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Factors Affecting *Ex vitro* Rooting in Micropropagated Shoots from Nodal Explants of *Terminalia arjuna*

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Authors' contributions

This work was carried out in collaboration among all authors. Author MC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors IDA and SA supervised the study. All authors read and approved the final manuscript.

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ABSTRACT

The present work was done with the aim to study the effect of rooting mixture and incubation temperature on *Ex vitro* rooting of *Terminalia arjuna*, an important multipurpose tree. The nodal explant collected from Ummaid garden Jodhpur was subjected for *In vitro* shoot proliferation on BAP supplemented modified MS medium. These shoots were *In vitro* multiplied on BAP (half concentration of BAP used in *In vitro* shoot proliferation) with low concentration of NAA supplemented medium. The individual shoots from *In vitro* multiplied shoots were pulse treated with IBA for 10 min. and transferred in different rooting mixture and incubation temperature for *Ex vitro* rooting. Analysis of data revealed that maximum 62.22% rooting was observed when the plantlet pulse treated with 984.25 µM IBA for 10 min were transferred on bottle containing vermiculite as rooting mixture and incubated at the temperature of 26°C.The optimization of *Ex vitro* rooting mixture and temperature conditions will be helpful in propagation of this important species rapidly in large scale.

Keywords: Micropropagation; Ex vitro rooting; rooting mixture; temperature.

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1. INTRODUCTION

Terminalia arjuna is handsome and evergreen tree with buttressed trunk. The tree attains a height of about 20- 26 m and girth about 3 m. The stem of the tree is mostly long and straight. The wood of this tree is very hard and brown. It is ornamental timber without any characteristic odor and taste. In India, it is mostly found in Bihar, Orissa, Madhya Pradesh, Gujarat, Maharashtra, Tamil Nadu, West Bengal and Punjab [1]. All parts of this tree (leaves, bark, gum, flowers and fruits) have medicinal properties. Leaves are sub-opposite, oblong and usually 10-15 cm long. Fresh bark of tree is green, grey or pinkish grey/ green and pinkish from inside in color. Bark which attains thickness up to 9 cm is generally light and spongy and exfoliating in large irregular sheet and its yield varies from 9 to 45 kgs per tree. A very nutritive brownish clean golden colored, transparent gum is obtained from bark of the tree. Fruits are green when young, but become brownish- black at maturity and are 2.5 - 3.5 cm long, fibrous woody, glabrous with 5 hard wings, striated with numerous curved veins. WHO estimates that approximately 80% of the developing world's populations meet their primary health care needs through traditional medicine [2]. Many different medicine systems like Ayurvedic, Unani and Siddhi exist in India. Ayurveda and Siddha are mainly of plant based medicine system. Therefore, the evaluation of rich heritage of traditional medicine is essential [3]. In this regard, one such plant is *Terminalia arjuna*. Arjun holds a reputed position in both Ayurvedic and Yunani Systems of medicine. It is widely used in the preparation of important ayurvedic formulations like arjunaishtam, Cintamanirasam, Laksagugula [4] and has antibacterial [5,6], hypolipidemic, anti-carcinogenic [7], antioxidant [8] and anti-inflammatory effects. Timber is locally used for carts, agricultural implements, water troughs, traps, boat building, house building, electric poles, tool-handles, jetty-piles and plywood. It is one of the major tannin yielding trees. Arjun wood is excellent firewood and produces good quality charcoal for producer gas. It is also used as feeding material for silk worm. Therefore, to improve the feeding material for the silk worm and quality of the silk product, it is necessary to propagate *T. arjuna* in large scale. It can be propagated through seed and cutting but these methods have some limits as low seed germination and difficulty in rooting from cuttings. Due to overexploitation to meet medicine requirement, lack of proper propagation and replenishing method its population is sharply decline. To conserve such economically and medicinally important plant species, other non-conventional method like micropropagation are playing key role. For any micropropagation method rooting is crucial step. Earlier, *ex vitro* rooting of *Terminalia arjuna* was reported by Gupta et al. [9] but they studied only hormonal effect on *Ex vitro* rooting. In 2018, we [10] also reported effect of different auxins on *ex vitro* rooting and this is the further study on factors influencing the *Ex vitro* rooting in *T. arjuna.*

