

**REGENERATION OF PLANTS FROM HYPOCOTYLE AND ROOT
EXPLANT OF ENDEMIC AND ENDANGERED TREE TERMINALIA
PALMATA BRANDIS OF TIRUMALA HILLS.**

R. Vinolya Kumari*

Lecturer in Botany, K.V.R Govt College (w), Kurnool, 518002.

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***Correspondence for
Author**

R. Vinolya Kumari

Lecturer in Botany, K.V.R
Govt College (w),
Kurnool, 518002.

ABSTRACT

Callus was induced from Root, Hypocotyle and Leaf explants of *Terminalia Palmata* on MS medium supplemented with 2, 4-D alone or in combination with the BAP or KN. Adventitious shoot buds were regenerated from hypocotyle and root explants from the callus tissue on the medium containing BAP or KN in combination with NAA. Generally hypocotyls explants were more responsive than root explants and developed shoots on MS + 1mg/l BAP+ 0.5 mg/l NAA. Whereas root explants develop shoots on MS + 1mg/l BAP + 0.05 mg/l NAA the shoot buds grew into shoot on MS + 2mg/l BAP + 0.5mg/l. Rooting was inducing when shoots were transferee to MS + 2mg/l

BAP. The regenerated plants were transfer to the soil.

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

INTRODUCTION

There is greater need to develop efficient methods for vegetative propagation of recalcitrant tree species. The family combretaceae represents no of economical important plant species requiring rapid clonal propagation method. The plants of genus *Terminalia*, comprising of 250 species, are widely distributed in tropical region,^[1] The genus *Terminalia* should be declared as national tree genus by the Government of India.^[2] In this *Terminalia pallida* is an important constituent in Triphala in are being used intensively in Indian system of Ayurvedic medicine. *Terminalia pallida* is endemic to Seshchallum hills in view for strong claim made by folklore about its therapeutic importance; *T.pallida* was taken up into the present investigation.

Although regeneration of plants from callus has been achieved in the number of woody.^[3,4,5,6,7&8] regarding morphogenesis and less in other members which are medicinally important part of the plants from callus cultures of the root and hypocotyl explants of *Terminalia Pallida*. As the regenerative potential was maximum in the root and hypocotyls explants, there was used as the explants material to determine the efficiency of the various media to induce callus and organogenesis.

MATERIAL AND METHODS

Seeds were collected from the Tirumala hills of Sechachallum hills. Seeds were mechanically separated from the seed coat and were surface sterilized for 10min in tween 80, then dipped in 70% alcohol for 3.5 min and subsequently in the 0.5% H₂O₂ for 2-3 min 3-4 times in the sterile distilled water. Seedlings of *Terminalia pallid* were micropropagated on MS medium in the 2 mg/l BAP. Hypocotyl and leaf explants were aseptically excised from 15-30 days old seedling. The explants were transferred to the MS medium with various concentration and combinations of auxins. And cytokinin and sucrose 3% is as the carbon source. The PH of the medium was adjusted to 5-6 by adding 0.8% agar before sterilization was observed within 15 days and callus was subculture one in every fifteen days well developed shoots were excised and transferred to rooting media for rooting. All the experiment was repeated at least thrice.

RESULT

CALLUS CULTURE

Within a week after placement of the explants (Hypocotyl, leaf and root) on MS medium callus formation was evident. Callus was initiated first along the cut surface, then alone the entire surface of the explants after 10-15days of cultures, depending on the auxin and cytokinin concentrations in the calls medium. MS medium with BAP and KN in the cultures medium failed to induce callus, even when the cultures were kept for prolonged periods. Most of the medium supplemented with the NAA, IAA, 2, 4-D alone or in combination with 0.5-2 mg/l BAP yielded the best callus in terms of growth rate and appearance and organogenesis (Table1). Healthy green callus was formed from hypocotyls and leaf followed by root (figer-1). Explants grow in light generally turned brown and callus formation was poor. Of the different concentrations tested 2mg/l 2,4-D alone or in combinations with 0.5-2mg/l BAP yielded the best callus in terms of growth rate and appearance with 0.5-2mg/l BAP yielded the best callus in terms of growth rate and appearance and organogenesis (Table-

