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Influence of different auxin treatment on *ex vitro* rooting in *in vitro* regenerated micro shoots of *Terminalia arjuna* (Arjun)

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Abstract
Terminalia arjuna is an important cardio tonic tree, commonly known as Arjun. The realistic aspect of micropropagation technology is its successful execution from lab to field conditions. Rooting of *in vitro* induced shoot is a critical factor in the micropropagation of woody tree species. The objective of present study was to find out the role of different auxin on *ex vitro* rooting of micropropagated plantlet of this tree species. For this, *in vitro* plantlets developed through micropropagation techniques were subjected to different hormonal regime (IBA, NAA & IAA), maximum 62.22% *ex vitro* rooting was achieved, when plantlets were pulse treated with 200 mg/l (984.25 µM) IBA for 10 min. The rooted plantlets were successfully hardened and acclimatized in poly house then under agro-shade house. These plants were planted in field condition and they showed 60% survival rate in the field conditions.

Keywords: *Terminalia arjuna,* auxin, *ex vitro* rooting and micropropagation

Introduction
Terminalia arjuna (Arjun) belongs to family Combretaceae, having approximately 200 species distributed around tropical and sub-tropical regions of the world. Around 24 *Terminalia* species were found in various parts of India (Srivastav *et al.*, 2003) [15]. Clinical evaluation of *T. arjuna* reveals that it can be use for the treatment of coronary artery disease, heart failure, hypercholesterolemia and also play an important role in the sericulture industry (Orwa *et al*., 2009) [7] and its leaves are ideal food for tasar silkworm (*Antheraea mylitta*). Timber is locally used for carts, agricultural implements, water troughs, traps, boat building, house building, electric poles, tool-handles, jetty-piles and plywood. Thus, it is an important multipurpose tree species. This species is under threat due to over-collection and destructive harvesting practices. Demand for medicinal plant based industry is increasing many fold along with world population. Seed germination of *T. arjuna* is only 50-60% in the natural condition and conventional propagation methods are not much successful. It is therefore necessary to find other ways to meet these requirements of medicinal products from this plant. For this, micropropagation technique is a powerful tool for rapid multiplication of selected genotypes.
During the past years, several micropropagation protocols of *T. arjuna* have been developed through seedling and nodal explant collected from mature tree (Pandey and Jaiswal 2002; Pandey *et al*. 2006; Gupta *et al*. 2014; Choudhary *et al*. 2015) [9, 6, 2]. Earlier reports reveals that rooting of the *in vitro* raised shoots is the most important step in tissue culture to develop a complete plant and all these protocols does not highlight the importance of factors that promote rooting response in this tree species. This is the first attempt made on the role of different auxin on *ex vitro* rooting of *T. arjuna*.

Material and methods
Plant Materials: The experiments were conducted at Plant Tissue Culture Laboratory, Forest Genetics Tree Breeding Division of Arid Forest Research Institute, Jodhpur. The cultures were established from nodal explants collected from mature lopped tree (10-20 years old) of *Terminalia arjuna* situated at ummaid garden, Jodhpur.
Plant nutrient media, culture initiation and multiplication: The modified MS medium (Half strength of NH_4NO_3 and KNO_3) + additives was used for *in vitro* shoot proliferation. The *in vitro* proliferated shoots from nodal explants were further multiplied on modified MS medium supplemented with 4.44 µM 6-Benzylamino purine (BAP) + 0.54 µM Naphthalene acetic acid (NAA) + additives (100 mg/l of ascorbic acid, 50 mg/l of citric acid, 50 mg/l of adenine sulphate and 25 mg/l of PVP). Medium was autoclaved at 15 psi for 15-20 min at 121 °C. Cultures were maintained at 25±2 °C temperature with 16 hours illumination with a light intensity of 1600 lux.

