulfuric acid (SA) for 25, 50, 75, 100 and 125 min, respectively. Seeds were scarred (ground) by using a Dremel®[5] Multi Pro variable speed (5,000 to 30,000 rpm) grinder Model 395. After the sulfuric acid treatments, the seeds were rinsed three times with tap water followed by deionized water. After pretreatments, seeds were sown in coconut peat (coir) and perlite (50/50 v/v) media in 40-cell trays. The experiment was carried out under greenhouse conditions with temperatures from 28 to 30 °C (day) and 18 to 20 °C (night) and a relative humidity of 80 to 85%. The trays were placed on a mist bench with overhead microsprayers with a mist frequency of 16 sec every 8 min from 6:30 AM to 7:00 PM. Germination percentage, germination index, germination energy, and mean germination time were determined for each treatment. Root, radicle, and hypocotyl length, leaf number and seedling (root, radicle, hypocotyl, stem and leaf) fresh and dry weights were also determined.

[5]Company or trade names in this publication are used only to provide specific information. Mention of a company or trade name does not constitute an endorsement by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

All seeds were tested for germination following published procedures (ISTA, 2005). Treatments were arranged in a completely randomized design with four replications per treatment. Each treatment had 20 seeds. The amount of germination was scored daily during the 12 day treatment period. A seed was considered germinated when the radicle appeared above the substrate (Khayatnezhad et al., 2010). The number of seeds germinated in each treatment was recorded on alternate days. The starting and finishing dates of germination were also recorded. At the end of the germination period, the germination percentage and germination rate (Maguire, 1962) were calculated by using the following equations:

$$\text{Germination Percentages (\%)} = \frac{\text{Number of germinated seeds} \times 100}{\text{Total number of seeds sown}}$$

Germination index (GI) and mean germination time (MGT) were calculated according to the following formulas (Maguire, 1962; Wang et al., 2004).

GI = Σ (Gt/Tt), where Gt is the number of seeds germinated on day t^{th} and Tt is the number of days up to t^{th} day and MGT = ΣTi Ni/ΣNi, where Ni is the number of newly germinated seeds at time T_i.

Energy of germination was determined as the percentage of germinating seeds six days after treatment (Karaguzel et al., 2002).

Analysis of variance (ANOVA) was carried out with SAS® software version 9.1 (SAS Institute, 2003). Significance between means was tested by LSD (P = 0.05).

Germination test results demonstrated the significant effects of the treatments used to overcome seed coat dormancy in tamarind seeds. Sulfuric acid (SA) for 25 min and mechanically (BSC+BC; BSC and BC) scarified seeds started germination three days post-sowing and reached a germination percentage (GP) of 92.5, 92.5, 87.5, and 85.0 percent and a germination index (GI) of 6.72, 3.84, 2.92, and 2.14 after nine days, respectively. Seeds in these treatments germinated significantly faster than control seeds and chemically scarified seeds with SA above 25 min (Table 1). The control seeds started

TABLE 1.—*Effect of various mechanical and chemical scarification treatments on seed germination, germination energy, germination index and mean germination time of* Tamarindus indica *L. (tamarind) seeds.*

Treatments	Germination Percentage (%)	Germination Energy (%)	Germination Index	Mean Germination Time (days)
Control	55.00 d[1]	0.00 c	1.11 f	11.53 a
BC[2]	85.00 bc	0.00 c	2.14 e	11.11 ab
BSC[3]	87.50 ab	2.50 c	2.92 d	10.32 ab
BSC + BC[4]	92.50 a	5.00 c	3.84 c	9.61 b
25 minute soaking in SA[5]	92.50 a	82.50 a	6.72 a	6.90 c
50 minute soaking in SA	80.00 c	40.00 b	5.23 b	7.63 c
75 minute soaking in SA	42.50 e	0.00 c	1.53 ef	10.11 ab
100 minute soaking in SA	30.00 f	0.00 c	0.94 f	10.42 ab
125 minute soaking in SA	27.50 f	7.50 c	1.09 f	11.13 ab
LSD	6.695	11.597	0.760	1.869

[1]Means followed by the same letter within a column are not significantly different according to LSD, P < 0.05.

[2]BC: Bottom cutting; [3]BSC: Both side cutting; [4]BSC + BC: Both side and bottom cutting; [5]SA: Sulfuric acid.

germination after 8 d and had GP and GI of only 55.00% and 1.11, respectively (Table 1). The germination of control seeds was significantly better than chemical scarification with 98% SA, at 75 min or longer, but the GP and GI were still less than 25 min SA, BSC+BC, BSC, and BC treatments.

The lowest germination rate (27.50%) was found in the 125 min SA treatment (Table 1). The highest (92.50%) was obtained with the BC+BSC, and soaking in sulfuric acid (SA) for 25 min treatments. The highest germination energy (82.50%) was found in the 25 min SA treatment followed by 50 min SA treatment. Germination energy was lowest in the control and in the other SA treatments. The germination index was highest in the 25 min SA (82.5) and the lowest in 75 and 100 min SA treatments (0.0) (Table 1).

