EFFECT OF PROVENANCES ON SEED GERMINATION, EARLY SURVIVAL AND GROWTH PERFORMANCE OF TAMARINDUS INDICA L. IN ETHIOPIA: A KEY MULTIPURPOSE SPECIES

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ABSTRACT

Effect of provenances on seed germination, early survival and growth performance of Tamarindus indica L. in Ethiopia were evaluated. Seeds were collected from Dello Mena, Guba, Kurmuk and Metema Weredas. In this study seed pre-sowing treatments, effects of dry heat and moist heat treatments on seed germination as well as effect of provenances on early survival and growth performance of T. indica seedlings were evaluated. There were 1,800-2,600 seeds contained within 1kg of T. indica seeds within 94-97% pure seeds among the four provenances. From 1kg of pure T. indica seeds 1,245-1,606 seedlings are raised at Laboratory. Nicked seeds showed the highest germination of 100% compared to other treatments within 12 months of storage in cold room having between 7 and 10.6% moisture content. Dello Mena, Kurmuk and Metema provenances had the highest germination percentage (100%) as compared to Guba provenance in producing viable seeds. The survival rate of seedlings recorded at the age of four varied from 81 to 94% among the provenances. Dello Mena provenance had the highest mean values of seedlings' shoot length, RCD, tap root length as well as fresh and dry leaves and roots weight. Guba provenance, in turn, scored the largest number of leaves, while Kurmuk had the largest number of secondary roots and the highest fresh and dry stems weight. This implies that Dello Mena provenance is the most recommended for raising T. indica seedlings in the Green House. This research output provides an evidence of the genetic variability among T. indica provenances and hence the potential for future tree improvement and domestication programme.

KEY WORDS/PHRASES:

germination, growth performance, T. indica

1.INTROUCTION

Tamarindus indica L. is a multipurpose tree species belonging to the family Fabaceae and genus *Tamarindus* (Thulin, 1989; Jøker, 2000; Arbonnier, 2004;). *T. indica* has various local names in

different languages including HUMER in Amharic, Somalinya and Tigrinya; ROQA in Amharic, Orominya and Somalinya; ARABEB in Tigrinya; and TAMARIND in English (Thulin, 1989; Fichtl and Admasu Adi, 1994; Arbonnier, 2004; Muok and Alem, 2011). It is an evergreen tree growing up to a height of 3-24(30) m (Thulin, 1989; Jøker, 2000) and a diameter of up to 1 m (Arbonnier, 2004). Jøker (2000) reported that the trees begin to produce fruits when they are 8-12 years old. The trees may be continued to produce fruits for 200 years. According to Arbonnier (2004) *T. indica* is common and widespread to vast areas stretching from Senegal to Eritrea, from Sierra Leone to Cameroon, from Ethiopia and Somalia to Mozambique, Madagascar, India, United States and Australia. In Ethiopia, it is widely distributed in grassland, woodland and *Combretum* bushland, frequently riparian habitats of Tigray, Gonder, Welo, Gojam, Shewa, Harerge, Ilubabor, Kefa, Gamo Gofa and Sidamo regions within altitudinal ranges of 0-1,500 m above sea level (Thulin, 1989). It grows on often on heavy, though well-drained soils or termite nests, where the rainfall ranging between 400-1,500 mm, with a very definite dry season (Arbonnier, 2004). *T. indica* also tolerates temperatures up to 47°C, but it is very sensitive to frost (Jøker, 2000). *T. indica* has diverse uses and values including poles, mortars and pestles, tool handles, timber and general construction work, fuel wood (firewood and charcoal), medicinal uses and livestock fodder (Thulin, 1989; Fichtl and Admasu Adi, 1994; Jøker, 2000; Arbonnier, 2004; Muok and Alem, 2011). The acid pulpy part of the fruits is used in cooking and for making a cooling. Jøker (2000) described that its fruit pulp has a high content of vitamin B and is eaten fresh or made into jam, chutney, juice or sweets. Flowers, leaves and seeds are also edible and valuable for human consumption (Jøker, 2000). Hence, the seeds are commonly sold on local markets and supermarkets. In addition, it serves as wind break and fire break, soil and water conservation and hence recommended for agroforestry systems. (Thulin, 1989; Fichtl and Admasu Adi, 1994; Jøker, 2000). Therefore, the present study aimed at investigating the effect of provenances on seed germination, early survival and growth performance of *T. indica* species in Ethiopia, which is socio-economically important and an ecologically feasible species.

