In-vitro propagation of *Tamarindus indica* L. through Seed Culture Technique towards Plantation Establishment

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ABSTRACT

Tamarindus indica L. known as Tamarind or Plum tree is a leguminous tree with multipurpose uses. Different parts of the tree have been documented to be used in treating different ailments such as fever, jaundice, diarrhea, etc. Seeds of *T. indica* were extracted from freshly collected fruits from the wild. The media used for this study were Murashuge and Skoog (MS) and Woody Plant Medium (WPM) supplemented with 30g sucrose. Plant Growth Regulators were added to the medium in different concentrations and pH adjusted to 5.8 before sterilization. The seeds were pre-treated with disinfectants such as 70% alcohol and 10% hypochlorite before being inoculated into both MS and WPM media supplemented with BAP and NAA. Treatment C with 2mg/L BAP and 2mg/L NAA gave the best result. Treatments F – J containing the WPM medium did not respond quickly like those with the MS medium.

Keywords: *T. indica*, *in-vitro*, propagation, hormone concentration, medicinal.

INTRODUCTION

Tamarindus indica L. belongs to the *Fabaceae* family and subfamily *Caesalpinioideae* (Stege et al., 2011). Tamarind, also known as "Sweet Angle" and "Plum Tree" is a leguminous tree (Soares et al., 2017). Parts of Tamarind can be used in treating certain disorders like stomach disorders, diarrhea. It has been implicated in the treatment of jaundice, colds, fever and as skin cleanser. It serves as food in most parts of the tropics (De Caluwé et al., 2019). Originally indigenous to tropical Africa but domesticated in a lot of Asian countries. Its production is minimal in Africa unlike Asian countries like China, India which produce them in large quantities (El-siddig et al., 2006; De Caluwé et al, 2019). Fruit pulp is used for seasoning, to flavor confection, curries and also used as major components of some juices and beverages (El-siddig et al., 2006). It can also be processed into jam and candies. The seeds are edible after soaking. It is also used in livestock feeds (El-siddig et al., 2006). The fruit contains B vitamins, tartaric acid, acetic acid, citric acid, formic acid, malic acid, and succinic acid, amino acids, invert sugar (25-30%), pectin, protein, fat, some pyrazines (trans-2- hexenal), and some thiazoles. This helps in hypolipidemic antioxidant, antifluorose, analgesic, hepatoregenerativa and antispasmodic activities. Leaves are sources of protein, lipid, fiber and vitamins like thiamine, riboflavin, niacin, ascorbic acid and β-carotene which have antiemetic activity and helps in protecting the liver, Tamarind kernel powder (TKP) which is an important material used in the textile, paper and jute industries (De Caluwé et al., 2019). The leaves and flowers are consumed as vegetables, used as stews, soups, salads, etc. (El-siddig et al., 2006; De Caluwé et al., 2019).

The tamarind propagation is predominantly by seeds. However, there are many studies concerning the asexual propagation by cutting, grafting and micropropagation (Huang et al., 2015; Mowobi et al., 2016). Regardless of type of propagation, the plant production in a commercial scale is incipient, being performed without any scientific character (Ferreira et al., 2008). In most countries where it is cultivated, it is done in scattered manner with less management techniques which have led to limited variety of resources. This cultivation method gives rise to low yields, poor quality and susceptibility to diseases. Studies on *T. indica* have always been focused on its economic importance, extraction and applications, few investigations are reported on its cultivation and propagation (Wang et al., 2014). Efforts have been made through grafting and tissue culture techniques to maintain good characters and keep a consistent growth state. However, very few investigations have been done on the tissue culture of *T. indica*. This study therefore sought to determine the better media strength and optimal combination of Plant Growth Regulators for the in-vitro propagation of *T. indica* between Woody Plant Medium and Murashige and Skooge medium.