There were significant differences in the mean germination time among the treatments (Table 1). The mean germination time was longest (11.53 d) in the control and shortest (6.90 and 7.63 days, respectively) in the SA at 25 and 50 minute treatments (Table 1).

The longest root length was observed in mechanical scarification treatments (Table 2) while the shortest root length (3.23 mm) was recorded in the 125 min SA soaking treatment. Radicle and hypocotyl length was statistically different among treatments. The longest radicle length was observed in 25 min SA soaking and the longest hypocotyl length with the BSC + BC treatments. On the other hand, the shortest radicle and hypocotyl lengths were observed with the 125 and 75 min SA treatments, respectively (Table 2). Sulfuric acid at 25 and 50 min were the most effective treatments for improving shoot length in tamarind (Table 2), whereas the shortest shoot lengths were observed in 100 and 125 min SA treatments. Stem diameter and leaf number were also recorded and found to be significantly different among treatments (Table 2). The highest stem diameter and leaf number were recorded with the BSC + BC treatment and the lowest in the 25 min (stem) and 125 min (leaf number) soaking in SA.

Root and radicle fresh weight were significantly higher with the SA 25 min treatment (Table 3). However, the hypocotyl fresh weight and total fresh weight were higher with the BSC, BSC+BC grinding treatments than with the SA treatments or the control (Table 3). Stem and leaf weight were highest in 25 min SA, BSC, and BSC+BC treatments. Overall, total dry matter, radicle weight, and stem plus leaf dry weight was significantly higher in the SA 25 min (Table 4); hypocotyl weight was highest in BSC and BSC+BC treatments, and root dry weight highest in all cutting treatments as well as 25 min soaking in SA. There was a highly significant reduction in root dry weight with SA above 50 min.

One type of dormancy in seeds is physical dormancy (also called exogenous dormancy). Seeds with physical dormancy have hard seed coats that do not allow the seed to absorb water (Nikolaeva, 1977). Seed coat dormancy is found in at least 15 plant families, including horticulturally important families such as the Fabaceae, Malvaceae, Cannaceae, Geraniaceae, and Convolvulaceae (Baskin and Baskin, 1998). Physical dormancy characteristics were reported in tamarind by El-Siddig et al., 2000 and in *Albizia* (Tigabu and Oden, 2001). Seed coat dormancy may be overcome by several presowing treatments such as gibberellic acid, dry heat, mechanical scarification, acids, and hot water (Velempini et al., 2003; Azad et al., 2006a; Pérez-García and Gonzalez-Benito, 2006).

Our results indicate that, mechanical scarification and sulfuric acid for 25 min significantly increased germination percentage and mean germination time (Table 1). On the other hand, applications of SA for periods longer than 25 min proved not a useful scarification treatment. Muhammad and Amusa (2003), Perez-Garcia and Gonzalez-Benito (2006) and Azad et al. (2010) used various treatments to enhance seed germination in tamarind, *Helianthus* and okra, and reported the highest (70%, 80%, 83%) germination rates in manually scarified seeds and lowest (30%, 14%, 43%) in the control seeds. How-

TABLE 2.—*Effect of various mechanical and chemical scarification treatments on root, radicle, hypocotyl and shoot length, stem diameter, and leaf number of* Tamarindus indica *L. (tamarind) seeds.*

Treatments	Root Length (mm)	Radicle Length (mm)	Hypocotyl Length (mm)	Shoot Length (mm)	Stem Diameter (mm)	Leaf Number
Control	10.56 d[1]	3.29 c	8.67 d	9.75 c	1.55 d	2.51 ef
BC[2]	14.51 c	3.68 b	9.39 c	10.47 b	1.63 c	3.49 b
BSC[3]	15.24 b	3.81 b	10.18 b	10.72 b	1.77 b	3.53 b
BSC + BC[4]	16.15 a	4.13 a	10.64 a	11.03 b	1.85 a	3.79 a
25 minute soaking in SA[5]	10.89 d	4.29 a	9.37 c	11.83 a	1.01 g	3.26 c
50 minute soaking in SA	8.60 e	3.71 b	8.51 d	11.76 a	1.04 g	3.05 d
75 minute soaking in SA	8.26 e	3.33 c	7.49 e	8.60 d	1.53 d	2.65 e
100 minute soaking in SA	5.88 f	3.28 c	7.50 e	7.13 e	1.45 e	2.43 fg
125 minute soaking in SA	5.59 f	3.23 c	7.55 e	7.25 e	1.34 f	2.29 g
$LSD_{\%5}$	0.649	0.220	0.455	0.592	0.067	0.164

[1]Means followed by the same letter within a column are not significantly different according to LSD, $P < 0.05$.
[2]BC: Bottom cutting; [3]BSC: Both side cutting; [4]BSC + BC: Both side and bottom cutting; [5]SA: Sulfuric acid.

