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Micropropagation of Terminalia Arjuna Roxb., from Nursery Plant Material

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ABSTRACT

An in vitro micropropagation system has been developed for Terminalia arjuna Roxb., an important Indian medicinal plant and non mulberry primary food plant of tropical tasar silkworm (Antheria mylitta Drury). Nodal segments obtained from one year-old nursery plant aseptically grown seedlings were used as explants. MS medium containing 2.0 mg/L^{-1} BAP was found most suitable for culture initiation. Although shoot multiplication was achieved on MS medium containing BAP and Kn, the maximum number of shoots was obtained with 1.5 mg/L^{-1}BAP. Best rooting response was observed on MS medium salts, 0.6% agar and 0.1 mg/L^{-1} IBA. Plantlets were hardened initially in culture room conditions and then transferred to misthouse.

Keywords: *Axillarys bud proliferation, Arjun, in vitro, rooting, shoot multiplication*

1. INTRODUCTION

Terminalia arjuna., commonly known as 'Arjun' is a large tree belongs to Combretaceae family (Fig.1). The fruits and bark posses antioxidant properties and form an important ingredient of many ayurvedic preparations. Conventional methods of multiplication of *Terminalia arjuna* have proved inadequate on account of hard seed-coat, heavy insect infestation of seeds and low survival rate of cuttings and, therefore, require alternative methods of propagation. In recent years, plant tissue culture techniques have been employed for multiplication of various tree species using seedling and mature explants. Very little literature of *Terminalia arjuna* micropropagation is available through some attempts are made on micropropagation of T. arjuna by Ramesh[5] *et al*., (2001); Tirkey[1] *et al*.,(2000); Nishi[17] *et al.*, (1998); Priyaranjan *et al.*, 1994. Tirkey *et al*., (1999, 2000); Some of the scientists (Nishi *et al*.,(1998); Ramesh *et al*.,2000) have been worked on separated tissue of *Terminalia arjuna* but there was no response on insect reared tasar food plants. However, Tirkey *et al*.(1999, 2000) reported for somatic embryogenesis of some selected genotypes, effect of antioxidant and absorbent in *Terminalia arjuna*. Recently a protocol for effective plant regeneration via somatic embryogenesis has been developed for *Terminalia arjuna* by Kumari *et al*., (1999).

Micropropagation of *Terminalia* species, by cotyledonary nodes of seedlings, has been reported previously by Pandey and Jaiswal[24] (2002) in *T.arjuna*, Shyamkumar *et al*., (2004) in *Terminalia chebula*, and Sadanandam[9] *et al*. (2005) in *T. bellerica*. So far, no method is available for *in vitro* propagation of this species from the mature tree explants, which is desirable for obtaining mass propagation of high yielding true-to type individuals. Thus, the present study was carried out to develop an efficient *in vitro*

protocol in micropropagation of selected nursery plant for *T. arjuna* from aseptically raised seedling explants.

2. MATERIALS AND METHODS

The fruits of *T.arjuna* were collected from natural growing around s.k.university campus (Anantapur. A.P) surroundings and some were seshachalam hills at Tirupati, in the month of March-May. Seeds were socked in plane water for 96 hours and kept in the mixture of sterile soil and sand (1:1) in the month of Aug- Sept., for seedling. After one month germinated seedlings were transplanted to polythene bags. These bags are filled with soil and vermiculture mixture (1:3) and were hardened mist chamber in gradually decrease humidity regimes. Then the hardened plants were successfully transferred to greenhouse. Nursery plants are in observation every day spraying Bavistine on leaves to avoid insects and caterpillars. We must facilitate water time to time of morning and evening up to six months. After that shoot, apical bud and nodal explants were collected from one year old nursery plant (Fig. 3). The selected explants were cut into 3-4 cm long segments after washing with tap water for half an hour and were treated with 2-3 drops of centrimide solution for 10-12 minutes. Afterwards these were soaked in 0.1% Bavistine (fungicide solution) along with few drops of centrimide solution for 30 minutes. Explants were again washed in sterilized double distilled water (SDDW) and were socked in 70% ethanol for 30 seconds and were agitated with few drops of Tween 20 centrimide solution for 30 min successively. Explants were then again washed in SDDW (at least 3-4 times) and agitated in 25% bleach solution containing 2-3 drops of Centrimide for 20 minutes. Finally, explants were washed with SDDW (again, at least 3-4 times or until all traces detergent, phenols was removed). After the necessary treatment the basal part of the explants was cut and remove with help of sharp scalpel and then implanted vertically in MS media with different concentrations of phytohormones BAP and Kn (0.5-5.0 mg/L^{-1}) were used individually for proliferation of shoots from seedling nodes. Auxins (NAA & IAA) were combined with optimum BAP concentration for shoot proliferation. Some experiments were repeated for shoot multiplication.

