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OPTIMAL SYSTEM OF FAST PROPAGATION FOR TAMARIND COTYLEDON NODES VIA TISSUE CULTURE

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Abstract

Using young tamarind cotyledon nodes as the test material, the effects of different basic culture media, hormone concentrations, and seedling states on bud induction and different hormones on root induction were studied. The results showed that the optimum medium for inducting the adventitious bud of tamarind is 6-BA 3.0 mg/l + NAA 0.5 mg/l. The most suitable seedling state for bud induction was the S3 seedling whose cotyledon exited the seed shell completely and whose true leaves were not open. The S3 seedling had the best adventitious bud induction rate of 100% and a proliferation coefficient of up to 4.1. In addition, the appropriate rooting medium was ½MS + IBA 2.0 mg/l.

Introduction

Tamarind (*Tamarindus indica* L.), also known as sweet angle and plum tree, is a leguminous tree. It is mainly produced in the various tropical regions of India, Sri Lanka, and Southeast Asia. However, China also has a small planting. Certain parts of the tamarind can be used in traditional medicine for treating colds, fevers, stomach disorders, diarrhea, and jaundice and as skin cleanser, and therefore, these parts are highly valued. Moreover, its pulp contains tartaric acid, sugar, pectin, and other ingredients rich in calcium, phosphorus, iron, and other elements (Doughari 2006). It has the highest calcium level among all fruits and is used in relieving summer-heat and as an appetizer. The seed contains 16.85 - 19.50% of protein rich in lysine, glutamic acid, aspartic acid, glycine and leucine (Bhattacharya *et al.* 1994). In addition, edible oil can be squeezed from the kernel seeds, and seed extracts can be used to absorb chromium and other heavy metals (Agarwal *et al.* 2006). The tamarind shell is rich in flavonoids, and its extract is safe to eat and can help decrease blood sugar. Furthermore, the immunomodulatory activity of tamarind polysaccharide can inhibit leukocyte migration and cell proliferation (Sreelekha *et al.* 1993), and it can protect corneal cells against UV (Raimondi *et al.* 2003). Therefore, it is largely used in the food industry and pharmacy. Finally, the tamarind does not require a large soil environment. Therefore, with its beautiful trunk and evergreen foliage, it is the ideal tree or ornamental tree to be planted next to roads and can protect the environment. As a valuable species, tamarind is worth further investigation and cultivation promotion.

In China, tamarind is mostly planted in a scattered manner with less cultivation and management, which leads to a limited variety of resources and its being wild or semi-wild (Luo *et al.* 2002). Moreover, this manner of planting gives rise to poor quality, low yields, and serious diseases. Furthermore, studies on tamarind have mainly concentrated on economic characters as well as extraction, development, and application (Dai *et al.* 2013, Luo *et al.* 2014); however, there have been only a few investigations on the cultivation and propagation of tamarind (Wang *et al.*

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2014, Li *et al.* 2014). In order to maintain good characters and keep a consistent growth state, asexual reproduction methods such as grafting and tissue culture have been explored (Zhao *et al.* 2005), but there has been just a few tissue culture investigations on tamarind. Explants came from cotyledon (Jaiwal and Gulati 1991), hypocotyl (Sonia *et al.* 1998), longitudinal section of the embryo axis with a cotyledon (LSEC), that is the cotyledon node (Mehta *et al.* 2000). In this study, the cotyledon nodes of tamarind were used as explant to study the effects of different basic culture media, hormones, and seedling states on bud induction and different hormones on root induction in order to obtain a rapid propagation technique of tamarind via tissue culture that can provide a technical basis for further investigations of tamarind breeding and genetic improvement methods.

Materials and Methods

Fresh plump tamarind seeds were acquired from sweet tamarind purchased from a super market and were used as the test material. The seeds were washed with detergent and running water. Then, they were placed on a clean bench and soaked in 70% alcohol for 30 sec and then in 2% sodium hypochlorite solution for 15 min before being washed with sterile water for 3 min for a total of three to five times. Finally, after 24 hrs of immersion in sterile water, the seeds were inoculated in ½MS culture medium (adding 30 g/l sucrose, 7 g/l carrageenan, pH 5.8, the same below) at a culture temperature of 25 ± 2°C with 16/8 photoperiod (light/dark).

