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MICROPROPAGATION OF ENDEMIC AND ENDANGERED TREE TAXA OFTERMINALIA PALLIDA BRANDIS OF TIRUMALA HILLS

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ABSTRACT

Terminalia pallid Bramdis is narrow endemic endangered and threatened medicinal tree taxa on Tirumala hills of Eastern Ghats. It is one of the oldest medicinal herb of India, is an ingredient of Ayurvedic drug "triphala" used for the treatment of hepatic disorders. This globally threatened plant is over exploited for its medicinal use. The present work is aimed to develop a protocol for micropropogation method for rapid and large scale propagation of the Terminalia pallid by using cotyledonary node explants of In vitro grow seedling. B5 is the most suitable medium for multiplication than WPM and MS medium. 5-6 shoot were on B5 medium containing 2 and 5mg/l BAP + 0.5 mg/l NAA. Rooting was best induced in shoot excised from proliferated shoot culture on MS 1mg/l BAP+2mg/l NAA. The rooted plant was successfully acclimatized in the soil.

KEYWORDS: KN – Kinetin, BAP – N6 – Benzyl Aminopurine ; IAA – Indole 3 – acetic acid; IBA – Indole butyric acid; NAA – 1 - Naphthalene acetic acid.

INTRODUCTION

Terminalia is a large genus of combretaceae, covering more than 250 species of which about 12 species are found in India. Being endemic to seshachalum hills in view for strong claim made by folk lore about its therapeutic importance, *T. pallida* was taken up in to the present investigation. *Terminalia* species are important ingredient in ayurvedic therapy called "Triphala". The folk lore claim that the fruit decoction of *T.pallida* used to bring down the fever i.e. antipyretic action. *T.pallida* leaf gall are used to cure diarrhea and dysentry which fruit paste is used extensively in dysentery, sores and as leaf have shown mild to good

influence on central nervous system by reducing reaction time and spontaneous motor activity and exhibit hypothermia. *T.pallida* showed 100% inhibition of mycelium growth of *sclerotium rolfsia* at 2000 pm.[1] Parts from all these leaves are fed to animals as a green fodder during lean period. Wood is valuable and used for making agricultures implements house construction and musical instrument. Fruit paste is applied as an ointment.

Invitro plant regeneration among the members of combretacea has been quite difficult. The explants source has been one of this important factor that greatly affected the frequently of plant regeneration. The results presented below provider a complete and rapid clonal propagation system for *T.pallida.* To our knowledge this is first report on *T.pallida.* Unfortunately there is no such report available for clonal propagation and seed germination studies of *T.pallida* in this paper we presented rapid multiplication of this medicinally important plant through high frequency axillary shoot proliferation from cotyledonary node explants followed.

MATERIALS AND METHODS

Mature dry fruits of *T.pallida* are collected from Tirumala hills of seshachalam forest. The fruit wall was mechanically removed with the help of stone. The viable seeds were washed under running tap water, and again washed with liquid detergent for 10-15 minutes. After dipping the seeds within 70% ethanol for 5 minutes and rinsing with the sterile distilled water. The seeds were gently agitated in 0.3% H2 02 for 3-5 minutes. The seeds are washed with sterilized distilled water and placed on ms medium supplemented with 2mg/l BAP+0.5 mg/l NAA Cotyledonary node explants were excised 15-30 days. *Invitro* seedlings and culture on different media MS,B5 & WPM containing different concentrations of BAP, KN (1-5mg/l) alone or in combination with the 0.5mg/l NAA, IBA & IAA for shoot multiplication and rotting 2% sucrose, 0.8% ager , 500mg/PVP added to the medium and PH was adjusted to 5.6 before autoclave. All cultures were maintained at 25+1^{o}c and 16 h photo period provided by white florescent light. There was a minimum of 25 individual explants per treatment and each treatment was repeated three times. The regenerated shoots were removed from the culture tubes and transferred to the garden soil and sand 3:1 ratio. The plants were covered with the polythene bags to maintain high humidity. The regenerated plant was successfully acclimatized on the soil.

