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A micropropagation protocol for mass multiplication of *Terminalia arjuna* - a valuable medicinal tree

Meena Choudhary[1*] Shipra Jaiswal[1] Rakhi Singh[1] Inder Dev Arya[1] Sarita Arya[1]

[1] Forest Genetics and Tree Breeding Division, Arid Forest Research Institute, Jodhpur, 342005, India.

* Author for correspondence: meenaseervi@gmail.com
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Abstract
A reliable and efficient micropropagation protocol was developed through axillary shoot proliferation from nodal explants of mature *Terminalia arjuna*. Season of explants collection and maturity of explants showed direct influence on bud-break. Nodal stem segments collected during the months of April and May gave best response. Nodal segments of fresh sprouts originated from lopped tree of *T. arjuna* were used as explants for establishment of *in vitro* culture. Surface sterilized explants produced optimum number of shoots through activation of axillary buds - on modified Murashige and Skoog's (M-MS) medium. Maximum (100%) *in vitro* shoot proliferation was obtained on M-MS medium supplemented with 8.86 µM BAP + additives (100 mg L^{-1} of ascorbic acid, 50 mg L^{-1} of citric acid, 50 mg L^{-1} of adenine sulphate and 25 mg L^{-1} PVP). Modified M-MS medium supplemented with 4.44 µM BAP + 0.54 µM NAA + additives was found to be best for shoot multiplication (11.38±0.26). After four week of culturing the *in vitro* regenerated shoots were rooted when pulse treated with 984 µM IBA for 10 min and transferred on hormone free half strength MS medium containing 100 mg L^{-1} activated charcoal. *In vitro* regenerated plants were transferred to field after gradual hardening and acclimatization procedure. Present method can be used for large scale commercial production of this medicinally important tree.
Key words: Micropropagation technique; Axillary bud culture; *In vitro* culture.

Introduction

Terminalia arjuna (Roxb.) Wt. & Arn. (Family: Combretaceae) is a large, evergreen tree with spreading crown and drooping branches. This species is commonly called of arjun, and is found throughout the tropical and subtropical region of the peninsular India. In the Indian subcontinent, natural forests of *T. arjuna* are reported in Bihar, Orissa, Madhya Pradesh, Gujarat, Maharashtra, Tamil Nadu, West Bengal and Punjab. This tree is valued for their leaves, bark, gum, flowers and fruits. It has antibacterial (Perumalsamy et al. 1998), antimutagenic, hypolipidemic, antioxidant and hypocholesterolaemic and anti-inflammatory effects. The bark of arjun contains many natural ingredients. *Terminalia* active constituents include cardenolide, triterpenoid saponins (e.g., arjunic acid, arjunolic acid, arjungenin, arjunglycosides), gallic acid, ellagic acid, oligomeric proanthocyanidins (e.g., OPCs), phytosterols, flavonoids (e.g., arjunone, arjunolone, luteolin), tannins, calcium, magnesium, zinc, and copper (Akhter et al. 2012).

The bark of *T. arjuna* is expectorant, diuretic and used widely in various heart problems like congestive heart failure, mitral regurgitation, cardiomyopathy, coronary artery disease, heart attack and ventricular failure. It is useful in fracture, hemorrhages, ulcers, blood disease, intoxications, excessive perspiration, asthma, tumors, leucoderma and false presentation of fetus. Regular use of *T. arjuna* bark improves pumping activity of heart and improves cardiac muscle strength so bark is used as ayurvedic remedy for cardiovascular disorders (Dwivedi 2007), thus making the *T. arjuna* unique amongst currently used medicinal plants. The leaves of this tree are fed to tasar silkworm (*Antheraea mylitta*) for rearing of tasar cocoon. The gum is very nutritive and energetic for heart. Fruit is used as tonic and deobstruent (Paarakh 2010).