The aseptic seedlings were removed from the clean bench, and the hypocotyl about 5 mm below the cotyledon was retained. After separating the two cotyledon nodes, removing the terminal bud, and cutting off a third to a half of the cotyledon in the distal part, the remaining cotyledon nodes with the proximal part upwards were obliquely stuck into the bud induction mediums (whose components are given in Table 1) for subcultures every two weeks. WPM (woody plant medium) and MS (Murashige and Skoog medium) were chosen as the basic media, and 6-BA and NAA as plant growth regulators. Eight treatments were conducted with each inoculated with 10 explants in triplicate (Table 1).

Table 1. Mediums with different basic mediums and plant growth regulation for bud induction.

Medium	Basic medium	6-BA (mg/l)	NAA (mg/l)
T1	WPM	1.0	0.5
T2	WPM	2.0	0.5
T3	WPM	3.0	0.5
T4	WPM	4.0	0.5
T5	MS	1.0	0.5
T6	MS	2.0	0.5
T7	MS	3.0	0.5
T8	MS	4.0	0.5

Four kinds of seedlings at different states were chosen from the above optimum bud induction media: S1, seedlings whose two cotyledons were un-shelled and un-separated; S2, seedlings whose cotyledons were half-shelled and where the terminal bud came out but did not exceed the cotyledon edge; S3, seedlings whose cotyledon exited the seed shell completely, and the true leaves were not open; and S4, seedlings whose cotyledon exited the seed shell completely, and the

true leaves were open. Each seedling of the four different states was inoculated with 10 explants in triplicate. The optimum state seedlings through bud induction were chosen after the budding percentages of all the seedlings were counted. Further culture of the multi-buds of optimum seedlings was conducted. The budding numbers per explant were counted in order to calculate the proliferation coefficient.

Root induction of the tamarind regeneration buds was carried out via a two-step rooting method. The first step was to promote root primordium growth by inducing buds in the medium (½ MS with IBA) through dark culture. The second step was to transfer the regeneration buds with root primordium into the same medium without hormone to grow well-developed roots. In order to investigate the effects of different hormone concentrations on root induction, media of different IBA concentrations (R1: 0.5 mg/l, R2: 1.0 mg/l, R3: 2.0 mg/l and R4: 3.00 mg/l) in the first step were prepared. Ten buds were induced in each treatment in triplicate, and the results were observed and counted. Data statistics and analysis were performed using Excel 2007.

Results and Discussion

The soaked seeds began to expand with an expansion of the seed coats in the shape of scale or villus. They began to germinate after about 8 days of inoculation in the ½ MS medium and were half-shelled or completely shelled after 15 to 20 days. During which time, the terminal buds sprouted and extended with a 100-germination rate. Moreover, the germination required more time since the tamarind seed coat contains more tannin and other phenolic compounds, which may inhibit seed germination and growth. It has been advised to remove the seed coat before inoculation so as to relieve the impact (Urmil *et al.* 2004); however, this would be a difficult procedure and would increase the probability of contamination.

Expansion and budding occurred in the junction of the basal proximal part and hypocotyl after seven days. Additionally, multi-buds formed in about 15 days, and regenerated buds of 2 - 3 cm grew in about 30 days. Different media resulted in different bud induction times and rates (Fig. 1). The average bud induction rate was above 46%, in which T3 had the highest rate. This indicates that 6-BA and NAA had good effects on the tamarind cotyledon nodes. The bud induction rate reached 93.3% in the medium (WPM + 6-BA 3.0 mg/l + NAA 0.5 mg/l), which was significant ($\alpha = 0.05$) compared to the other treatments. In contrast, the lowest bud induction rate was 46.7% in the medium (MS + 6-BA 4.0 mg/l + NAA 0.5 mg/l). The WPM culture medium was more suitable for the regeneration of the tamarind cotyledon nodes than the MS culture medium. Moreover, the bud induction rates increased with increasing 6-BA concentration within a certain range and began to decrease after the 6-BA concentration exceeded 3.0 mg/l. As a result, T3 (WPM + 6-BA 3.0 mg/l + NAA 0.5 mg/l) was the optimal bud induction culture medium.