2. MATERIALS AND METHODS

For present study nodal explants (2.0 to 2.5 cm) containing axillary buds were collected from green, healthy, looped lateral branches of single mature tree of *Terminalia arjuna* situated at Ummaid garden, Jodhpur. After pre disinfection with Bavistin and streptomycin and surface sterilization with 0.1% HgCl2, the axillary bud break and shoot proliferation was achieved on MMS (Modified MS) medium supplemented with 8.88 µM BAP + additives (100 mg/l of ascorbic acid, 50 mg/l of citric acid, 50 mg/l of adenine sulphate and 25 mg/l PVP). These shoots were then cut into clump of three shoots and cultured on MMS medium augmented with 4.44 µM BAP + 0.54 µM NAA + additives. The culture vessels for induction and multiplication was kept in culture room for 4 weeks at 25 ± 2ºC temperature and 16 hrs light conditions. The shoots of 2-3 cm were separated from *In vitro* multiplied shoot clump and then pulse treated with 984.25 µM IBA for 10 min for *Ex vitro* rooting.

2.1 Effect of Rooting Mixture

The pulse treated shoots were then transferred to autoclaved screw cap glass bottles having different rooting mixture like soilrite, vermiculite, sand and their combination (1:1) for *Ex vitro* rooting and moistened with half strength MS salts. These bottles containing micro shoots were initially incubated in culture room for 3-4 weeks and then transferred to polyhouse.

2.2 Effect of Incubation Condition

To assess the effect of incubation condition on *Ex vitro* rooting, the jam bottles containing vermiculite and pulse treated shoots were incubated at different temperature (22°C, 26°C, 30°C) in BOD incubator for 4 weeks.

2.3 Statistical Analysis

Total 15 replicate were used for each treatment and each treatment repeated three times. The data of each experiment were recorded after 4 weeks. The resultant data were analyzed through one-way analysis of variance (ANOVA) using Statistical Packages for Social Sciences Software (SPSS 17.0). The results are expressed as mean ± SE of three experiments. The significance difference between means were assessed by Duncan's multiple range test (P = 0.05).

3. RESULTS AND DISCUSSION

3.1 Effect of Rooting Mixture

In order to investigate the effect of rooting mixture on *Ex vitro* rooting, the pulse treated shoots were transferred to autoclave bottles containing different rooting mixture (soilrite, vermiculite, sand and their combination). These rooting media showed varied effect on rooting response. Among all the rooting mixture tested vermiculite was found to be the best rooting mixture which showed maximum 62.22% rooting with 2.92 root numbers and 4.25 cm root length (Fig. 1C). The roots developed in vermiculite had numerous secondary root hairs. Minimum 17.77% shoots rooted on autoclaved bottle containing sand (Fig. 1A).

To study the effect of combination of rooting mixture on *ex vitro* rooting, best responding rooting mixture vermiculite was tried in combination with soilrite or sand (Fig. 1D). It was observed that 55.55% shoots *ex vitro* rooted when shoots, pulse treated with 984.25 µM IBA, were transferred on bottles containing vermiculite + soilrite (Table 1 & Fig. 1E).