MATERIALS AND METHODS

Explant Preparation: Fresh *T. indica* seeds were extracted from sweet tamarind obtained from the wild. The seeds were washed with detergent and running water. They were then disinfected by soaking in 70% alcohol for 3 to 5 min and then rinsed with sterile distilled water. The seeds were then immersed in 10% sodium hypochlorite solution for 15min with few drops of Tween 20 before being rinsed with sterile distilled water three times.

Media preparation: Full strengths of both the Woody plant and Murashige and Skoog medium were used for this study. They were supplemented with 30g sucrose and some Plant Growth Regulators (PGRs), and pH adjusted to 5.8 and 7g agar added before sterilization. The PGRs used in this experiment were Naphthalene acetic acid (NAA) and Benzyl amino purine (BAP). The Plant Growth Regulators were added in the ratio below in Tables 1 and 2.

Table 1: MS Medium with Plant Growth Regulators

Treatments	BAP mg/L	NAA mg/L
A	0.0	0.0
B	1	1
C	2	2
D	3	1
E	2	1

Table 2: WPM Medium with Plant Growth Regulators

Treatments	BAP mg/L	NAA mg/L
F	0.0	0.0
G	1	1
H	2	2
I	3	1
J	2	1

A volume of 7ml of each treatment was dispensed into 17ml test tubes with caps. The media were sterilized at 121^{o}C for 15mins at 1 atmos. The seeds were inoculated into both and transferred to the growth room with temperature of 20 ± 2°C with 16/8 photoperiod (light/dark). There were 5 replicates per treatment. It is a completely randomized design experiment.

Parameters assessed: These included the followings; day of radical emergence, germination rate and shoot height (cm). The mean values of these parameters were subsequently taken. The data collected were converted to means, and analyzed using Mean and Descriptive Statistics.

RESULTS AND DISCUSSION

The results showed no germination in tubes containing the WPM media at 8DAI (Days after inoculation) in all the treatments while there was radical emergence in treatments B, C, D and E of the tubes containing the MS medium with Plant Growth Regulators (PGRs) at 8DAI. However, there were growths in all the treatments containing MS medium except the control (treatment A). At 15DAI, there were growths in 3 tubes (Tables 3 and 4).

Table 3: Mean shoot height (cm) of plantlets in MS medium

Treatment	8DAI	15DAI	21DAI	28DAI
A	0	1.91	3.33	5.13
B	1.91	3.33	5.13	6.1
C	2.03	3.45	5.22	6.63
D	2.01	2.43	4.01	6.55
E	2.01	2.43	4.01	6.41

Table 4: mean shoot height (cm) of plantlets in WPM medium

Treatment	8DAI	15DAI	21DAI	28DAI
F	0	2.01	2.43	4.01
G	0	1.91	3.33	5.13
H	0	2.03	3.45	5.22
I	0	1.93	2.9	3.91
J	0	2	3.1	3.83

Treatments F, I and J had one growth each in all replicates while there were two tubes with germination in each of treatments G and H. At 21st DAI, treatments A-E (MS medium) showed significant germination in all while treatments F- J (WPM) had three to four germination out of the 5 replicates. However, at the 28th DAI, all the tubes had germinated (Figures 1 and 2).