Ex vitro rooting: For *ex vitro* rooting, individual micro-shoots (2-3 cm in length) were harvested from *in vitro* multiplied shoot clumps and washed gently in distilled water to remove all traces of medium attached and pulse treated with different concentration of auxins IBA (50, 100, 200 & 500 mg/l or 246.06, 492.13, 984.25, & 2460.63 µM), NAA (50, 100, 200 & 500 mg/l or 268.53, 537.06, 1074.11 & 2685.28 µM) or IAA (50, 100, 200 & 500 mg/l or 285.39, 570.78, 1141.55, 2853.88 µM) for 10 min. These pulse treated shoots were then transferred to screw cap glass autoclaved bottles containing vermiculite, moistened with half strength MS salts and transferred to poly house.

Experimental design and data analysis: In present research work, randomized block design (RBD) was used for statistical analysis. Resultant data were analyzed through one-way analysis using Statistical Packages for Social Sciences Software (SPSS 14.0) for all the studied parameters i.e. rooting percentage, mean root number and mean root length. Means of all factor were compared using Duncan Multiple Range Test (DMRT) at 0.05% probability.

Result

Different auxins (IBA, NAA or IAA) were tested at different concentration to induce *ex vitro* rooting. Among all the auxins tried for *ex vitro* rooting, IBA produced maximum number of roots as compared to NAA or IAA. When *in vitro* raised shoots were pulse treated with 50 mg/l (246.06 µM) to 500 mg/l (2460.63µM) IBA for 10 min, maximum 62.22% rooting with 2.96 mean root numbers and 4.28 cm mean root length was achieved on 200 mg/l (984.25 µM) IBA (Table 1). Any increase or decrease in IBA concentration resulted in reduced rooting potential of *in vitro* regenerated shoots. The roots developed with IBA treatment were thick and bearing numerous secondary root hairs as compared to NAA and IAA (Fig. 1A).

Shoots treated with different concentration of NAA i.e. 50 mg/l (268.53 µM) to 500 mg/l (2685.28 µM) for 10 min also induced *ex vitro* roots. The *ex vitro* rooting response gradually increased up to 100 mg/l (537.06 µM) NAA and later it started to decline. Maximum 44.44% rooting response was observed on 100 mg/l (537.06 µM) NAA with 2.40 root numbers and 3.70 root length (Table 2, Fig. 1B). At higher NAA (500 mg/l; 2685.28 µM) concentration, callus was developed at the base of the shoot and only 22.22% rooting was observed.

When shoots were pulse treated with IAA 50 mg/l (285.39 µM) to 500 mg/l (2853.88 µM) for 10 min, results revealed that IAA was least responsive in terms of percentage rooting and root numbers as compared to IBA and NAA. The optimum concentration of IAA for *ex vitro* rooting was 200 mg/l (1141.55 µM) where 37.77% shoots rooted (Table 3, Fig. 1C).

Table 1: Effect of auxin (IBA) on *ex vitro* rooting of *Terminalia arjuna*. Data recorded after 4 weeks of experiment.

IBA (mg/l;µM)	Rooting %	Mean root number	Mean root length (cm)
Control	0.00±0.00c	0.00±0.00e	0.00±0.00e
50.00 (246.06)	31.11±0.06b	1.21±0.11d	3.32±0.18c
100.00 (492.13)	44.44±0.07ab	2.20±0.16b	3.87±0.10b
200.00 (984.25)	62.22±0.07a	2.96±0.16a	4.28±0.13a
500.00 (2460.63)	40.00±0.07b	1.83±0.19c	2.62±0.13d
Mean	35.56±0.03	1.41±0.11	2.32±0.16
ANOVA (Analysis of Variance)			
df	4.0	4.0	4.0
F-value	12.30	126.77	410.21
P-value	0.00	0.00	0.00

Values within the column with similar superscript are not significantly different at p≤*0.05 level* as determined using Duncun's multiple range test. A value represents mean ± standard error.

Table 2: Effect of auxin (NAA) on *ex vitro* rooting of *Terminalia arjuna*. Data recorded after 4 weeks of experiment.