The culture medium containing 3% sucrose was solidified with 0.8% agar. The pH of the media was adjusted to 5.8$\pm$0.2 with 1 N NaOH or 0.5 N HCl before adding with agar autoclaving. Media poured in culture vessels were steam sterilized by autoclaving at 1.4 Kg/cm^2 for 20 minutes. The cultures were incubated under controlled conditions of temperature at 26$\pm$2^o under 16 hr photoperiod and 2000 lux with florescent tubes. Humidity 60-70% was provided.

RESULTS

The epicotyledonary and cotyledonary (after removal of cotyledons) nodes when inoculated on MS medium containing BAP and Kn in the range 0.5-5.0 mg/L^{-1} showed enhanced shoot proliferation. BAP at its 1.5 mg/L^{-1} concentration evoked best response. MS and WPM both medium containing with BAP (2mg/L^{-1}) gives the same results in the shoot initiation. Incorporation of NAA or IAA improved bud proliferation but the shoots remained stunted. When explants were inoculated on various media containing 1.5 mg/L^{-1} BAP, MS medium elicited best response, followed by WPM, SH, B_5 and White's medium.

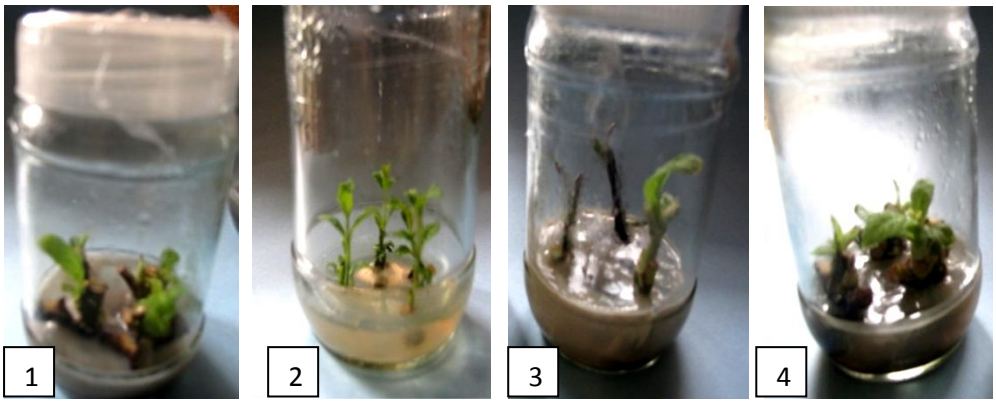
Shoot after their initial proliferation on medium containing 1.5 mg/L^{-1} BAP were subcultured on fresh medium after every 21 days. When shoot cultures were inoculated on various media for multiplication, the maximum number of shoots (10.4) was obtained on SH medium but stunting and yellowing shoots were observed, which intensified on subsequent subcultures on the same fresh medium. Better growth and average shoot was, however, obtained on MS medium and average shoot length remained highest on it (Table 1). All other media were found unsuitable for shoot multiplication. Consequently, in all subsequent experiments, MS medium was used.