The revival of interest of the wide spread belief that green medicine is healthier than synthetic products. Nowadays, there is manifold increase in medicinal plant based industries due to increase in the interest of use of medicinal plants throughout the world. *T. arjuna* are being over-exploited because of their medicinal and economic importance leading to loss of valuable genetic resources. Thus it is important to apply conventional as well as non conventional propagation technique. *T. arjuna* is propagated either through seed or through cutting. The conventional techniques of propagation in arjun include sexual propagation through seeds or vegetative propagation by cuttings and air layering. All these methods have certain pitfalls which further limit their application for conservation programme. Seed germination is only 50-60%, also there is a lack of proper macropropagation method as the cutting do not root well. Therefore there is an urgent need for application of non-conventional methods for conservation of germplasm. Micropropagation is an important method for cloning such economically and medicinally useful trees and thus overcomes some of the hurdles in conventional methods.

During the past years, a regeneration protocol for *T. arjuna* have been reported both from seedling and mature tree explants (Pandey and Jaiswal 2002; Pandey et al. 2006; Gupta et al. 2014). However, protocol developed earlier was not so efficient, as number of shoot regenerated and rooting response was very low. Success of micropropagation protocol depends on the field transferred of tissue culture raised plantlets. Plantlets produced in earlier studies were successfully acclimatized under greenhouse conditions however their transfer to field conditions was not shown.

The present study was carried with the aim of rapid *in vitro* mass propagation of *T. arjuna* by micropropagation technique and their successful establishment in field conditions.

Material and methods

Explants preparation and surface sterilization

Newly emerged shoots were harvested from both lopped and non lopped branches of 10-20 year-old trees of *T. arjuna* growing in Jodhpur, Rajasthan, India throughout year from January to December. Nodal segments containing axillary bud were collected from healthy tree and washed under running tap water for removal of dust particles. These explants were treated with Tween-80 for 10 min and rinsed

with autoclaved distilled water for three to four times. To remove the fungal and bacterial contamination explants were treated with 0.1% (w/v) Bavistin and Streptomycin for 15 min. Explants were surface sterilized with 0.1% $HgCl_2$ for 8 min under laminar air flow bench and rinsed three to four times with autoclaved distilled water. To reduce phenolic exudation, explants were kept in pre chilled sterile antioxidant solution of 100 mg L^{-1} of ascorbic acid, 50 mg L^{-1} of citric acid and 25 mg L^{-1} PVP for 10-30 min prior to inoculation on nutrient media.

Nutrient media and culture conditions

Murashige and Skoog (1962) medium was modified by reducing the strength of NH_4NO_3 and KNO_3 salts to half and was added with additives (100 mg L^{-1} of ascorbic acid, 50 mg L^{-1} of citric acid, 50 mg L^{-1} of adenine sulphate and 25 mg L^{-1} PVP), 3% sucrose and 0.8% agar-agar (w/v) for culture initiation. This media was referred in text as modified MS medium (M-MS). All culture media were adjusted at pH 5.8 and followed by autoclaving at 1.0 kgf cm^{-2} pressure and 121°C for 15 min. All the cultures were aseptically inoculated and manipulated under laminar air flow bench. These cultures were maintained in an aseptic culture room having a temperature of 26±2°C, 16 h of photoperiod with a light intensity of 1600 lux by cool white fluorescent tube.

Axillary bud break and in vitro shoot proliferation

To study the effect of cytokinin and auxin on *in vitro* shoot proliferation, surface sterilized explants were inoculated on modified MS medium supplemented with different concentration of BAP (0, 2.22, 4.44, 8.86, 13.32 and 17.76 µM) alone or in combination with auxin, i.e., NAA (0.27, 0.54 and 1.34 µM) or IAA (0.29, 0.57 and 1.43 µM).

In vitro shoot multiplication

The *in vitro* raised shoots were excised from mother explants and further multiplied on M-MS medium supplemented with different concentration of BAP (2.22, 4.44, 8.86, 13.32 and 17.76 µM) alone or in combination with auxin, i.e., NAA (0.27, 0.54 and 1.34 µM) or IAA (0.29, 0.57 and 1.43 µM). A propagule of three shoots was used for *in vitro* shoot multiplication. The number of shoot cultured and number of shoot obtained is reflected as multiplication rate or multiplication fold.