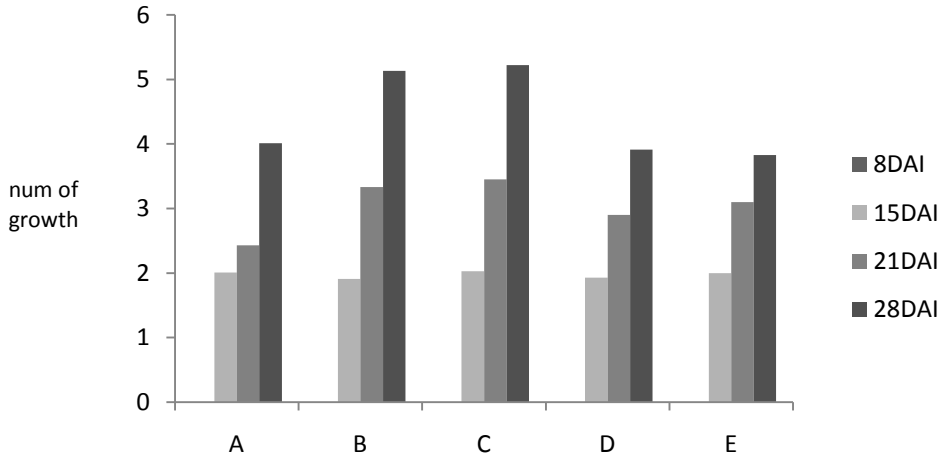

Fig. 1: Showing the growth response of *T. indica* in Murashige and Skoog (MS) medium.

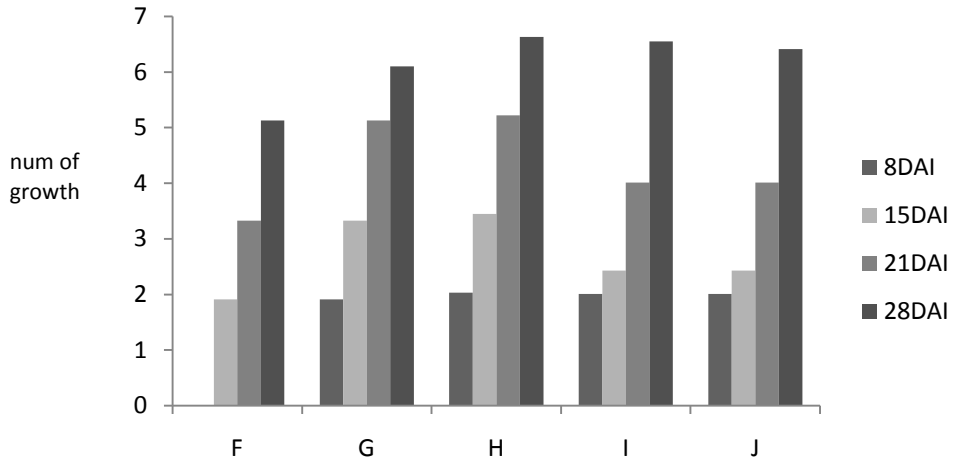

Fig. 2: Showing the growth response of *T. indica* in Woody Plant Medium (WPM).

Treatments A, B, C, D and E germinated faster than treatments F, G, H, I & J, this is in line with the work carried out by Asonibare et al. (2016) in which different media were used to compare the rate of radical emergence of *Piliostigma Thonningii*. This is also similar to the work carried out by Maku *et al.* (2014), which showed that different hormonal concentrations have effect on the number of leaves per seedling. The nutritional composition of MS medium was responsible for the better rate of growth (Asonibare et al., 2016). MS medium also contains KI, KNO_3, and $CoCl_2$ which are absent in the WPM medium (Frit industries, 2010). In Photosynthesis, potassium regulates the opening and closing of stomata, and therefore regulates CO_2 uptake. Potassium triggers activation of enzymes and is essential for production of Adenosine Triphosphate (ATP). ATP is an important energy source for many chemical processes taking place in plant issues. Nitrogen is used by plants for lots of leaf growth and good green color. Phosphorous is used by plants to help form new roots, make seeds, fruit and flowers. It's also used by plants to help fight disease. Potassium helps plants make strong stems and keep growing fast. With these elements abundantly present more in the MS medium, growth response was better than that of the WPM medium.

CONCLUSION

Tamarindus indica L. tree has various economic and medical importance. Every part of the species is very useful. Plantation establishment of this species should therefore be encouraged so that its existence will not become threatened. The results obtained from this study showed that Plant Growth Regulators at low concentrations should be adopted for the in-vitro propagation of *T. indica.* Healthy plantlets were obtained from this propagation method. From this study, it can be concluded that for optimum results in the in-vitro propagation of *T. indica*, MS medium should be employed with the right concentration and combination of Plant Growth Regulators (PGRs). The addition of PGRs in the right concentration has significant difference in the in-vitro propagation of *T. indica.*

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