In corporation of BAP or Kn into MS medium supported multiplication of shoots in culture. BAP proved to be a better choice than Kn and the maximum number of shoots was obtained on its 1.5 mg/L^{-1} concentration (Table 2). When NAA or IAA was used in combination with BAP (1.5 mg/L^{-1}). a variety of responses were observed. At higher concentrations (0.5 mg/L^{-1} and above) of both the auxins, callusing was observed. Shoot multiplication was improved in presence of 0.5 mg/L^{-1} NAA, and 0.25 and 0.5 mg/L^{-1} IAA (Table 2), combined with 1.5 mg/L^{-1} BAP. However, in presence of auxins, shoots remained stunted and the length did not improve even after repeated subculture on the same fresh medium. Therefore, for shoot multiplication, MS medium containing 1.5 mg/L^{-1} BAP was considered most appropriate.

Auxins (IAA, IBA & NAA) in different concentrations (0.1-1.0 mg/L^{-1}) induced rooting when incorporated in the medium containing ¼ MS salts (Table 3). Callusing was observed on all auxins used at their higher concentrations (0.5 mg/L^{-1} and above). Best rooting response (60%), however, was observed on medium containing 0.1 mg/L^{-1} IBA where 1.36 roots measuring 2.62 cm (average) were formed (Fig. 1). Combining IBA (0.1 mg/L^{-1}) with NAA or IAA increased the percentage of rooting but the roots formed were short, thick and without laterals. Elongation of shoots was accompanied with rooting in any of the experiments.

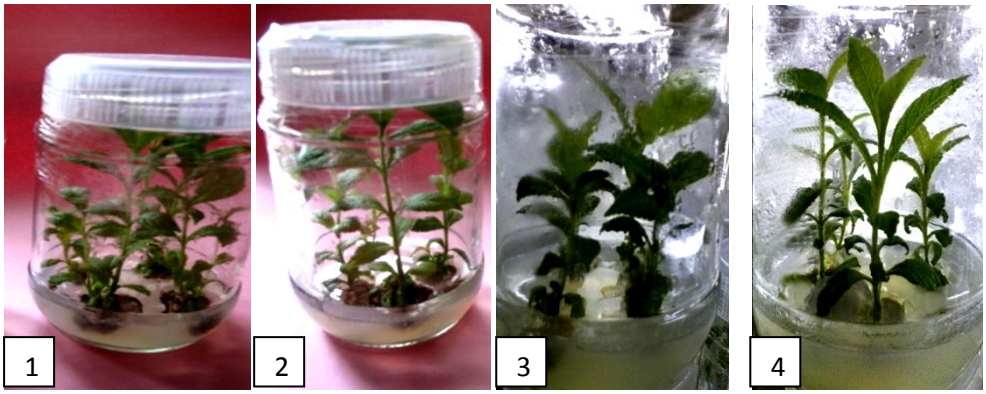
In vitro rooted plantlets were initially hardened in culture room conditions where leaved expanded. After 3 wk, the plantlets were shifted to mist house. There was an increase in length of shoots and new leaves emerged which expanded quickly transplanted to polythene bags (Fig. 4).

1. A mature tree of *Terminalia arjuna* growing in natural habitat 2. Fully matured Dry fruits 3.*T.arjuna* one year old nursery plant 4. Hardened, Acclimatized *T.arjuna* plantlets