In vitro rooting

For *in vitro* rooting shoots of 2-3 cm length were excised from main shoot clump and pulse treated with different concentration of IBA (246; 492; 984 and 2,460 µM) or NAA (268.5; 537.0; 1,074.0 and 2,685.0 µM) for 10 min. The number of *in vitro* shoots produced roots was reflected as rooting percentage. The number of roots obtained per shoot was also counted and referred as root number.

Hardening and acclimatization of plantlets

In vitro rooted plantlets were carefully removed from culture vessels and washed under running tap water to remove agar without damaging the roots. These rooted plantlets were transferred to bottles containing autoclaved vermiculite moistened with half strength MS medium without organics components. The bottles were capped and initially placed in the culture room itself for 3-4 weeks and then transferred to mist chamber near the pad section at relative high humidity of 85-90% and low temperature of 30±2°C. The plastic caps of glass bottle were gradually loosen to reduce relative humidity in the bottle and finally removed. Plants were shifted to polythene bag containing sand:soil:FYM (Farmyard manure) in 1:1:1 ratio and placed in the mist chamber for four to five weeks. These hardened plants after mist chamber stage were shifted to agro net shade house for acclimatization to outer environmental condition. During hardening, shoots elongated and leaves expanded.

Statistical analysis

All the experiments were conducted with 15 replicate per treatment. Each experiment was repeated three times. Observations were recorded after 4 weeks. The results are expressed as mean±SE of three experiments. The data were analyzed statistically using General liner Model (GLM) multi variance factor analysis and one way analysis of variance (ANOVA), and the significance difference between means were assessed by Duncan's multiple range test ($P < 0.05$).

Results

Axillary bud break and in vitro shoot proliferation

For the establishment of aseptic cultures from a mature tree, selection of suitable explants was a serious constraint. In the present investigation, aseptic culture of *Terminalia arjuna* was successfully established by collecting the shoot sprouts (flushes) that regenerated from lopped tree during active growth phase in the month of April and May. Season of explants collection played an important role in the culture establishment. The explants (i.e., nodal segments containing axillary bud) harvested during the month of April and May showed maximum bud break response with minimum contamination which was helpful in culture establishment (Fig. 1).

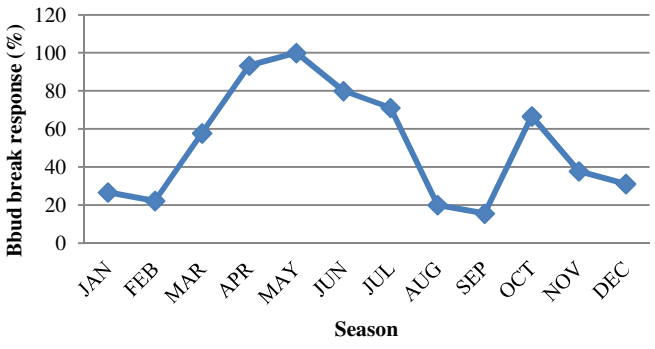

Figure 1. Effect of different season on *in vitro* shoot induction response of *Terminalia arjuna* explants on modified M-MS medium supplemented with 8.86 µM BAP.

During the culture establishment, problem of phenolic exudation was encountered as the cut end of all explants exhibited browning in the culture media and subsequently the entire explant necrosed and died. This problem of phenolic exudation was overcame by keeping the explants in a solution of (chilled 4°C) ascorbic acid (100 mg L^{-1}), citric acid (50 mg L^{-1}) and PVP (25 mg L^{-1}) for 20 min.