TABLE 3.—*Effect of various mechanical and chemical scarification treatments on root, radicle, and hypocotyl fresh weights, stem and leaf fresh weight and total fresh weight of* Tamarindus indica *L. (tamarind) seedlings.*

Treatments	Root Fresh Weight (g)	Radicle Fresh Weight (g)	Hypocotyl Fresh Weight (g)	Stem and Leaf Fresh Weight (g)	Total Fresh Weight (g)
Control	0.058 cd[1]	0.058 de	0.208 c	0.610 c	0.934 c
BC[2]	0.085 b	0.088 bc	0.240 b	0.768 b	1.181 b
BSC[3]	0.085 b	0.085 c	0.320 a	0.803 ab	1.293 a
BSC + BC[4]	0.083 b	0.100 ab	0.303 a	0.783 ab	1.269 a
25 minute soaking in SA[5]	0.103 a	0.113 a	0.185 d	0.805 a	1.206 b
50 minute soaking in SA	0.068 c	0.093 bc	0.163 e	0.540 d	0.864 d
75 minute soaking in SA	0.060 cd	0.065 d	0.173 de	0.460 e	0.758 e
100 minute soaking in SA	0.045 e	0.050 e	0.165 de	0.418 f	0.678 f
125 minute soaking in SA	0.050 de	0.033 f	0.140 f	0.413 f	0.636 f
$LSD_{\alpha 5}$	0.0112	0.0136	0.0214	0.0354	0.0581

[1]Means followed by the same letter within a column are not significantly different according to LSD, $P < 0.05$.
[2]BC: Bottom cutting; [3]BSC: Both side cutting; [4]BSC + BC: Both side and bottom cutting; [5]SA: Sulfuric acid

TABLE 4.—*Effect of various mechanical and chemical scarification treatments on root, radicle, and hypocotyl dry weights, stem and leaf dry weight and total dry weight of Tamarindus indica L. (tamarind) seedlings.*

Treatments	Root Weight (g)	Radicle Weight (g)	Hypocotil Weight (g)	Stem+Leaf Weight (g)	Total Dry Weight (g)
Control	0.030 bc[1]	0.015 f	0.053 c	0.123 f	0.221 fg
BC[2]	0.038 ab	0.030 bcd	0.065 b	0.153 cd	0.286 c
BSC[3]	0.040 a	0.033 bc	0.078 a	0.188 b	0.339 b
BSC + BC[4]	0.043 a	0.035 b	0.085 a	0.198 b	0.361 b
25 minute soaking in SA[5]	0.045 a	0.043 a	0.065 b	0.265 a	0.418 a
50 minute soaking in SA	0.028 c	0.023 e	0.053 c	0.163 c	0.267 cd
75 minute soaking in SA	0.025 cd	0.028 cde	0.058 bc	0.148 cd	0.259 de
100 minute soaking in SA	0.018 de	0.025 de	0.050 c	0.143 de	0.236 ef
125 minute soaking in SA	0.013 e	0.023 e	0.038 d	0.128 ef	0.202 g
$LSD_{\%5}$	0.0078	0.0073	0.0083	0.0174	0.0274

[1]Means followed by the same letter within a column are not significantly different according to LSD, $P < 0.05$.
[2]BC: Bottom cutting; [3]BSC: Both side cutting; [4]BSC + BC: Both side and bottom cutting; [5]SA: Sulfuric acid

ever, this study demonstrated that application of 98% SA for 25 min and grinding (BSC + BC) had the highest germination rate (92.5%) and a germination time of 6.90 and 9.61 days, respectively. Muhammad and Amusa (2003) reported that SA at 50% and 60 min induced the highest germination percentage (78.8%) and the shortest average germination time (15 days). In this study, the highest germination percentage was obtained with mechanical scarification and 25 minute sulfuric acid treatments, and the shortest mean germination time was found by using sulfuric acid for 25 min. Muhammad and Amusa (2003) reported that if tamarind seeds are dipped in concentrated or diluted sulfuric acid and then rinsed in water, there is no subsequent problem (i.e., stunt growth, damage to the root) in terms of germination.

This study has demonstrated that mechanical and acid (sulfuric acid) scarification treatments are effective methods for enhancing tamarind germination. These techniques can be implemented by farmers, ornamental nurseries and forestry authorities in many regions of Africa, India, Asia, Mexico, and Central America where there is a high demand to establish tamarind orchards or re-establish forested areas, particularly in dry or semi-arid regions of Africa. The study proved that seed treatments are beneficial to overcome seed coat dormancy in tamarind seeds.

In conclusion, application of 98% SA for 25 min and mechanical scarification (grinding) of the seed significantly improved germination of tamarind seeds. This finding indicates that dormancy in tamarind seeds is due to the hardness and impermeability of the seed coat. Removing part of the pericarp is sufficient to break dormancy, but in practice it is difficult to do to a large number of seeds. Therefore, for practical purposes we recommend 25 min SA treatment for enhancing seed germination in tamarind seeds.

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