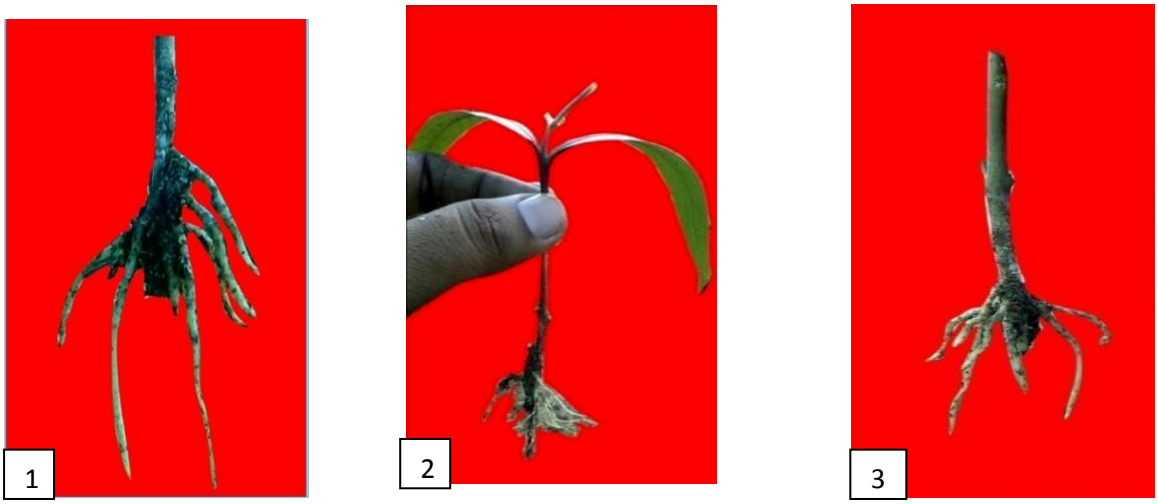


Shoot initiation of multip from nodal explants of *Terminalia arjuna* cultured on different media fortified with various hormones

1. WPM + 2mg/L^{-1} BAP, 2. WPM+2mg/L^{-1} Kn +0.5mg/L^{-1} IAA, 3.MS+2mg/L^{-1} Kn, 4.MS+2mg/L^{-1}BAP.



Shoot multiplication from nodal explants of *Teminalia arjuna* cultured on different media fortified with various hormones
1.WPM+2mg/L^{-1} Kn+0.5 IAA, 2. WPM+2mg/L^{-1} BAP+0.5mg/L^{-1} NAA, 3. B_5+1mg/L^{-1} BAP,
4. MS+1.5mg/L^{-1} BAP



Rooting of *in vitro* regenerated shoots in *Terminalia arjuna* 1.Rhizogenesis on MS+0.1mg/L^{-1} IBA,
2. Root initiation from *Terminalia arjuna* on MS+0.1mg/L^{-1} IBA+1.0mg/L^{-1} NAA,
3. Root initiation on MS+0.1mg/L^{-1}+1.0mg/L^{-1} IAA.

Aseptically grown *Terminalia arjuna* 2 months old plants

DISCUSSION

Seedling derived explants, being juvenile, are frequently used for micropropagation as they are easy to establish in culture. The most widely used method of *in vitro* plant propagation is the stimulation of axillary bud development. In presence of cytokinins, bud dormancy is broken and axillary branches proliferate. In *Terminalia arjuna*, MS medium containing 1.5 mg/L^{-1} BAP was the best for culture initiation. MS medium frequently used for micropropagation of large number of plants. We have found that *T.arjuna* cultures grew better on MS medium in comparison media. Cytokinins are believed to induce bud break and shoot proliferation. In *T.arjuna* 1.5 mg/L^{-1} BAP was most suitable for shoot multiplication. Enhanced shoot multiplication by addition of auxin along with cytokinin has been reported in some plants. We also observed improvement in shoot multiplication by incorporation of NAA (0.5 mg/L^{-1}) and IAA (0.25 mg/L^{-1} and 0.5mg/l) in the medium along with BAP (1.5 mg/L^{-1}) but the shoots remained stunted and the length did not improve even on repeated subculture of shoots on the same fresh medium.

IBA has been widely used as root induction hormone under *in vitro* and in vivo conditions. We also found positive role of IBA during *in vitro* rooting. In *T.arjuna*, 0.1 mg/L^{-1} IBA proved to be the best for *in vitro* rooting. The *in vitro* rooted plants were hardened first under controlled conditions of culture room and then shifted to misthouse where they exhibited enhanced growth successfully transferred to field and were gives 100% survival rate.

Table 1-Effect of various nutrient media on shoot multiplication in *Terminalia arjuna*

Media	Av.no. of shoots	Av. length of shoots(cm)
MS	8.50± 0.71^{b}	1.70±0.14^{a}
SH	10.40±0.52^{a}	0.90±0.12^{b}
WPM	8.20± 1.14^{b}	0.80±0.27^{c}
B_5	4.00± 0.00^{c}	0.76±0.13^{c}
WM	4.00± 0.00^{c}	0.50±0.00^{d}

Table-2: Effect of Cytokinins and their interaction on shoot proliferation from nodal explants of nursery plant of *Terminalia arjuna* on MS medium