Ex vitro rooting has more advantages over *in vitro* rooting as it is low cost method and rooting and acclimatization take place simultaneously. The plants obtained through *ex vitro* rooting of *in vitro* developed shoot have a well developed root system and better subsequent growth and development [11]. According to Baskaran and Staden [12] *Ex vitro* rooting could enhance the chances of survival of plantlets in the field conditions. Rooting media play a crucial role in *Ex vitro* rooting by providing physical support, retention of moisture, providing drainage and aeration. In present study among all the *Ex vitro* medium tested vermiculite favored highest rooting. This is because vermiculite has a relatively high cation exchange capacity and thus can hold the nutrient in reserve and later release them. Yan et al. [13] reported that vermiculite enhanced the growth and development of some plant at early stage. Vermiculite as a rooting mixture was also used for different plant species like *Albizia procera* [14], *Petrocarpus santalinus* [15] and *Tylophora indica* [16].

3.2 Effect of Incubation Conditions

The temperature at which bottles containing pulse treated shoots were incubated also effect the rooting percentage as well as root numbers and health of shoots. When pulse treated shoots with 984.25 µM IBA were incubated on different temperature (22°C - 30°C) in BOD incubator the maximum 62.22% rooting was obtained on 26°C. On 26°C temperature shoot remained green and healthy. On lower temperature (22°C), shoots remained green but showed minimum 6.66% rooting. At increased temperature (30°C), only 24.44% rooting was obtained and the health of shoots deteriorated with increased leaf fall (Table 2).

Table 1. Effect of rooting mixture on *Ex vitro* rooting of *T. arjuna* shoots

Rooting media	Rooting %	Mean root number	Mean root length (cm)
Sand	17.77 ± 0.05^{b}	1.37 ± 0.15^{c}	1.18 ± 0.09^{d}
Soilrite	51.11 ± 0.08^{a}	2.30 ± 0.13^{b}	3.58 ± 0.06^{b}
vermiculite	62.22 ± 0.07^{a}	2.92 ± 0.13^{a}	4.25 ± 0.06^{a}
Vermiculite + Soilrite	55.55 ± 0.07^{a}	2.44 ± 0.11^{b}	3.98 ± 0.19^{ab}
Vermiculite + Sand	28.88 ± 0.06^{b}	1.69 ± 0.17^{c}	2.74 ± 0.15^{c}
Mean	43.11 ± 0.03	2.36 ± 0.08	3.57 ± 0.10
Analysis of Variance			
df	4	4	4
F-value	7.25	13.99	49.64
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 rooting mixture on *Ex vitro* rooting of *Terminalia arjuna*
(A) Sand; (B) Soilrite; (C) Vermiculite; (D) Vermiculite + Sand; (E) Vermiculite + Soilrite

Table 2. Effect of incubation conditions on *Ex vitro* rooting of *T. arjuna* shoots

Temperature	Rooting %	Mean root number	Mean root length (cm)
22°C	6.66 ± 0.04^{c}	1.00 ± 0.00^{b}	2.36 ± 0.08^{c}
26°C	62.22 ± 0.07^{a}	3.00 ± 0.13^{a}	4.01 ± 0.07^{a}
30°C	24.44 ± 0.06^{b}	1.72 ± 0.19^{b}	3.24 ± 0.08^{b}
Mean	31.11 ± 0.04	2.52 ± 0.14	3.69 ± 0.09
Analysis of Variance			
df	2	2	2
F-value	22.04	21.76	38.94
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

The incubation conditions of autoclaved bottles containing pulse treated shoots also effect the *ex vitro* rooting in *T. arjuna*. For this, bottles were kept at different temperature in incubator. Maximum *Ex vitro* rooting was obtained at 26°C temperature. At lower and higher temperature above 26°C, rooting response drastically decreased. Similarly, Shekhawat and Manokari [17] also obtained *Ex vitro* rooting in *Couroupita guianensis* on 25-28°C temperature.

4. CONCLUSION

As it is well known that micropropagation cannot be an efficient method until it is successfully established in field conditions. In view of this, *ex vitro* rooting of *In vitro* plantlet is critical step in micropropagation. Therefore, present study concluded that by optimizing the different conditions of *ex vitro* rooting, mass production of *Terminalia arjuna* can be done.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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