2. MATERIALS AND METHODS

2.1 Seed collection, handling and processing

Fruits of *T. indica* were collected from mother trees stands at a distance of 100m apart from Metema Wereda, Amhara Regional State; Guba and Kurmuk Weredas, Benishangul-Gumuz Regional State as well as Dello Mena Wereda, Oromia Regional State, Ethiopia. The collected fresh fruits were put into perforated plastic bags and safely transported to Forestry Research Center (FRC), Ethiopian Institute of Agricultural Research (EIAR). Seeds were separated from fruits and seeds which had been attached by insects or decayed ones were excluded, extracted and cleaned in FRC seed processing room following the procedures by FAO (1985). Finally, the seeds were placed in perforated plastic bags and stored in cold room at $+5^0$c until the experiment was started. This enables to maintain the viability of seeds and prevent from insect attack.

2.2 Determination of purity analysis, moisture content and seed weight per kg of seeds

The purity analysis, moisture content and seed weight per kg of *T. indica* seeds were determined at FRC laboratory following the methods by FAO (1985).

2.3 Pre-sowing seed treatments

The effect of seed pre-sowing treatments on the germination of *T. indica* seeds were assessed by conducting three major pre-sowing treatments. Accordingly, seeds were subjected to nicking, pouring hot water (80^0c) over seeds for 5 minutes & then soaked in cold water for 24hr and control (without treatment or intact seeds) treatments in order to improve the rate and percentage germination of seeds. To investigate the effect of nicking on germination of seeds, a small portion of the seed coat was carefully removed at the side of the hilum using the sharp edge of a Scissor until a small hole was remained for imbibitions of water and Oxygen. Great care was taken to avoid/not to damage seed embryo and the emerging radicle as well.

2.4 Dry heat treatment

In this test, seeds were treated with dry heat (seeds placed in Glass Bottle without water) in preheated dry oven at a range of different temperatures (60, 80 and 100^0c) and duration (15, 30 and 60minutes) for each range. The test had also a control (without heat treatment) with the same number of seeds and replications.

2.5 Moist heat treatment

Seeds were treated with moist heat in preheated dry oven at a range of different temperatures (60, 80 and 100^0c) and duration (15, 30 and 60minutes) for each range. Moist heat is similar to dry heat but the only difference is in moist heat seeds were placed in Glass Bottle containing water and treated in dry oven. The test had also a control (without heat treatment) with the same number of seeds and replications.

2.6 Daily germination assessment of seeds

All germination tests were conducted in such a way that all treatments had 100 seeds in 4 replicates of 25 seeds each. The filter paper was kept moist with distilled water as much as possible throughout the entire experimental period. Seeds were inspected everyday starting from the 2^{nd} day after sowing. All germinated seeds were counted and removed daily in order to avoid the double counting of seeds. A seed was considered as germinated when the radicle was penetrated out from the seed coat and clearly appeared visually. The daily germination count was continued until no more seeds had been germinated.

2.7 Seedling raising in the laboratory and transplanting on polyethylene pots

Treated seeds with best germination percentage of *T. indica* (*i.e*., nicked seeds) were sowed in the Laboratory on Plastic Petri-dishes, where sand used as a substrate. Following this, 100 polyethylene pots having 10cm height and 8cm diameter for each provenance were filled with 2 hand sand and 1 hand forest soil mix (2:1 Ratio) to raise the seedlings. Accordingly, using a Randomized Complete Block Design (RCBD), 100 polyethylene pots in 5 replications of 20 polyethylene pots each were designed in the Green House. Hundred germinated seedlings having 2-4 leaves were transplanted to polyethylene pots in the Green House from three different provenances (*i.e*., Dello Mena, Guba, Kurmuk and Metema Weredas). Seedlings survival was supervised until two weeks and the dead seedlings were replaced by other normal seedlings. Watering and weeding was done regularly (morning and evening) when it is necessary. Finally, data on early survival and mortality rate as well as growth performance of seedlings were carried out on four months after the completion of transplanting in the polyethylene pots.

2.8 Early survival and mortality rate of seedlings

Early survival and mortality rate of *T. indica* seedlings were counted among the four provenances.

2.9 Early growth performance of seedlings

The shoot length/height of each *T. indica* seedling was measured using ruler in centimeter (cm), while root-collar diameter (RCD) was measured using seed caliper in millimeter (mm) among the four provenances. In line with this, the number of leaves in pairs of each seedling was counted.