PGRs/	Concentrations mg/L^{-1}	No. of Shoots	Shoot Length in (cm)
BAP	0.00	3.00±0.00^{f}	0.50±0.00^{f}
	0.05	5.00±1.22^{ef}	0.76±0.14^{ef}
	1.0	5.60±0.55^{e}	0.98±0.04^{c}
	1.5	9.00±0.71^{bc}	1.70±0.14^{a}
	2.0	7.00±1.58^{d}	0.88±0.08^{cd}
	5.0	3.00±0.00^{f}	0.50±0.07^{ef}
Kn	0.00	3.00±0.00^{f}	0.50±.00^{ef}
	0.05	4.00±1.00^{f}	0.66±0.15^{de}
	1.0	4.60±0.55^{ef}	0.68±0.13^{d}
	1.5	5.20±0.84^{ef}	0.96±0.18^{c}
	2.0	6.00±1.58^{de}	1.26±0.32^{b}
	5.0	8.00±1.58^{ed}	1.54±0.32^{a}
	0.0	9.00±0.71^{bc}	1.70±0.14^{a}

BAP + NAA (1.5mg/L^{-1})	0.5	8.60±0.89^{c}	0.50±0.00^{ef}
	1.0	9.40±0.55^{bc}	0.48±0.04^{f}
	1.5	13.20±0.84^{a}	0.48±0.04^{f}
	2.0	4.20±0.45^{f}	0.44±0.99^{fg}
	5.0	3.60±0.55^{f}	0.40±0.00^{fg}
BAP + IAA (1.5mg/L^{-1})	0.0	9.00±0.71^{bc}	1.70±0.14^{a}
	0.5	5.80±1.48^{d}	0.40±0.00^{fg}
	1.0	10.20±2.07^{b}	0.50±0.00^{ef}
	1.5	12.80±0.84^{a}	0.68±0.16^{d}
	2.0	6.40±0.89d^{e}	0.48±0.04^{f}
	5.0	4.00±0.00^{f}	0.30±0.00^{g}

Table-3: Effect of various Auxins on rooting from nodal explants of a nursery plant of *Terminalia arjuna* on MS medium

PGRs/	Concentrations	No. of Roots	Concentrations	Root Length in(cm)
IBA	0.1	1.31±0.31^{abc}	0.1	1.54±0.49^{a}
	0.25	1.38±0.37^{abc}	0.5	1.20±0.20^{bcde}
	0.5	1.35±0.23^{bc}	1.0	1.09±0.12^{ef}
	0.75	1.35±0.48^{abc}	1.5	1.09±0.12^{ef}
	1.0	1.23±0.33^{abe}	2.0	1.09±0.12^{ef}
IAA	0.1	1.08±0.19^{c}	0.1	1.12±0.26^{e}
	0.25	1.31±0.31^{bc}	0.5	1.47±0.44^{ab}
	0.5	1.31±0.31^{abc}	1.0	1.44±0.40^{abcd}
	0.75	1.31±0.31^{abc}	1.5	1.38±0.36^{abcde}
	1.0	1.31±0.31^{abc}	2.0	1.18±0.19^{bcdef}
NAA	0.1	1.08±0.19^{c}	0.0	1.15±0.33^{abc}
	0.25	1.23±0.33^{bc}	0.5	1.17±0.23^{bcdef}
	0.5	1.15±0.33^{c}	1.0	1.04±0.10^{f}
	0.75	1.15±0.33^{c}	1.5	1.04±0.10^{f}
	1.0	1.15±0.33^{c}	2.0	1.03±0.06^{f}

PGRs		No.of roots			Root length(cm)		
IBA + (0.1mg/L)	Concentrations	0.1	0.5	1.0	0.1	0.5	0.1
	NAA	1.69±0.41 a	1.59±0.57 ab	1.38±0.37 abc	1.33±1.19 abde	1.13±0.12 def	1.08±0.08 ef
	IAA	1.38±0.37 abc	1.33±0.19 abc	1.13±0.19 abc	1.13±0.12 cef	1.13±0.19 abcdef	1.46±0.30 abc

All data represent average of five replicates

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