RESULTS AND DISCUSSION

Among the different explants (hypocotyl, roof, leaf, shoot) tested; only cotyledonary node explants showed positive morphogenetic response and readily developed multiple shoot and *T.pallida.* Cotyledonary node produce multiple shoots following incubation on medium containing 1-5 mg/l BAP or KN on different media (MS,B5, WPM). But best growth obtained on B5 medium. (Table-2, 3) In all these medium BAP lower than 1-5mg/l was more effective (Table-1). When cotyledonary node explants cultured on B5 medium supplemented with 1-5 mg/l BAP or KN alone or in combination with (0.5-1 mg/l) NAA or IAA differentiate maximum number of shoot buds. Though explants cultured on these two cytokinins, the best growth recorded only on BAP. When cotyledonary node explants cultured on B5 medium containing 2mg/l BAP + 1 mg/l NAA the explants differentiated 5 to 7 axiallary shoot per explants (Fig-1). Almost a similar no of shoots were obtained on the medium containing, 5mg/l BAP + 1mg/l NAA. But more no of shoots were responded in 5mg/l BAP + 0.5 mg/l NAA than 2mg/l BAP + 1 mg/l NAA (Table-1) these shoots were well dark green and without evidence of defoliation. Sub culturing of these shoots on to medium containing 5mg/l BAP + 0.5 mg/l NAA resulted in an increase in shoot mass growth. These micro shoots were small, further more it was difficult to separate them from the explants. When cotyledonary node explants were placed on ms medium containing 1mg/l NAA and 5mg/l BAP on approximately 60% of explants shows little callus with 5-6 shoots were formed within 5 weeks. In the presence of 0.5 mg/l NAA with 5mg/l BAP the number of multiple shoots obtained were nearly double the no of obtained on the medium containing 0.5mg/l IAA with 5mg/l BAP (Table-2). Replacement of BAP with KN resulted in less shoot multiplication. Cultures of cotyledonary node explants an MS and WPM medium containing 2 & 5mg/l BAP +0.5 mg/l NAA generally resulted in 2-4 shoots. These explants also showed positive response in 2 mg/l BAP + 0.5 mg/l IAA. . Among the different explants tested only those of the cotyledonary node showed best morphogenetic response and readily developed multiple shoots from explants. Among these B5 medium was focused to be the best medium for shoot induction in *T.pallida.*

This shoot induction in *Anogeissus pendula.*[2], *A. Sericea vara sericea*[3] *wrightia tintoria*[4] *Acacia nilotia*[5], *Eucalyptus tercticornis*[6] *Sesbania.*[7] KN was less effective in inducing shoot buds[8] and [9] also report this for shoot differentiation. The combination of auxin and cytokinin seams to be positive for induction of multiple shoot and elongation of shoots in *T.pallida.* More number of 5-6 shoots were formed at higher concentration of BAP 2 or 5

mg/l with 0.5 mg/l NAA and only 3-5 shoot regenerated to 4-6 cm in *T.pallida* some produce compact callers at the base of the explants. Similar results were obtained in *Eucalyptus*[10], *Dalbergia latifolia*[11], *Pterocarpus santalinus*[12] *sygigium* alternifolium[13] *Citrullus vulgaris.*[14]. However KN was found to be second best cytokinin for shoot multiplication.

Figur 1: Induction of multiple shoots from Cotyledonary node explants of Terminalia pallida cultured on different Media fortified with 1-5 mg/lBAP+0.5mg/lNAA.

TABLE- 1: Effect of growth hormone combinations on multiple shoot inuction of cotylendonary noe explants of t. Pallida on b5 medium

Hormone Concentration (mg/l)	% of explants showing shoot buds	Shoot No.Per explant ± S.E	Shoot length(cm)
BAP + NAA			
1 + 0.5	30	2.5 ± 0.5	5.2±0.4
2 + 0.5	54.8	5.8±0.6	6.2±0.8
3 + 0.5	38	3.8±0.2	5.3±0.9
4 + 0.5	39	4.5 ±0.1	4.8±0.7
5 + 0.5	62.5	5.1±0.7	5.8±0.9
BAP + NAA			
1 + 0.5	18	1.9±0.7	5.1± 0.5
2 + 0.5	22	2.2±0.9	5.2± 0.9
3 + 0.5	18	1.2±0.7	5.1± 0.8
4 + 0.5	12	1.3±0.8	5.6±0.7
5 + 0.5	35	2.8±0.9	5.6±0.3
KN + NAA			
1 + 0.5	27	1.8±0.9	5.1±0.2
2 + 0.5	28	2.1±0.7	5.2± 0.4
3 + 0.5	18	1.1± 0.5	5.1± 0.5
4 + 0.5	22	1.5 ± 0.5	4.9±0.7
5 + 0.5	39	1.9± 0.5	5.2±0.9