2.10 Seedlings' leaf, stem and root fresh and dry weight estimation

Once the necessary data on survived seedlings, seedling shoot length and RCD recorded, 30seedlings were randomly sampled from each provenance for this experiment. Accordingly, each seedling was uprooted one by one and the tap-root length was measured using ruler in cm. The number of secondary roots associated with the tap root was counted. Then each seedling's leaves were separated from the stem and counted. Likewise, the stem was cut from root at the soil level and all parts put separately in to the coded Manila Envelope (*Kaki Wereqet* (Amharic)). Immediately, fresh weight of each seedling's leaves, stem and roots were measured using the sensitive analytical balance with four Digits (0.0000) in gram (g). Following this, each seedling's leaves, stem and roots with their respective envelopes placed in to the dry-Oven at a temperature of 73^0c for 24hours. Finally, envelops were removed out from the dry-Oven and each seedling's leaves, stem and roots dry weight was measured soon not to absorb moisture and affect the weight. The process was continued in the same way until insignificant weight difference was observed between dry weights by measuring randomly drawn samples.

2.11 Statistical data analysis

The germination percentage data were first arcsine transformed before statistical analysis to fulfill normality (Gomez and Gomez, 1984). Collected data were analysed and evaluated by using percentages. In addition, the statistical significance difference was determined by one-way Analysis of variance (ANOVA) test at 95%, 99% or 99.9% confidence interval and multiple comparison of Least Significance Difference (LSD) to show significance difference among the treatments using SPSS Version 20.0 Computer Software Programme.

3.RESULTS AND DISCUSSION

2.10 Purity analysis, moisture content and seed weight/kg

The research output reported that the purity analysis of *T. indica* seeds ranged from 94-97% among the four provenances. Dello Mena provenance had the highest purity percentage (97%) among the provenances, while Kurmuk provenance had the least (94%). Its percentage moisture content from the average of the two samples found to be 7-10.6% for storage in cold room at $+5^0$C among the four provenances. The overall seed weight test after purity analysis indicated that there were 1,324-1,656 seeds are contained within one kilogram (kg) of pure *T. indica* seeds among the provenances. But, Jøker (2000) reported that there are 1,800-2,600 seeds are found in

one kg. This, in turn, has a mean thousand seed weight of 604-755g. Therefore, from one kilogram (kg) of pure *T. indica* seeds 1,245-1,606 seedlings are raised at Laboratory.

2.11 Pre-sowing seed treatment

The germination percentage of *T. indica* seeds recorded at the end of the experiment ranged between 0% and 100% within 12 months of storage in cold room within 7 and 11% of moisture content. Thus, the seeds had longer viability as well as survival value and hence stored for longer periods. This showed that *T. indica* seeds have Orthodox seed storage behavior as also reported by Jøker (2000) and Muok and Alem (2011). Nicked seeds showed the best germination (100%), followed by the control (88%). On the contrary, pouring hot water (80^0c) over seeds for 5 minutes & then soaked in cold water for 24hr had 0-88% germination. The statistical test of One-way ANOVA also showed that there was a highly statistical significant difference ($P<0.001$) among treatments with a highest mean value of 85.19 $\pm$ 5.94 for nicked seeds. Germination was fastest and uniform in nicked seeds than the rest treatments. This showed that *T. indica* seeds require a proper seed pre-sowing treatment for better germination of seeds. The importance of seed treatment was similarly reported by earlier studies of Demel Teketay and Mulualem Tigabu (1996) and Jøker (2000). Out of the total 28 days of germination period (4 weeks), most of the seeds germinated within the 1^{st} week starting from the 5^{th} days after sowed and completed within 14 days for nicked seeds. But, the seed germination of other treatments started on the 2^{nd} week and extended until the 4^{th} week after sowed. Similarly, nicking of seeds also successfully improve the germination of seeds as described by Tilahun Gebre Medhin and Legesse Negash (1999).

2.12 Effect of provenances on seed germination

The results of *T. indica* seed germination in the laboratory indicated that except Guba (97%) provenance, Dello Mena, Kurmuk and Metema provenances had a germination of 100%. Therefore, all the provenances had the highest germination percentage in producing potential viable seeds. The statistical test also showed that there was no a significant difference ($P<0.05$) among the provenances.

2.13 Dry heat treatment

The experimental result showed that there was a highly statistical significant difference among the different dry heat treatments ($P<0.001$) with a highest mean value of 51.98$\pm$3.09 at 80^0c for 15minutes and the least mean value of 40.90$\pm$ 10.27 at 100^0c for 15minutes. On the other hand, totally no germination was recorded at 100^0c for 60minutes out of the 9 dry heat treatments. This revealed that appropriate dry heat treatments trigger the germination of *T. indica* seeds. Moreover, the germination of *T. indica* seeds was increased from 60^0c to 80^0c and declined to 100^0c. Therefore, heat generated from forest fire in dryland areas act as one of the factors stimulating seed germination. This result was also similarly reported by earlier studies of Demel Teketay (1996), Demel Teketay and Granström (1997) and Abeje Eshete *et al*. (2012).

