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IN VITRO RESPONSE BY *Terminalia arjuna* GENOTYPES DURING MICROPROPAGATION

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KEYWORDS

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Modified MS medium

Plant Growth Regulator

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ABSTRACT

Terminalia arjuna is an important tree of the medicinal and sericulture industry, commonly known as Arjun. It's bark is rich in secondary metabolites makes this plant highly valuable in medicine industry to treat cardiovascular disease. Overexploitation due to high demand in medicine, low seed germination, limitations of the conventional method of propagation push this plant towards being endangered. To conserve germplasm of such tree species and meet the requirement in medicinal industry, some non-conventional propagation method like micropropagation has been developed. The present work highlighted the effect of three genotypes (G-1, G-2, and G-3) on tissue culture of *T. arjuna* situated at Jodhpur, Rajasthan, India. *In vitro* shoot proliferation was achieved on a modified MS medium enriched with BAP + additives. Among the tested genotypes, Genotype -1 showed maximum bud break response (100%) followed by G-3 (93.33 %) and G-2 (86.66%). Further multiplication of these shoots on modified MS medium containing BAP + NAA + additives gave 11.38±0.26 (G-1), 9.44±0.21 (G-2) and 10.22±0.32 (G-3) shoots. *In vitro* rooting was done by pulse treatment with IBA for 10 min prior to transfer on hormone free half strength MS medium containing 0.1% activated charcoal. Maximum *in vitro* rooting was obtained in G-1 (80%) followed by G-3 (71.11%) and G-2 (68.88%). In the present study, it was observed that optimum growth in all three genotypes required different doses of Plant Growth Regulator. Thus, by identifying and multiplying the best performing genotypes the gap between demand and supply of such medicinal plant can be fulfilled.

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1 Introduction

Terminalia arjuna (arjun) is most valuable multipurpose tree species belonging to the flowering plant family Combretaceae and commonly known as Arjuna, Koha, Kahu, Arjan, White Marudah, White Murdh, etc. It comprising around 250 species distributed in the tropical region of the world (Chauhan et al., 2008). It is commonly distributed in India, Burma, Mauritius, and Sri Lanka (Kapoor et al., 2014). In India, *T. arjuna* is about 60–80 feet large tree with a buttressed trunk and horizontally spreading crown and drooping branches.

This tree species is highly prized for their natural ingredients present in the bark which are useful in cardiac disorder (Shengule et al., 2019) and cancer treatment (Singh et al., 2017). It is also showed hypocholesterolemic, antibacterial (Bano et al., 2020), antimicrobial, antitumoral, antioxidant, anti-inflammatory (Sarma et al., 2019), antiallergic and antifeedant, antifertility, and anti-HIV activities (Bachaya et al., 2009). The leaves of *T. arjuna* are ideal food for the Tasar silkworm *Antheraea mylitta*. Therefore, Arjun also plays an important role in the sericulture industry. These trees are recommended for reclamation of saline, alkaline soils, and deep ravines. It is also used for agroforestry (Sarvade et al., 2017), social forestry programs, soil conservation, and wasteland afforestation.

Today this medicinal plant is facing a serious threat because of the rapid loss of natural habitats and overexploitation of plants for medicinal purposes. Thus, there is the need to apply conventionally as well as non-conventional propagation techniques. To get the sustainable supply of *T. arjuna*, it is necessary to raise them on a mass scale in plantations. Vegetative propagation is beset with problems. Therefore, micropropagation holds a key to achieve mass propagation in a short period.

As it is well known, genotype plays a major role in all phases of vegetative propagation (Land & Cunningham, 1994). The number of papers dealing with the micropropagation of *T. arjuna* either using seedling nodal explants or nodal explants from the mature tree (Pandey & Jaiswal, 2002; Pandey et al., 2006; Gupta et al., 2014). Choudhary et al. (2015) and Choudhary et al. (2018) reported the complete protocol of *T. arjuna* micropropagation and the effect of different auxins on *ex vitro* rooting, respectively. It is the further study on the effect of three genotypes viz., G-1, G-2, and G-3 on *in vitro* axillary bud break, *in vitro* shoot multiplication, and *in vitro* rooting of *T. arjuna*. Thus, by propagating the best responding genotype on large scale, the demand of such tree species can be met.