Shoot induction was observed when nodal explants (Fig. 1A) containing axillary bud were inoculated on M-MS medium supplemented with BAP. Maximum axillary bud break response (100%) and number of shoots (5.80±0.16) were produced within 4 weeks on M-MS medium supplemented with 8.86 µM BAP (Table 1, Fig. 2B). BAP was supplemented with NAA or IAA to study the effect of cytokinin/auxin interaction on axillary bud induction. It was observed that the interaction of BAP with auxins was not synergistic for *in vitro* shoot proliferation. Among the combination of BAP (4.44-8.86 µM) and NAA (0.27-1.34 µM) or IAA (0.29-1.43 µM) tried, 8.86 µM BAP with 0.27 µM NAA+ additives supported optimal 84.44% axillary bud break response with 5.15±0.16 axillary shoots per explant. Increased in NAA concentration with BAP more than 0.27 µM resulted in decreased bud break percent. Bud breaking was observed in 80% of nodal explants when cultured on medium having 8.86 µM BAP + 0.29 µM IAA + additives.

Nodal segments containing axillary bud were also cultured on MS medium but after 2-3 repeated subculture shoots became yellow and died. When nodal segments containing axillary bud were cultured on half concentration of NH_4NO_3 and KNO_3 salts in M-MS medium - plus additives like ascorbic acid, citric acid, adenine sulphate and PVP - it resulted in green, healthy and thick shoots with good *in vitro* shoot proliferation.

Figure 2. Micropropagation of *Terminalia arjuna* from nodal explants of mature tree. (A) Nodal explants of *T. arjuna*. (B) Shoot induction from nodal explants on M-MS medium + 8.86 µM BAP. (C) Shoot multiplication on M-MS medium + 4.44 µM BAP + 0.54 µM NAA. (D-E) *In vitro* rooting in pulse treated shoot with 984 µM IBA. (F-G) Tissue culture raised plantlets.

Table 1. Effect of cytokinin (BAP) alone or in combination with auxins (NAA/IAA) supplemented in modified M-MS medium on *in vitro* shoot induction from nodal explants of *Terminalia arjuna*.

BAP (µM)	NAA (µM)	IAA (µM)	ABBR (%)	MSN	MSL (cm)
control	-	-	0.00	0.00 ± 0.00^{l}	0.00 ± 0.00^{h}
2.22	-	-	62.22	1.60 ± 0.13^{k}	0.49 ± 0.02^{g}
4.44	-	-	75.56	3.00 ± 0.16^{fg}	0.61 ± 0.01^{f}
8.86	-	-	100.00	5.80 ± 0.16^{a}	0.85 ± 0.03^{a}
13.32	-	-	80.00	4.50 ± 0.10^{bc}	0.71 ± 0.01^{cd}
17.76	-	-	60.00	2.03 ± 0.12^{ijk}	0.58 ± 0.02^{f}
4.44	0.27	-	68.88	2.83 ± 0.18^{gh}	0.71 ± 0.02^{cd}
4.44	0.54	-	57.78	2.53 ± 0.18^{ghi}	0.68 ± 0.02^{de}
4.44	1.34	-	53.33	2.25 ± 0.21^{ij}	0.61 ± 0.02^{f}
8.88	0.27	-	84.44	4.71 ± 0.17^{b}	0.84 ± 0.02^{a}
8.88	0.54	-	75.56	4.08 ± 0.18^{cd}	0.81 ± 0.02^{ab}
8.88	1.34	-	64.44	3.58 ± 0.22^{de}	0.76 ± 0.02^{bc}
4.44	-	0.29	62.22	2.46 ± 0.22^{hi}	0.68 ± 0.02^{de}
4.44	-	0.57	53.33	2.16 ± 0.19^{ij}	0.62 ± 0.02^{ef}
4.44	-	1.43	51.11	1.86 ± 0.15^{jk}	0.60 ± 0.01^{f}
8.88	-	0.29	80.00	4.44 ± 0.15^{bc}	0.79 ± 0.02^{ab}
8.88	-	0.57	64.44	3.75 ± 0.21^{de}	0.76 ± 0.01^{bc}
8.88	-	1.43	57.78	3.46 ± 0.20^{ef}	0.70 ± 0.02^{cd}

Mean value followed by the same latter are not significantly different according to Duncan's multiple range test at $P < 0.05$. Data were recorded after 4 weeks of culture. Data presented as: mean±SE. ABBR - axillay bud break response, MSN - mean shoot number, MSL - mean shoot length.