1). Callus was greenish yellow nodular in the beginning and turns brown if allowed to grow in same medium for more than 5-6 weeks. When leaf, hypocotyls and root callus was excised and transfer to MS medium with different concentrations BAP or KN (1-2 mg/l) with 0.5 mg/l NAA green nodular callus was produced from explants (figer-2). But shoot organogenesis was evident from hypocotyls and root explants but in leaf no shoot organogenesis were produced in BAP or KN concentrations higher than 3 mg/l or lower than 0.5mg/l. When explants were cultured on medium in order to induce callus formation, in most cases the explants turn brown. Large quantity of dark brown exudates was secreted into the medium. The browning of explants could be prevented by the addition of 500 mg/l PVP to the medium Callus was apparent on the wound surface of the explants between 3-6 weeks after transfer to the culture medium. Of the three explants used, hypocotyls formed callus earlier and this callus continued to grow faster (Figer-1). This callus could probably provide suitable material for the initiation of organogenesis. Callus grown on 0.5-2mg/l NAA or 2, 4-D in combination with 0.5-2mg/l BAP or KN was yellow or green and had a more compact texture.



FIGURE: 1 Induction of callus from hypocotyle, leaf and root explants on MS medium containing 2,4-D

1. Hypocotyle

2. Leaf

3. Root

ORGANOGENESIS

When hypocotyle explants of *Terminalia pallid* were cultured on MS medium containing 0.5-1 mg/l NAA with 0.5 mg/l BAP callus arises from the base of explants tissue within 15 days of cultures. The processes of differentiation involved the gradual loss of organized vascular system, disappearance of epidermal layer, proliferation of tissues mass by cell division in increasingly in isolated location. Unorganized callus was formed to have developed between the third and six month of cultures. Within a week after placement of the explants on MS

medium callus formation was evident. The callus was white or greenish and initially proliferation in the combination of auxins and cytokines at the cut ends of explants resulting in a nodular like appearance. The earlier stage of *de novo* bud induction occurred upon transfer of callus of 3-6 month age on MS medium contains 0.5 mg/l NAA with 1mg/l BAP in hypocotyle explants (figure b). Transfer of induce callus and buds to hormones free medium permitted maturation and further development of shoot buds. It was during these passages that buds become recognized on the surface of callus. Most cultures were competent to this stage, but also induce callus. Some cultures exhibited on improvement in developmental competence as well as continued induction of new buds when subculture on MS+ 0.5 NAA+2 mg/l BAP or 0.5mg/l IAA+ 1mg/l BAP (figure 2). Repeated subculture of shoot buds in the medium permitted continuously induction of shoot buds from hypocotyle explants. Shoot bud explants development accompanied by 10-15 small shoot in MS+0.5 mg/l NAA+2mg/l BAP.

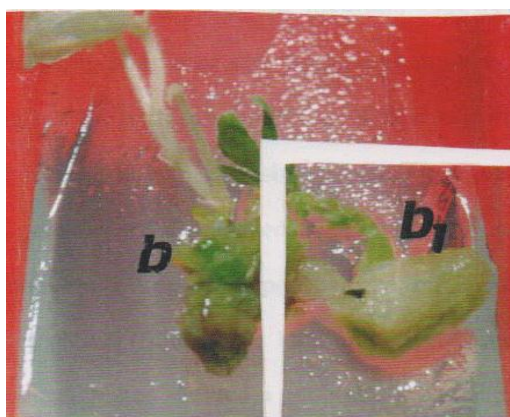


Fig:2 Induction of shoot buds from Hypocotyle explants on MS medium with 0.5mg/l NAA+1mg/l BAP

B: MS medium with 0.5mg/l NAA+2mg/l BAP.