As one of the most commonly used cytokinins in plant tissue culture, the superior inductive effects of 6-BA have been demonstrated in numerous studies. The results of Jaiwal (1991) and Sonia (1998) also showed that 6-BA had good inductive effects on the explants of *Tamarindus indica* L. Similarly, the results in this study demonstrated that the use of 6-BA + NAA can help in inducing the regeneration of tamarind cotyledon nodes. Moreover, by comparing the WPM basic culture medium with the MS basic culture medium, the results showed that the WPM medium had better inductive effects than the MS medium.

The bud induction rates and proliferation results of the cotyledon nodes under four different seedling states in optimal culture media exhibited the following results: the bud induction rates followed the sequence S3 > S2 > S4 > S1 (Figs 2, 3). In addition, the bud induction rate of the cotyledon nodes under optimal seedling state on medium could achieve up to 100% with a

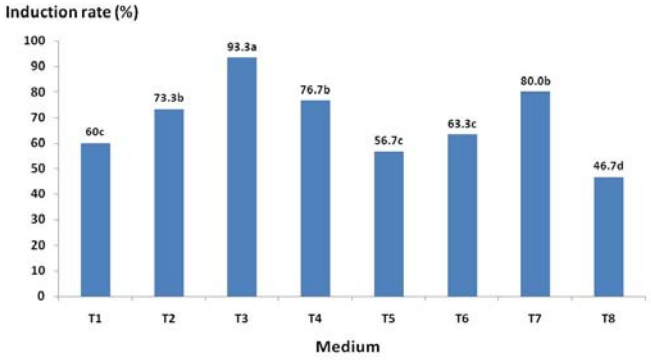

Fig. 1. Bud induction rates in different basic mediumswithvaryinghormone concentrations (α = 0.05)

proliferation coefficient of up to 4.1, and the average proliferation coefficient was 3.6. The S1 seedlings whose cotyledon nodes were too young to adapt to an *in vitro* environment had a lower bud induction rate, and the S4 seedlings whose cotyledon nodes were too old so that hypocotyl part connecting the cotyledons was lignified to a great extent had a lower bud induction rate as well. Therefore, the S3 seedling whose cotyledon exited the seed shell completely and did not have open true leaves was the most suitable seedling state for bud induction.

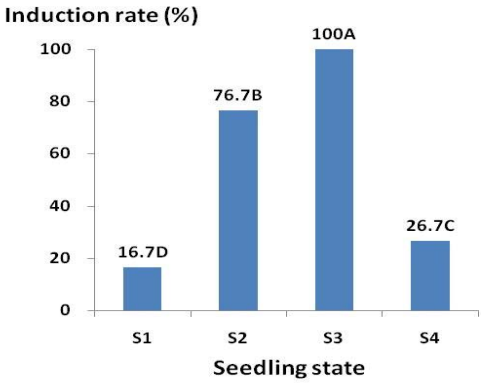

Fig. 2. Bud induction rates for different seedling states (α = 0.01).

The seedlings of the four different states showed differences in their seedling ages, sizes, shapes, endogenous hormone of explants, and adaptation towards the external environment, which in turn influenced the adventitious bud induction rates. In this study, the seedling ages of S1, S2, S3 and S4 were 12, 15, 20 and 25 days, respectively. The S3 state at 20 days produced the best effect as an explant, which was inconsistent with the study results of 12 days (Sonia 1998) and 15 days (Mehta 2000). This inconsistency might be relevant to the species or to habitat differences. Furthermore, consistent results were shown in the bud induction rate and proliferation coefficient.

