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

Meena Choudhary¹*, Inder Dev Arya¹ and Sarita Arya¹

¹Genetics and Tree Improvement Division, Arid Forest Research Institute, Jodhpur-342005, India.

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.

Article Information

DOI: 10.9734/CJAST/2020/v39i4131123

Editor(s):
(1) Dr. Tushar Ranjan, Bihar Agricultural University, India.

Reviewers:
(1) Miguel Jordan Zimmermann, Catholic University and Universidad Mayor, Chile.
(2) Gloria Irma Ayala Astorga, Dictus-Universidad De Sonora, México.

Complete Peer review History: http://www.sdiarticle4.com/review-history/63868

Received 04 October 2020
Accepted 10 December 2020
Published 21 December 2020

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 Ummeid 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.
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 Ayurveda, 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 arjunaisham, Cintamanirasam, Laksagugula [4] and has antibacterial [5,6], hypolipidemic, anticarcinogenic [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 ± 2°C 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).

| Rooting media        | Rooting %      | Mean root number | Mean root length (cm) |
|----------------------|----------------|-----------------|-----------------------|
| Sand                 | 17.77 ± 0.05a  | 1.37 ± 0.15c    | 1.18 ± 0.09c          |
| Soilrite             | 51.11 ± 0.08a  | 2.30 ± 0.13d    | 3.58 ± 0.06b          |
| vermiculite          | 62.22 ± 0.07a  | 2.92 ± 0.13a    | 4.25 ± 0.06a          |
| Vermiculite + Soilrite | 55.55 ± 0.07a | 2.44 ± 0.11b    | 3.98 ± 0.19ab         |
| Vermiculite + Sand   | 28.88 ± 0.06b  | 1.69 ± 0.17c    | 2.74 ± 0.15c          |
| 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 Duncan’s multiple range test. A value represents mean ± standard error.
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 ± 0.04c  | 1.00 ± 0.00a     | 2.36 ± 0.08b          |
| 26°C        | 62.22 ± 0.07a | 3.00 ± 0.13a     | 4.01 ± 0.07a          |
| 30°C        | 24.44 ± 0.06b | 1.72 ± 0.19b     | 3.24 ± 0.08b          |
| Mean        | 31.11 ± 0.04  | 2.52 ± 0.14      | 3.69 ± 0.09           |

Analysis of Variance

|          | df | F-value | P-value |
|----------|----|---------|---------|
|          | 2  | 22.04   | 0.00    |
|          | 2  | 21.76   | 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 affect 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.

ACKNOWLEDGEMENTS

The authors are thankful to Council of Scientific and Industrial Research (CSIR), New Delhi for the financial support and Director, Arid Forest Research Institute (AFRI), Jodhpur, India for providing lab facilities to carry out this research.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Choudhary M, Jaiswal S, Singh R, Arya ID, Arya SA. Micropropagation protocol for mass multiplication of *Terminalia arjuna* – a valuable medicinal tree. Advances in Forestry Science. 2015; 2:1-6. DOI: 10.34062/afs.v2i1.2107
2. Bannerman RH. Traditional medicine in modern health care. World Health Forum. 1982;3(1):8-13.
3. Padmaa MP, Leena JP, Angelin. Genius Salacia. A comprehensive review. Journal of Natural Remedies. 2008;8:116-131.
4. Damodaran SK, Yenamandra SP. On the ethnomedical significance of the Arjun tree, *Terminalia arjuna* (Roxb.) Wight & Arnott. Journal of Ethnopharmacology. 1987; 20:173-190.
5. Perumalsamy, Ignacimuthu RS, Sen H. Screening of 34 Indian medicinal plants for antibacterial properties. Journal of Ethnopharmacology. 1998;62:173-182.
6. Singh DV, Gupta MM, Kumar TRS, Saikia D, Khanuja SPS. Antibacterial principles from the bark of *Terminalia arjuna*. Current Science. 2008;94(1):27-29.
7. Nagpal A, Meena LS, Kaur S, Grover IS, Wadhwa R, Kaul SC. Growth of suppression of human transformed cells by treatment with bark extracts from a medicinal plant *Terminalia arjuna*. *In vitro* Cellular and Developmental Biology- Animal. 2000;36: 544-547.
8. Gupta R, Singhal S, Goyal A, Sharma VN. Anti-oxidant and hypocholesterolaemic effects of *Terminalia arjuna* tree-bark powder: A randomized placebo-controlled trail. Journal of Association of Physicians, India. 2001;49231-49235.
9. Gupta AK, Harish, Rai MK, Phulwaria M, Agarwal T, Shekhawat NS. *In vitro* propagation, encapsulation, and genetic fidelity analysis of *Terminalia arjuna*: A cardioprotective medicinal tree. Applied Biochemistry and Biotechnology. 2014; 173 (6):1481-1494.
10. Choudhary M, Gehlot A, Arya ID, Arya S. Influence of different auxin treatment on *ex vitro* rooting in *In vitro* regenerated micro shoots of *Terminalia arjuna* (Arjun). Journal of Pharmacognosy and Phytochemistry. 2018;7:3079-3082.
11. Benmahiou B, Dorion N, Harche MK, Daguin F. Micropropagation and *Ex vitro* rooting of pistachio (*Pistacia vera* L.). Plant Cell Tiss Organ Cult. 2012:108:353–358. DOI: 10.1007/s11240-011-0040-6
Baskaran P, Staden JV. Rapid *In vitro* micropropagation of *Agapanthus praecox*. South African Journal of Botany. 2013; 86:46-50.
12. Yan H, Liang C, Yang L, Li Y. *In vitro* and *Ex vitro* rooting of *Siratia grosvenorii*, a traditional medicinal plant. Acta Physiol Plant. 2010;32:115-120. DOI: 10.1007/s11738-009-0386-0
13. Swamy SL, Ganguli JL, Puri S. Regeneration and multiplication of *Albizia procera* Benth. through organogenesis. Agroforestry Systems. 2004;60(2):113-121.
14. Rajeswari V, Paliwal K. *In vitro* shoot multiplication and *Ex vitro* rooting of *Petrocarpus santalinus* L.: An endangered leguminous tree, In: 11th IAPTC & B congress on biotechnology and sustainable agriculture 2006 and beyond, Beijing, China. 2006;13-18.
15. Faisal M, Ahmad N, Anis M. An efficient micropropagation system for *Tylophora indica*: An endangered, medicinally important plant. Plant Biotechnology Report. 2007;1:55-61.
16. Shekhawat MS, Manokari M. *In vitro* propagation, micromorphological
studies and Ex vitro rooting of threatened species. Physiology and cannon ball tree (*Couroupita guianensis* Aubl.): A multipurpose species. Physiology and Molecular Biology of Plants. 2016;22:131-142.

© 2020 Choudhary et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/63868