2.14 Moist heat treatment

The statistical test indicated that no significant difference ($P<0.05$) among the different moist heat treatments with a highest mean value of 50.08$\pm$ 11.25 at 60^0c for 30minutes. In contrary, the least mean value (40.91$\pm$ 5.20) was recorded at 100^0c for 15minutes. The germination of *T.*

indica seeds was increased along the minutes for 80 and 100^0c. This showed that appropriate moist heat treatments trigger the germination of *T. indica* seeds. Therefore, moisture generated

from forest fire in dryland areas serve as a favourable environmental condition to break seed dormancy and stimulate seed germination as also reported by Demel Teketay (1996) and Demel Teketay and Granström (1997).

2.15 Seedlings' survival and mortality rate determination

T. indica seedlings' survival rate in the Green House indicated that the survival percentage of the seedlings among the four provenances were different. Generally, the highest survival percentage of seedlings was recorded in Kurmuk provenance, which accounted about 94% of survival rate, followed by Metema provenance with 89%. On the other hand, Guba provenance was characterized by the highest mortality rate (19%) of seedlings from all the rest. This revealed that the long-term yield of plantation per unit area can be affected by the mortality or survival rate of seedlings (Negash Mamo and Mebrate Mihretu, 2005; Girma Shumi *et al*., 2012). This might be due to various environmental factors such as the size and effect of polyethylene pots, soil, moisture and temperature in the Green House, availability of water, insect and pest and other determinant factors. Therefore, in the Green House Kurmuk and Metema provenances are more recommended for raising large number of *T. indica* seedlings with the best survival potential more than other provenances.

2.16 Seedlings' early growth performance rate determination

The result output indicated that Dello Mena provenance had the highest mean values of *T. indica* seedlings' shoot length (13.20 cm), RCD (0.24 mm) as well as tap root length (20.30 cm). This indicated that Dello Mena provenance had the highest growth performance and adaptation capacity than the rest provenances. Similarly, Guba provenance scored the largest number of leaves (53) on the stems of seedlings, while Kurmuk had the largest number of secondary roots (54) associated with the primary roots. The statistical test showed that there was a significant difference ($P<0.05$) among provenances for the mean values of seedlings' RCD, root length and number of leaves. On the contrary, no significant differences ($P<0.05$) was observed for the mean values of seedlings' shoot length and number of roots.

In general, the seedlings' longer tap root length and higher number of secondary roots might increase the surface area for efficient absorption of water and nutrients from the soil and provides physical support of the plant (Silvana, 1998; Shiferaw Alem *et al*., 2010). Higher shoot length and RCD values also important for better survival rate and higher growth performance with better adaptation capacity of the seedlings at field conditions. In turn, a large number of seedlings' leaves might contribute to a higher rate of photosynthesis, which in turn resulted in a higher growth rate as also reported by Shiferaw Alem *et al*. (2010).

2.17 Seedlings' fresh and dry shoot and root weight (Biomass) estimation

In all the mean values of fresh and dry weight *T. indica* seedlings' parameters, Dello Mena provenance had the highest fresh and dry leaves and roots weight values. Similarly, Kurmuk provenance had the highest fresh and dry stems weight values. On the other hand, the least fresh leaves, dry leaves and stems; fresh stem and roots as well as dry roots weight values were recorded for Guba, Metema and Kurmuk provenances respectively. Likewise, the seedlings'

root/shoot ratio showed a similar trend in all the seedlings' fresh (0.4) and dry (0.6) weight parameters. The statistical test also showed significant differences ($P<0.05$) except the dry stem and roots weight values among the four provenances.

4.CONCLUSION AND RECOMMENDATION

In general, raising high quality and large number of seedlings in the Green House plays a significant role for better survival, growth performance and adaptation of seedlings at field conditions. The present study indicated that the hard seed coat of *T. indica* seeds prevents the imbibitions of water and oxygen and hence inhibits the germination of seeds. In order to get high, rapid and uniform germination of seeds, seeds require appropriate seed pre-sowing treatment methods. As a result, nicking the seeds (100%) greatly improved seed germination as compared to other treatments. The effect of provenance on seed germination further revealed that Dello Mena, Kurmuk and Metema provenances had the highest germination percentage as compared to Guba provenance in producing viable seeds, which indicates their potential as a seed source. On the other hand, the result of *T. indica* seedlings' survival and growth performance rate in the Green House indicated that Kurmuk provenance had the highest survival rate (94%), followed by Metema provenance (89%). On the other hand, Guba provenance was characterized by the highest mortality rate (19%) of seedlings from all the rest. Dello Mena provenance had the highest mean values of seedlings' shoot length, RCD, tap root length as well as fresh and dry leaves and roots weight. Guba provenance scored the largest number of leaves, while Kurmuk had the largest number of secondary roots associated with the primary roots and the highest fresh and dry stems weight. This implies that Dello Mena provenance is the most recommended for raising *T. indica* seedlings in the Green House.