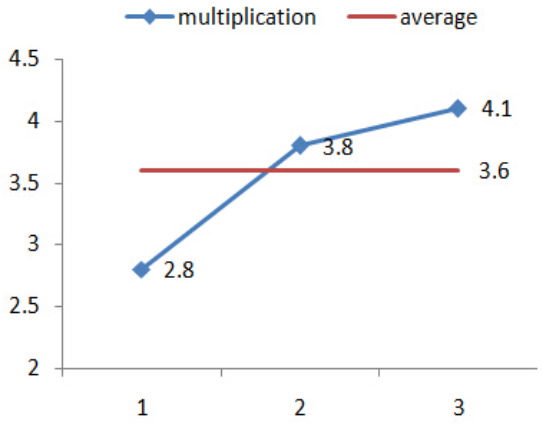

Fig. 3. Multiplication factor statistics under optimal seedling states.

The roots of the regeneration buds cultured on root induction media with IBA began to expand after seven days and grew erumpent root primordia. Different media resulted in different root primordial growth times, and they followed the sequence R4 > R3 > R2 > R1. After the root primordium appeared, the buds were transferred to medium supplemented with ½ MS. By the eighth day, short roots began to appear and then slowly increased. The root induction rates as well as the root numbers, lengths, and shapes were different for the different treatments (Figs 4 and 5). The root induction rates followed the sequence R3 > R2 > R1 > R4. Roots induced by the R1 and R2 treatments were few in number, and only one to two roots were present, whereas the R4 treatment induced numerous roots with a short length and a black color. In contrast, the R3 treatment induced numerous roots with a normal shape and robust seedling growth. The result showed that IBA was suitable for the root induction of tamarind. A low IBA concentration had poor induction results, and a high IBA concentration resulted in toxic seedlings, which affected the root growth and absorption of cultured seedlings. The two-step rooting method was adopted to avoid the toxic effects of growth hormone on the plants. As a conclusion, the optimal culture medium for root induction is R3, ½MS + IBA 2.0 mg/l.

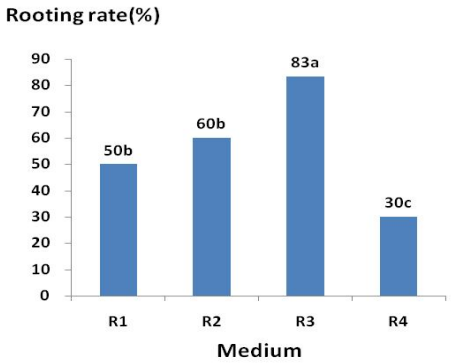

Fig. 4. Effects of different hormone concentrations on root induction.

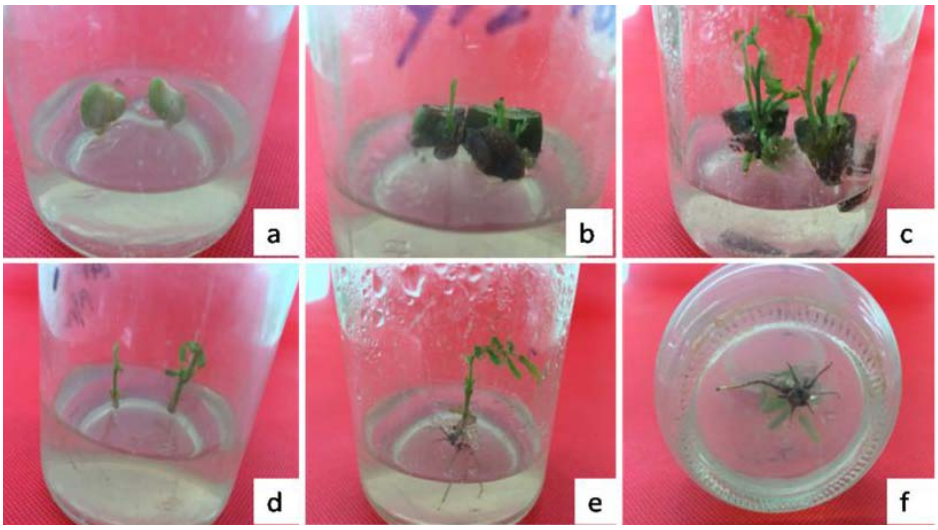

Fig. 5. Regeneration of tamarind cotyledon nodes. a: Explants of cotyledon nodes. b: Adventitious buds on bud induction medium began to appear from the junction of the proximal part and hypocotyl. c: Proliferation and elongation of multiple shoots on medium supplemented with WPM + 6-BA 3.0 mg/l + NAA 0.5 mg/l; d: Root induction culture of regeneration buds. e and f: Root grew from the base of regeneration buds and into complete tissue culture seedling.