NAA (mg/l;µM)	Rooting %	Mean root number	Mean root length (cm)
Control	0.00±0.00c	0.00±0.00d	0.00±0.00d
50.00 (268.53)	33.33±0.07ab	1.06±0.06c	2.98±0.13b
100.00 (537.06)	44.44±0.07a	2.40±0.16a	3.70±0.06a
200.00 (1074.11)	31.11±0.06ab	1.57±0.13b	2.85±0.09b
500.00 (2685.28)	22.22±0.06b	1.20±0.13c	2.42±0.09c
Mean	26.22±0.03	0.94±0.10	1.75±0.15
ANOVA (Analysis of Variance)			
df	4.0	4.0	4.0
F-value	7.13	131.25	825.69
P-value	0.00	0.00	0.00

Values within the column with similar superscript are not significantly different at p≤*0.05 level* as determined using Duncun's multiple range test. A value represents mean ± standard error.

Table 3: Effect of auxin (IAA) on *ex vitro* rooting of *Terminalia arjuna*. Data recorded after 4 weeks of experiment.

IAA (mg/l;µM)	Rooting %	Mean root number	Mean root length (cm)
Control	0.00±0.00b	0.00±0.00d	0.00±0.00d
50.00 (285.39)	22.22±0.06a	1.10±0.10c	3.13±0.12b
100.00 (570.78)	28.88±0.07a	2.00±0.00b	3.41±0.14a
200.00 (1141.55)	37.77±0.07a	2.29±0.20a	3.63±0.10a
500.00 (2853.88)	31.11±0.06a	1.35±0.13c	2.16±0.10c
Mean	24.00±0.03	0.95±0.10	1.64±0.16
ANOVA (Analysis of Variance)			

df	4.0	4.0	4.0
F-value	5.60	132.48	650.43
P-value	0.00	0.00	0.00

Values within the column with similar superscript are not significantly different at p≤*0.05 level* as determined using Duncun's multiple range test. A value represents mean ± standard error.

Fig 1: Effect of different auxin on *ex vitro* rooting of *in vitro* regenerated micro-shoots of *T. arjuna* A: Rooting with treatment of 200 mg/l (984.25 µM) IBA, B: rooting with treatment of 100 mg/l (537.06 µM) NAA and C: rooting with treatment of 200 mg/l (1141.55 µM) IAA.

Discussion

The realistic aspect of micropropagation technology is its successful execution from lab to field conditions. Rooting of *in vitro* produced shoot is a critical factor in the micropropagation of woody tree species **(**Durkovic and lux, 2010**).** Rooting in the external environment is an aid for simultaneous hardening and acclimatization of plantlets and decreased the micropropagation cost as well as the time from laboratory to field conditions (Pruski *et al.* 2000) [12]. For *ex vitro* rooting, micro shoots were given pulse treatment with higher concentrations of auxins for short duration and the best results were obtained through pulse treatment of 200 mg/l (984.25 µM) IBA for 10 min. Treated micro shoots were subsequently transferred to autoclaved bottles containing vermiculite and kept under controlled conditions. The effectiveness of IBA as compared to other auxins on rooting has been reported earlier in many medicinal tree species (Arora *et al*., 2010; Phulwaria and Shekhawat 2013; Rathore *et al.* 2013) [1, 10, 13, 14]. IBA is now used as a commercial rooting growth hormone for many plant species worldwide (Epstein and Luduig-Muller, 1993) [5]. *Ex vitro* rooting has been successfully utilized in the establishment of a regeneration system of several woody plants including *Sarcostemma acidum* (Rathore and Shekhawat, 2012) [3], *Leptadenia pyrotechnica* (Dagla *et al*., 2012) [3] and *Terminalia bellirica* (Phulwaria *et al*., 2012) [11].

Conclusion

In the present research work, a successful protocol has been developed for the rapid and large scale multiplication of *T. arjuna*. *Ex vitro* rooting, of *in vitro in vitro* regenerated micro-shoots improve hardening and acclimatization process of the plant and also increases the chances of survival in the field conditions. It will help to overcome the major constraints in successful *in vitro* multiplication of plant, i.e. hardening and acclimatization, less time, labor, and cost of *in vitro* propagation.

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