***In vitro* shoot multiplication**

Once the cultures were established *in vitro* shoots were further multiplied on cytokinin alone or in combination with auxin supplemented media. *In vitro* shoots were tried for multiplication it was found that cluster of shoots gave good multiplication rate. Single and paired shoots did not multiply rapidly resulting in reduced multiplication rate. *In vitro* shoots (a clump of 3 shoots) when subcultured for 4 weeks in the medium yielded significant multiplication of *in vitro* shoots. Medium supplemented with 2.22-17.76 µM BAP alone or in combination with 0.27-1.34 µM NAA or 0.29-1.43 µM IAA brought 1-3 fold *in vitro* shoot multiplication. Subculturing of *in vitro* regenerated shoots on modified M-MS medium supplemented with 4.44 µM BAP resulted in 2 fold *in vitro* shoot multiplication with 2.01± 0.06 cm shoot length. Any further increase in BAP concentration in medium resulted in decreased multiplication response. Auxin alone did not influence shoot multiplication but when auxin was used in combination with cytokinin it slightly enhanced *in vitro* shoot multiplication response. Cytokinin and auxin supplemented medium showed synergistic response for *in vitro* shoot multiplication. When auxin and cytokinin were used in combination, 2-3 fold *in vitro* shoot multiplication was observed (4.44 µM BAP + 0.54 µM NAA) with development of 11.38±0.26 shoots of 3.17 ±0.07 cm shoot length (Table 2, Fig. 2C). *In vitro* shoots responded differently on various concentration and combination of plant growth regulators in medium (Table 2). Addition of NAA along with BAP in the medium was found effective as compared to IAA for *in vitro* shoot multiplication and production of healthy shoots. Any increase in NAA (0.54 µM) concentration in combination with BAP showed tendency of callus formation in the cultures reduced with *in vitro* shoot multiplication.

***In vitro* rooting**

For *in vitro* root induction, *in vitro* raised shoots (2-3 cm long) were aseptically excised individually from the propagule of *in vitro* shoot and pulse treated with IBA (246-2,460 µM) or NAA (268.5-2,685 µM) for 10 min prior to transfer on hormone free half strength MS medium containing 100 mg L^{-1} activated charcoal. Root induction

was started within 10 days of subculturing. Pulse treatment with 984 µM IBA proved to be the most effective for 80% rooting of microshoots than that of any other concentration tried (Table 3). Medium containing the mentioned concentration showed the highest percentage of rooting with 4.00±0.15 mean number of roots per shoots and the mean root length was found to be 4.39±0.17 (Table 3, Fig. 2D-E). However, it was observed that increasing the concentration of the mentioned hormones more than 984 µM IBA affected growth of roots and ultimately the rooting percentage. Variation in number of roots developing at the base of each shoot was recorded with variation in IBA concentration. A varied effect of NAA was observed by pulse treating the shoots at different concentration. The different concentration of NAA used for inducing roots, a concentration of 537 µM NAA resulted in 64.44% *in vitro* root formation.

Table 2. Effect of cytokinin (BAP) alone or in combination with auxin (NAA/IAA) supplemented in modified M-MS medium on *in vitro* shoot multiplication of *Terminalia arjuna.*