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REFERENCES

[1] Abeje Eshete, Demel Teketay, Mulugeta Lemenih and Frans Bongers (2012). Effects of resin tapping and tree size on the purity, germination and storage behavior of Boswellia papyrifera (Del.) Hochst. seeds from Metema District, northwestern Ethiopia. Forest Ecology and Management 269: 31-36.

[2] Arbonnier, M. (2004). Trees, shrubs and lianas of West African dry zones. Wageningen, The Netherlands. CIRAD-MARGRAF-MNHN. 573pp.

[3] Demel Teketay (1996). Germination ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethiopia. Forest Ecology and Management 80: 209-223.

[4] Demel Teketay and Mulualem Tigabu (1996). The effect of pre-sowing seed treatments, temperature and light on the germination of Tamarindus indica L., a multipurpose tree. Journal of Tropical Forestry 12(2): 73-79.

[5] Demel Teketay and Granström, A. (1997). Germination ecology of forest species from the highlands of Ethiopia. Journal of Tropical Ecology 14: 793-803.

[6] FAO (1985). A guide to forest seed handling with special reference to the tropics. FAO Forestry paper 20/2. DANIDA Forest seed center, Denmark. 379pp.

[7] Fichtl, R. and Admasu Adi (1994). Honeybee flora of Ethiopia. Margraf Verlag, Weikersheim, Germany. 510pp.
[8] Girma Shumi, Yigremachew Seyoum, Wendsen Melak, Nesibu Yahya, Negash Mamo and Miftah Fekadu (2012). Provenance Effects on Early Survival and Growth of Juniperus procera at Kulumsa,
Arsi Zone. In: Forestry and Forest Products: Technologies and Issues. Proceedings of the National Workshop on Forestry Research Technologies Dissemination 29 - 31 May 2012, Hiruy Hall, EIAR, Addis Ababa, Ethiopia,pp. (Wubalem Tadesse, Getachew Desalegn and Abraham Yirgu, eds.).
[9] Gomez, K. A. and Gomez, A. A. (1984). Statistical procedures for Agricultural research. 2nd Edition, John Wiley & Sons, New York. 680pp.
[10] Jøker, D. (2000). Tamarindus indica L. Seed Leaflet, No. 45, October 2000. Danida Forest Seed Centre, Krogerupvej, Humlebaek, Denmark. 2pp.
[11] Muok, B.O. and Alem, S. (2011). Tamarindus indica, tamarind. Conservation and Sustainable Use of Genetic Resources of Priority Food Tree Species in sub-Saharan Africa. Bioversity International (Rome, Italy). Leaflet, 7pp.
[12] Negash Mamo and Mebrate Mihretu (2005). Provenance effect on early survival and growth performance of Juniperus procera at Jibli, Awi zone. Ethiopian Journal of Natural Resource 7(2): 203-217.
[13] Shiferaw Alem, Wubalem Tadesse, Guzman, P. and Gil, L. (2010). Provenance Variation in Germination and Seedling Growth of Eucalyptus Globulus. In: Eucalyptus Species Management, History, Status and Trends in Ethiopia. Proceedings from the Congress held in Addis Ababa. September 15th-17th, 2010, pp.240-245, (Gil, L., Wubalem Tadesse, Tolosana, E. and López, R., eds.).
[14] Silvana, N. (1998). Understanding root systems to improve, seedling quality. HortTechnology 8(4): 1-6.
[15] Thulin, M. (1989). Fabaceae. In: Flora of Ethiopia. Vol. 3, pp. 49-251, (Hedberg, I. and Edwards, S., eds). The National Herbarium, Biology Department, Science Faculty, Addis Ababa University, Addis Ababa, Ethiopia and the Department of Systematic Botany, Uppsala University, Uppsala, Sweden. 659pp.
[16] Tilahun Gebre Medhin and Legesse Negash (1999). The effect of different pre-sowing seed treatments on the germination of Boswellia papyrifera, a key dryland tree. Ethiopian Journal of Natural Resources 1(1): 37-55.