B1: MS medium with 0.5mg/l IAA+ 1mg/l BAP

Root explants of *Terminalia pallid* when cultured on MS medium containing 2 mg/l of 2, 4-D profuse friable white or light green callus was produced, But scanty callus were observed with IAA, NAA, IBA (figure 1). Direct shoot bud proliferation in root explants was observed when cultured on medium supplemented with various concentration of IAA, NAA (0.01-1 mg/l) in combination of BAP (0.5-2mg/l) (figure 3). Maximum number of shoot buds was induced on medium containing 0.05mg/l NAA or 0.5mg/l IAA+ 1mg/l BAP (Table 1). When these explants were cultured on MS medium containing 0.5 mg/l NAA+2mg/l BAP, they produced maximum number of 5-10 shoots of adventitious shoot buds. If explants are

cultured on different combinations of auxins and cytokines, it proliferated further become more nodular and differentiated into shoot buds. If that is significant that the shoots produced in study arose from bud production *de nove* rather by enhanced Axillary branching. The production of shoot from only a very smaller region at the base of the root explants in *Terminalia pallida*.

Shoots from different explants growing on different concentrations of auxins and cytokinin were isolated from culture vessels and transferred to MS medium containing different auxins (1-2 mg/l NAA, IBA, IAA) single or in combinations with cytokinin 1-2BAP & KN. A combination of NAA 2mg/l + BAP 1mg/l showed a significant increase in root number (Fig 4). Frequent subculture in rooting medium improve root number and length.



Fig:3 Induction of shoot buds from Root explants on MS medium containing 0.05mg/l NAA+1mg/l BAP.

Table: 1. Morphogenetic response of root and hypocotyl explants of terminalia pallida on ms medium containing different hormonal concentrations after 60 days inoculation.

S.NO	GROWTH HORMONES (mg/l)	ROOT	HYPOCOTYLE
1	2mg/l 2,4-D	callus	callus
2	2mg/l IAA	callus	callus
3	2mg/l NAA	Callus	callus
4	2mg/l IBA	Callus	callus
5	0.5 NAA+ 1 BAP	Callus +shoot buds	Callus +shoot buds
6	0.05 NAA+1BAP	Callus + shoot buds	Callus +shoot buds
7	0.5 IAA+ 1 BAP	Callus + shootbuds	Callus + shootbuds+ multiple shoots
8	0.5 NAA+2BAP	Callus + shootbuds+ multiple shoots	Callus + shootbuds+ multiple shoots
9	0.5NAA+2KN	Callus	callus
10	1NAA+2KN	Callus + shootbuds	Callus + shootbuds

DISCUSSION

Callus formation was observed after 15 days from the cut surface of the different explants i.e., leaf, hypocotyls and root (Figer-1). Auxin type and concentration significantly influenced callus formation. Successful callus induction depends on the choice of the explants composition of the nutrient medium and hormonal balance besides genotype. Explant cultured on medium containing 2-5mg/l 2, 4-D showed maximum response. A mass of friable yellow, green or white callus was observed within 3 weeks. Addition of 2, 4-D at higher concentration above 5mg/l inhibited callus growth. Although virtually all explants produced the same callus but there is considerable difference between explants type in the formation of this callus. Hypocotyls formed the callus earliest and this callus grew faster in MS medium containing 2mg/l 2, 4-D + 0.5mg/l BAP followed by leaf and root explants. Most of the other growth regulators combinations that induce rapid callus growth were induced either in 2mg/l IAA, NAA and IBA single or in combination with 2-5mg/l BAP, KN. When explants were cultured on auxin and cytokinines combination media a hard dark nodular callus was formed. If left on the same medium it proliferate further, become more nodular and differentiated *de nove* shoot buds. 2, 4-D in combination with BAP could also produce green compact callus in *Capsicum annum*,^[9] and *Aegle marmelose*.^[10] The organized structures within the callus in many passages developed adventitious shoots in spatial manner^[11] In an earlier study^[12&13] most of the shoots were regenerated formless in 3-6 months old callus in *Kalmia* spp. This suggests that there may be a temporal relationship between the callus and the organized tissue explants, which could also support the adventitious shoot buds. The type of auxin seems to be a determinate for organogenesis. Organogenesis from hypocotyls and root was observed in the presence of IAA or NAA in induction medium, never with IBA or 2, 4-D. The lack of formation of organogenesis from explants exposed to 2, 4-D, which could result from an inhibitory effect of this compound, were observed. This response was similar to the of *Ipomoea batatas*.^[14] The lack of effect of IBA was not surprising because this type of auxin is rarely reported to be related with organogenesis.