BAP (µM)	NAA (µM)	IAA (µM)	MSN	MSL (cm)
Control	-	-	3.71±0.11^{j}	0.95±0.02^{g}
2.22	-	-	5.38 ± 0.15^{fgh}	1.17 ± 0.19^{efg}
4.44	-	-	8.00 ± 0.31^{bc}	2.01 ± 0.06^{b}
8.86	-	-	6.63 ± 0.19^{d}	1.29 ± 0.06^{cde}
13.32	-	-	5.08 ± 0.15^{ghi}	1.07 ± 0.04^{efg}
17.76	-	-	4.44 ± 0.14^{i}	0.96 ± 0.04^{g}
2.22	0.27	-	5.89 ± 0.22^{ef}	1.30 ± 0.05^{cde}
2.22	0.54	-	6.47 ± 0.16^{de}	1.51 ± 0.05^{c}
2.22	1.34	-	5.05 ± 0.20^{ghi}	1.10 ± 0.04^{efg}
4.44	0.27	-	8.50 ± 0.17^{b}	1.81 ± 0.07^{b}
4.44	0.54	-	11.38 ± 0.26^{a}	3.17 ± 0.07^{a}
4.44	1.34	-	6.89 ± 0.25^{d}	1.45 ± 0.08^{cd}
2.22	-	0.29	4.83 ± 0.20^{hi}	1.10 ± 0.05^{efg}
2.22	-	0.57	5.64 ± 0.19^{fg}	1.22 ± 0.05^{def}
2.22	-	1.43	4.47 ± 0.19^{i}	1.01 ± 0.04^{fg}
4.44	-	0.29	6.38 ± 0.26^{de}	1.43 ± 0.05^{cd}
4.44	-	0.57	7.52 ± 0.30^{c}	1.87 ± 0.07^{b}
4.44	-	1.43	4.55 ± 0.20^{i}	1.19 ± 0.04^{efg}

Mean value followed by the same latter are not significantly different according to Duncan's multiple range test at $P < 0.05$. Data presented as: mean±SE. MSN - mean shoot number, MSL - mean shoot length.

Hardening and acclimatization of plantlets

In vitro rooting was completed in 3-4 weeks. During this period healthy plantlets with good roots and shoot system were developed. *In vitro* rooted plantlets were hardened and acclimatized prior to field transfer (Fig. 2F-G). The 90% *in vitro* rooted shoots were hardened and acclimatized successfully under greenhouse conditions which is higher than previously reported (75%) plant acclimatized in *T. arjuna.* When these plants were transferred to field conditions, 100% plants survival was obtained.

Discussion

A protocol has been successfully developed for micropropagation of mature *Terminalia arjuna*. Management of source plant and season influenced the efficiency of *in vitro* shoot proliferation. We evaluated two different sources of explant. Explants were collected both from lopped and non lopped branches of tree. The axillary shoots collected from lopped tree responded better to tissue culture because lopping of tree removed the inhibitory substances and allowed rejuvenation and regeneration of invigorated shoot sprouts. The explants derived from the non lopped tree were difficult to sterilize as they carry recalcitrant microbes. This type of observation was also reported in *Azadirachta indica* (Arora et al. 2010) and *Salvadora persica* (Phulwaria et al. 2011). During the course of present study, it was found that maximum bud break was occurred in the nodal explants if these were taken during the months of March and April from mother tree which were lopped back during November - January of the preceding year. Effect of season on *in vitro* shoot proliferation is also reported in woody plants such as *Maytenus emarginata* (Rathore et al. 1992), *Prosopis cineraria* (Shekhawat et al. 1993), *Terminalia bellirica* (Phulwaria et al. 2012), *Delbergia sisso* (Arya et al. 2013) and *Azadirachta indica* (Gehlot et al. 2014).

Table 3. Effect of auxins pulse treatment for 10 min on *in vitro* rooting of *Terminalia arjuna*. Pulse treated shoots were transferred on hormone free half strength MS medium containing 100 mg L^{-1} activated charcoal.

IBA (µM)	NAA (µM)	RO (%)	NRDS	ROL (cm)
0	-	0.00	0.00±0.00^{f}	0.00±0.00^{e}
246	-	37.78	1.52±0.17^{d}	3.57±0.12^{bc}
492	-	62.22	2.50±0.12^{bc}	3.83±0.11^{b}
984	-	80.00	4.00±0.15^{a}	4.39±0.17^{a}
2,460	-	48.88	2.04±0.13^{cd}	3.65±0.13^{bc}
-	268.5	51.11	1.78±0.19^{d}	3.32 ± 0.10^{cd}
-	537.0	64.44	2.62±0.20^{b}	3.75 ± 0.11^{bc}
-	1,074.0	35.55	1.68±0.19^{d}	3.26 ± 0.17^{cd}
-	2,685.0	26.66	1.00±0.00^{e}	3.07 ± 0.16^{d}

Mean value followed by the same latter are not significantly different according to Duncan's multiple range test at $P < 0.05$. Data presented as: mean±SE. RO - rooting, NRDS - number of roots developed per shoot, ROL - root length.