Excised shoot were cultured on MS medium supplemented with the IBA, NAA & IAA individually or in combination with BAP induced roots within 30 days (Table-4). Of these auxins tested NAA was most effective in inducing roots without callus. About 80% of the excised shoot developed root within 20-30 days. The percentage of rooting was markedly enhanced by supplementing the medium with a combination of 2mg/l NAA with 0.5-1 mg/l BAP (Fig-2). 91% of the shoots developed rooting within 30-60 days the combined presence of these hormones favored root induction in *Frazinus angustifolia.*[15]

Table -2: Effect Of Growth Regulators On Invitro Shoot\Formation Of Leonary Node Explants Of Terminalia Pallida On Ms Medium

Hormone Concentration (mg/l)	% of explants showing shoot buds	Shoot No.Per explant ± S.E	Shoot length (cm)
BAP + NAA			
1 + 0.5	42	1.8±0.4	4.8±0.6
2 + 0.5	58	2.8±0.3	5.2±0.2
3 + 0.5	42	1.9±0.1	4.5±0.6
4 + 0.5	40	2.1 ±0.4	4.0±0.4
5 + 0.5	68	3.1±0.8	4.7±0.5
BAP + NAA			
1 + 0.5	38	1.5±0.4	2.8± 0.4
2 + 0.5	22	2.6±0.6	3.8± 0.5
3 + 0.5	12	2.1±0.4	3.2± 0.8
4 + 0.5	18	2.6±0.6	3.8±0.7
5 + 0.5	38	2.8±0.8	4.1±0.9

Table- 3: Multiple Shoot Induction From Cotyleonary Node Explants Of Terminalia Pallida On Wp Medium Containing Different Growth Hormones

Growth Regulators (mg/l)	% of explants showing shoot buds	Shoot No. Per explant ± S.E	Shoot length (cm)
BAP + NAA			
1 + 0.5	27	1.9±0.6	4.5±0.7
2 + 0.5	17	1.4±0.5	4.2±0.2
3 + 0.5	15	1.6±0.7	3.2±0.9
4 + 0.5	8	1.5±0.8	3.7±0.8
5 + 0.5	31	2.6±0.9	4.9±0.7
BAP + NAA			
1 + 0.5	18	1.5±0.8	3.7± 0.9
2 + 0.5	15	1.7±0.9	3.9± 0.9
3 + 0.5	9	1.1±0.7	3.2± 0.6
4 + 0.5	10	0.9±0.5	3.4±0.7
5 + 0.5	17	1.2±0.5	3.7±0.7

In the present study and in the[16] and[17], application of auxins was essential for adventitious root formation. NAA was found to be more effective than IBA or IAA. Roots were also produced on MS medium containing 1mg/l NAA and BAP or Kn. These roots were regenerated from callus of the cut end of shoots. This was also reported in Anogeissus[2&4] and Terminalia bellerica.[18] Repeated subculture onto fresh proliferation medium progressively improved rooting frequency. This corresponds with result in Apple[19&20] and guava.[21] Repeated subcultureing may change the physiological state and gradually regenerate the shoots, which in turn promotes better rooting.[22, 23&24]

Fig2*:* Adventitious root induction from micro propagated shoots of Terminalia pallida on MS medium with 2mg/lNAA+1mg/l BAP

TABLE -4*:* EFFECT OF HORMONES ON ROOT INDDUCTION OF INVITRO GROWN SHOOTS EXPLANTS OF TERMINALIA PALLIDA

Hormones (mg/l)	No. of roots/ shoot	Length of root	Morphogenesis	Growth of shoot (cm)
MS + IBA (1mg/l)	2	6-8	Roots produced were not branched, no callus at the end of the shoot	3-4
MS + IBA (2mg/l)	1	5-6	Slender root with lateral, no callus at the end of the shoot	4-5
MS + BAP 1 (1mg/l) + IAA (2mg/l)	20	2-5	Callus produced from cut ends of the shoots and strong roots were produced from callus	2-5
MS + BAP (1mg/l) + NAA (2mg/l)	30 - 50	1 - 2	Roots produced from cut end of the shoot and they were week	4-5
MS + IBA + BAP (2mg/l)+ 0.5 NAA IAA (2mg/l)	1	5-7	No callus is produced at cut end of the shoot roots with laterals	5-6

The regenerated plants from *Invitro* culture were transferred to the garden soil and sand 3:1. They are maintained under high humidity in culture room for 8-10 weeks. Plants were transferred to the natural conditions where plants show normal shoot and leaf structure like natural plant.

SUMMARY AND CONCLUSION

In summary concentration of B5 medium containing 2 and 5mg/l BAP + 0.5 mg/l NAA is best for multiple shooting and MS basal medium containing 1mg/lBAP+2mg/l NAA is best for rooting.

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