In the present work we have demonstrated that through organogenesis shoot can be initiated on hypocotyls and root explants using low concentration of auxin and cytokinins. NAA supplementation stimulated callus formation from hypocotyls and root explants which had low plantlet formation capacity. On the other hand, BAP enhanced the formation of detectable adventitious shoot primordial, indicating that the cytokinin might stimulate an increase in the number and size of shoot primordial. But increase in the BAP concentration in

the medium caused shoots primordial to become enlarged and lead to the development of more number of shoots (Table-1). Shoot buds were obtained from explants derived calli after five to twelve weeks. Generally dark green nodular appeared before shoot buds emergence. Regeneration of organized shoots from callus culture was also reported earlier in *Albizia lebbbeck*,^[15] *Dalbergia latifolia*,^[16] *Albizia lebbbeck* ^[17] and *Citrus miti*.^[18] In *Brassica* plant regeneration was achieved in hypocotyls explants on NAA and KN.^[19] The production of shoot buds formed adjacent to each other in de novo fashion from the proximal end is also reported by Soya been cotyledonary explants placed on shoot induction medium.^[20] NAA and BAP combinations also induced shoot buds in root explants. In root explants the balance between the auxin and cytokinin determined the development of callus, roots and shoots. Effective shoot bud induction and subsequent plant regeneration have been achieved in medium containing 0.05 mg/l NAA +2mg/l BAP in *T. pallida*. Similar observations were made in *Anogeissus sericia*.^[21] Inductions of shoot buds from explants were also reported by *Aeschynomene sensitive*.^[22] *Nothofagus alpine*,^[23] *Albizia julibrissin*.^[24] and *Eucalyptus camaldeulensis*.^[25] All the explants produced large quantity of phenolics, which diffuses into the medium. The surfaces of the medium turned black by leachates from the callus. Addition of 500mg/l PVP and 0.5 mg/l activated charcoal in the medium and repeated subculture of explants can prevent the browning of the explants to new medium.^[26&27] Frequent explants transfer has been widely used to prevent tissue browning in black berry shoot tips^[28] Improved *In Vitro* development has been demonstrated for several plant species cultured on medium containing activated charcoal to absorb inhibitory compounds such as phenolics.^[29] These methods were used successfully in cashew experiments.



Fig 4: Profuse root formation from regenerated shoot on MS+2mg/l NAA+1mg/lBAP.

In the present study in application of auxins was essential for adventitious root formation. IBA was found to be more effective than NAA or IAA. Roots were produced on MS medium containing 1mg/l NAA and BAP or KN 2Mg/l. These roots were regenerated from calls of the cut end of the shoots. This was also reported in *Anogeissus* species^[30&31] and *Terminalia bellarica*^[32] Repeated subcultureing onto fresh proliferation medium progressively improved rooting frequency. This corresponds with results in apple^[33&34] and Guva^[35] Repeated sub culturing may change the physiological state and gradually regenerate the, which in turn promotes better rooting^[36, 37&38]

SUMMARY AND CONCLUSION

From the present preliminary results it can be conclude that with appropriate media *Terminalia pallid* can be micropropagated by using root, hypocotyle explants. Thus micropropagation may be ultimate source of selected trees in reforestation.

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