In the present study, excessive exudation of phenolic compounds made the initial establishment of cultures very difficult. However, pretreatment of explants, prior to inoculation, with chilled antioxidant solution was beneficial for culture establishment. Similar results to overcome phenolic exudation has been reported in *Syzygium cumini* (Rathore et al. 2004) and *Terminalia arjuna* (Pandey et al. 2006).

During the present investigation, effect of cytokinin (BAP) alone or in combination with auxins (NAA and IAA) was evaluated for *in vitro* shoot proliferation. Cytokinins induce bud break by activation of meristems and cause shoot proliferation. Addition of auxin with cytokinin did not have stimulatory effect on *in vitro* shoot proliferation. The stimulating effect of BAP on bud break and multiple shoot formation has also been reported in *Terminalia bellirica* (Ramesh et al. 2005), *Citrus limon* (Rathore et al. 2007), *Lawsonia inermis* (Ram and Shekhawat 2011), *Syzygium cumini* (Naaz et al. 2014).

The concentration of the salts of basal medium were also found to effect *in vitro* shoot proliferation and shoot multiplication in *T. arjuna*. Pandey and Jaiswal (2002) has also been reported low salt concentration for shoot proliferation and multiplication in *T. arjuna*.

Cytokinins are usually required for *in vitro* shoot multiplication but in certain instances, addition of lower concentration of auxins can have stimulatory effect on shoot multiplication. The requirement of auxin and cytokinin in the medium for shoot multiplication vary from species to species. Species with higher level of auxins required no auxins, whereas those with lower endogenous level required external supply of auxins. In the present investigation on *T. arjuna* incorporation of auxin in the BAP supplemented medium increased the *in vitro* shoot multiplication. Maximum *in vitro* shoot multiplication by addition cytokinin along with of low levels of auxin is also reported in many woody tree species such as *Terminalia bellerica* (Rathore et al. 2008), *Acacia chundra* (Rout et al. 2008) and *Rauvolfia tetraphylla* (Faisal et al. 2012).

Auxin concentration and the type of auxins also played a significant role for *in vitro* root induction and development of root length. Variation in number of roots developing at the base of each shoot was recorded with variation in IBA concentration. In the present study, maximum *in vitro* rooting was obtained when *in vitro* shoots were pulse treated with IBA and transferred on hormone free half strength MS medium containing 100 mg L^{-1} activated charcoal. A varied effect of NAA was observed on *in vitro* rooting. Increased and decreased concentration of NAA from optimal concentration showed decreased rooting response. Among the two auxins studied, IBA was found to be more effective than NAA. Rooting response with IBA was also reported by many researchers in *Celastrus paniculatus* (Rao and Purohit 2006) and *Anogeissus sericea* (Yusuf 2005).

Conclusion

A reproducible and efficient micropropagation protocol has been developed using nodal explants of a mature *T. arjuna*. Maximum bud break response was observed when explants were collected during April - May months. The best *in vitro* shoot induction response was obtained on 8.86 µM BAP. Medium supplemented with 4.44 µM BAP + 0.54 µM NAA was found optimal for *in vitro* shoot multiplication. The *in vitro* shoots were pulse treated with 984 µM IBA auxin for 10 min and transferred on hormone free half strength MS medium containing 100 mg L^{-1} activated charcoal gave maximum rooting response. The significance of the present protocol is further increased as the *in vitro* maintained shoots provide a regular supply of shoots for rooting and field transfer without any demand for fresh explants material. This simple regeneration system developed for *T. arjuna* from nodal explants in the present study can be used for large-scale rapid multiplication of this important medicinal tree.

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