Tamarind cotyledon nodes were used as explants to study the effects of different basic culture medias, hormones, and seedling states on bud induction and different hormone concentration son root induction in this paper. Through this tissue culture technique, seven to eight virus-free plantlets per seed (two explants) of the same age were obtained in about 10 weeks. This method offers numerous advantages: it is easy to operate, occupies a small area, and is not affected by the season or environment. Moreover, the breeding efficiency is high, and a large number of consistently-grown seedlings can be obtained in a short term as compared with the traditional seed breeding method.

Tissue culture is the technological basis of the genetic improvement of crops and is an important part of constructing a genetic transformation system. The establishment of a tamarind tissue culture technique could provide reliable technical reserves for the future application of genetic improvement and breeding.

References

Agarwal GS, Bhuptawat HK, Chaudhari S 2006. Biosorption of aqueous chromium (VI) by *Tamarindus indica* seeds.Bioresource Technol. **97**(7):949-956.

Bhattacharya S, Bal S, Mukherjee RK, Bhattacharya S 1994. Functional and nutritional properties of tamarind (*Tamarindus indica*) kernel protein. Food Chem. **49**(94):1-9.

Dai Jianjv, LuoHuiying, Liu Haigang 2013. A comparative study of 15 individual *Tamarindus indica* fruits from different ecological regions in Yunnan. China South F **04**:78-80.

Doughari JH 2006. Antimicrobial activity of *Tamarindus indica* Linn.Trop J. Pharm. Res. **5**(2): 597-603

Jaiwal PK, Gulati A 1991. *In vitro* high frequency plant regeneration of a tree legume *Tamarindus indica* (L.). Plant Cell Rep. **10**(11):569-73.

Li Huiduan, Cui Xu 2014.Extraction of general flavone on *Tamarindus indica* shell and scavenging hydroxyl

radical activity. Southern Agri. Sci. **05**:844-849.

Luo Jingping, Yan Junhua 2002. Survey on wild resources of *Tamarindus Indica* and Ecological Planting Regionalization in Yunnan. J. YnSciTech. **02**:20-22

LuoHuiying, Dai Jianjv, Liu Haigang, DuanYuetang, Ma Kaihua, QuWenling, ShaYucang 2014. Economical character evaluation of different types of *Tamarindus Indica* fruits. Trop. Agri. Sci. **03**:39-44.

Mehta UJ, Krishnamurthy KV, Hazra S 2000. Regeneration of plants via adventitious bud formation from mature zygotic embryo axis of tamarind (*Tamarindus indica* L.). Curr. India. **78**(10):1231-1234.

Raimondi L, Lodovici M, Guglielmi F, Banchelli G, Ciuffi M, Boldrini E., Pirisino R. 2003. The polysaccharide from *Tamarindus indica* (TS-polysaccharide) protects cultured corneal-derived cells (SIRC cells) from ultraviolet rays. J. Pharm. Pharmacol. **55**(3):333–338.

Sonia PKJ, Gulati A, Dahiya S 1998. Direct organogenesis in hypocotyl cultures of *Tamarindus indica*. Biol. Plantarum. 41**(3)**:331-337.

Sreelekha TT, Vijayakumar T, Ankanthil R,Vijayan KK, Nair MK 1993. Immunomodulatory effects of a polysaccharide from *Tamarindus indica*. Anti-cancer drug. **4**(2):209-212.

Urmil J. Mehta, Shaila M. Barreto, SulekhaHazra 2004. Effect of thidiazuron in germinating tamarind seedlings. In Vitro Cell Devan. **40**(3):279-283

Wang Jie, Wang Huixian, Wang Ling 2014. Optimization of extraction process of *Tamarindus indica* Pectin and antioxidant activity. Guangzhou Chem. **13**:52-55.

Zhao Yihe, Yang Shiyu, Li Kun 2005. Research and development of *Tamarindus indica* Worldwide. J YnAgri Univer. **01**:65-72.

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