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Rapid Clonal Propagation of *Tamarindus indica* (L) Using Explants from Adult Trees

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Abstract: *Tamarindus indica* (Tamarind) is a wild tropical tree species, over exploited commercially in third world countries but remains unimproved and neglected. Tissue culture has the potential to propagate elite genotypes of trees. Micropropagation of *Tamarindus indica* was achieved through adventitious shoots or axillary bud proliferation from nodes of adult trees (10-15 years old) on MS medium with BA, Kin. Proliferation of shoots continued on the medium with reduced levels of growth regulators and vitamins. Rooting was obtained with IBA. Nodal explants from young shoots responded better in comparison with the explants from mature shoots in the early stages of establishment of cultures and rooting of shoots.

Key words: *Tamarindus indica, in vitro*, adventitious shoots, regeneration and rooting

INTRODUCTION

Tamarindus indica (Tamarind) is a tropical tree legume species yielding fruits with sour taste, the pulp of which is used in many preparations in asian cooking. The leaves, fruits and seeds are used in herbal medicine. The trees also yield valuable timber used in making furniture and high quality firewood. *Tamarindus indica* is grown extensively but unattended in India. The unimproved wild trees are continuously exploited to meet the growing commercial demand. The major limitation in the improvement of tamarind has been lack of prioritized research agenda and the progress made on the scattered research has been unimpressive. Lack of improved cultivars, rapid and easy methods of germplasm production are the real constraints (Gunasena and Hughes, 2000). Tamarind is naturally propagated through seeds, though it can also be propagated rarely through cuttings to a limited extent. Genetic improvement of the species for higher biomass production is beset with problems due to its long generation time, heterozygous nature due to cross- pollination. Since adult trees are difficult to propagate through conventional techniques using cuttings, biotechnology can play an important role in the program of improvement. Therefore, micro propagation of elite genotypes occurring in natural stands is the most practical option to improve productivity in this neglected tree species.

Micropropagation of *Tamarindus* species has been reported earlier from the adaxial surface of the cotyledons of 12 day old seedlings with BAP (Jaiwal and Gulati, 1991); shoot tip explants from *in vitro* grown seedlings with IAA and IBA (Kopp and Nataraja, 1990) and hypocotyl segments (Sonia *et al.*, 1998). In all these cases, the explants used were obtained from seedlings or from juvenile material. However, there are no reports yet on the regeneration from the explants obtained from the mature trees. Usually, the explants obtained from mature trees, tend to release polyphenols in the medium leading to poor establishment, stunted growth and slow multiplication. This study provides information on the *in vitro* regeneration of *Tamarindus indica* using the explants obtained from the young branches of adult trees and compares the explant response in terms of release of polyphenols, establishment of cultures and other micropropagation parameters.

MATERIALS AND METHODS

Nodes from young shoots and mature shoots were collected from 15-20 years old trees of *Tamarindus indica*. 20-23 mm explants with preformed lateral or terminal buds were washed in running tap water for 2-3 h followed by partial drying for 5-10 min in a petri plate. The surface sterilization was accomplished with 0.05% $HgCl_2$ for 15 min followed by 3-4 rinses in sterile distilled water. Three media are used viz. Initiation medium, Multiplication medium and Rooting medium. All of these have MS composition (Murashige and Skoog, 1962) but differ in growth regulators and vitamins. Several trails were made with varying concentrations of the hormones and only those concentrations are reported here which gave positive response. Explants were inoculated onto initiating medium supplemented with benzyladenine (BA) - 2 mg l^{-1}, kinetin (KIN) - 2 mg l^{-1} and activated charcoal 0.5%. Shoot multiplication was done on multiplication medium with reduced levels of growth regulators and vitamins: 1 mg l^{-1} BA, 0.5 mg l^{-1} KIN. After proliferation, individual shoots 2-3 cm long, were separated and cultured on rooting medium consisting of

half strength MS medium with 5 mg l^{-1} of indole butyric acid (IBA). In all media 3% sucrose was used. Cultures were incubated at 25±2°C with a photoperiod of 16 h light 8 h darkness. Light intensity was 2000 lux. The plantlets were potted in sterilized potting mixture (3 parts sand: 1 part soil: 1 part compost) in polythene bags and acclimatized in greenhouse and irrigated when required. Standard error is given to indicate the variation among the means of three consecutive experiments based on 50 replicates.

RESULTS AND DISCUSSION

The process of micropropagation of *Tamarindus indica* involves thee stages viz. enlargement and sprouting of lateral buds, proliferation of shoots and rhizogenesis. After running several preliminary experiments on the response of explants to different concentrations of growth regulators, the present combination and concentrations were chosen. The protocols were established for high frequency micropropagation of *Tamarindus indica* involving enhanced formation of adventitious shoots from the nodal segments of the branches from matured trees. Pre-existing lateral buds from nodal segments of both young and mature shoots, sprouted after 4-5 weeks after inoculation in the initiation medium. Release of polyphenols was noted in both types of explants initially which has greatly affected culture establishment. Exudation of polyphenols into the medium could be avoided to a large extent by washing the explants in running water and then drying them partially. This step was crucial for the initial establishment of the cultures. Addition of charcoal significantly reduced the polyphenol leaching. Sprouting of buds was far more frequent in explants from younger branches than in older branches. The sprouted cultures were transferred to fresh medium for further establishment. In 2-3 weeks, shoots (2-4) developed on each explant. When the individual shoots were separated and subcultured on multiplication medium, they developed multiple shoots in three weeks. Once the cultures were established, the source of explants has no influence on the rate of proliferation of shoots. Profuse rooting was observed within three weeks of transfer of the shoots onto rooting medium. The frequency of rooting was much higher in explants derived from young shoots than in mature shoots (Table 1). The acclimatization and rate of survival of plantlets was significantly greater in shoots regenerated from younger explants.

Table 1: Effect of explant on culture responses in *Tamarindus indica*

Source	Culture response on initiation medium (Mean % ± S.E.)	Culture response on rooting medium (Mean % ± S.E.)	Survival (Mean % ± S.E.)
Explants from mature shoots	25±1.9	32±3.7	39±2.5
Explants from young shoots	59±1.5	62±2.3	56±1.2

The importance of selection of appropriate explant for *in vitro* culture has been recognized in many tree species for the establishment of primary cultures and subsequent development and successful regeneration. We have observed that in *Tamarindus,* the apical portions of mature shoots and the zone near the floral and apical meristems were more suitable, since these regions are relatively more juvenile and have greater potential for regeneration. Nevertheless, the young shoots are understandably more juvenile than any part of living tree and the results of our study agree with this general concept.

It is clear from the results that the source of explant plays a major role in the initial establishment of cultures, in regeneration, in subsequent rooting ability, in the acclimatization and survival of the plantlets. Use of young shoot explants could be crucial for large scale micropropagation. With this study the effect of explant source on rooting and final establishment of plantlets has been recognized in *Tamarindus.* Jaiwal and Gulati (1991) have shown that regenerated shoots obtained from seedling cotyledon explants established readily with 70% success. In our study the response of young shoots in general was better than that of explants from mature branches from the trees. While explant source does affect initial response pattern, it is not clear how it would influence many remotely related aspects of regeneration such as multiplication ratio and subsequent rooting. The protocol described here can be routinely used for mass propagation of adult *Tamarindus* clones, although some adjustments may be necessary to achieve better survival percentages in specific clones. In conclusion, it is advantageous to use explants from young shoots from trees for micropropagation of *Tamarindus.*

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