2 Materials and Methods

Nodal segments containing axillary bud were collected from three different mature disease-free lopped trees of *T. arjuna* situated at Ummaid garden (Genotype-1), AFRI campus (Genotype-2), and AFRI nursery (Genotype-3), Jodhpur, Rajasthan, India (Figure 1). The detail of the three genotypes of *T. arjuna* are given in Table 1. These explants were pre-disinfected with a 0.1% (w/v) Bavistin and streptomycin for 15 min, followed by surface sterilization with 0.1% $HgCl_2$ for 8 min. Explants were dipped in pre-chilled sterile antioxidant solution of 100 mg/l of ascorbic acid, 50 mg/l of citric acid, and 25 mg/l PVP for 10-30 minutes before inoculation on medium to reduce phenolic exudation.

MS medium (Murashige & Skoog, 1962) was modified by reducing the strength of NH_4NO_3 and KNO_3 salts to half and was added with additives (100 mg/l of ascorbic acid, 50 mg/l of citric acid, 50 mg/l of adenine sulphate, and 25 mg/l PVP), 3% sucrose and 0.8% agar-agar (w/v) for culture initiation. The pH was adjusted at 5.8 and autoclaving of the medium was done at 15 psi for 20 minutes at 121° C temperature. All the culture vessels after inoculation and culture manipulation were maintained in an aseptic culture room at 26±2ºC temperature and 16 h of photoperiod with 1600 lux light intensity.

Figure 1 Three selected genotypes of *T. arjuna*: G-1 from Ummaid Garden; G-2 from Arid Forest Research Institute (AFRI) Campus; G-3 from AFRI nursery, Jodhpur, India.

Table 1 Description of *Terminalia arjuna* trees selected for micropropagation

Particular parameter	Genotype- 1	Genotype- 2	Genotype- 3
Area of collection	Ummaid garden, Jodhpur	AFRI Campus, Jodhpur	AFRI Nursery, Jodhpur
latitude	N 26.29194˚	N 26.22738˚	N 26.23316˚
longitude	E 73.03439˚	E 73.03174˚	E 73.02153˚
Altitude	205 m	235 m/t m	222 m
Physiological parameter	Healthy plant with emergence of new shoots		
Height (meter)	20.0	26.5	15.0
Grith (cm)	150.0	170.0	100.0
Plant crown diameter (m)	12.0	13.0	9.0
Flowering	April - July		
Fruiting	June onwards		
Synchronization in fruiting	Within three months after fruiting initiation		

For *in vitro* bud break response and shoot proliferation, nodal explants from all three genotypes were cultured on Modified MS medium supplemented with BAP (2.22, 4.44, 8.88, 13.32, 17.76 µM) and additives. The *in vitro* proliferated shoots were excised from nodal explants of each genotype and a group of three shoots were cultured on MS medium supplemented with BAP (2.22-8.88 µM) in combination with NAA (0.27-1.34 µM) for *in vitro* shoot multiplication. The individual shoots from *in vitro* multiplied shoot clump of three genotypes were pulse treated with different concentrations of IBA (246, 492, 984, 2460 µM) for 10 min and then transferred to the hormone free half strength of MS medium containing 0.1% activated charcoal.

All the experiments were conducted with 15 replicates per treatment. Each experiment was repeated three times. Observations were recorded after 4 weeks for axillary bud break, multiplication, and rooting experiments. Resultant data were analyzed through General liner Model (GLM) multi variance factor analysis and 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 significant difference between means was assessed by Duncan's multiple range test ($P< 0.05$).

3 Results

3.1 Axillary bud break and *in vitro* shoot proliferation

The nodal explants of three genotypes culture on 8.88 µM BAP + additives revealed that G-1 gave best 100 ± 0.00% bud break with 5.80 ± 0.16 axillary shoot proliferation (Figure 5A) followed by G-3 (80 ± 0.06% bud break with 3.72 ± 0.16 axillary shoot; Figure 5C) and G-2 (71.11 ± 0.07% bud break with 3.50 ± 0.17 axillary shoot; Figure 5B). It was also observed that the optimal hormonal requirement for G-2 and G-3 was 13.32 µM BAP which supported 86.66 ± 0.05% and 93.33 ± 0.04% bud break response, respectively (Figure 2). The explants cultured on a hormone-free medium did not give bud break response in all three genotypes. Increased BAP concentration beyond optimal level resulted in a decline in both bud break percentage and axillary shoots formation in all three genotypes.

3.2 *In vitro* shoot multiplication

The shoots cultured from different genotypes showed variation in shoot multiplication potential. Here also genotype-1 gave the best multiplication response followed by genotype-3 and genotype-2 (Figure 3). The optimal hormonal requirement of G-1 and G-3 was 4.44 µM BAP + 0.54 µM NAA, which supported the development of 11.38 ± 0.26 shoots with 3.17 ± 0.07 cm shoot length and 10.22 ± 0.32 shoots with 2.32 ± 0.11 cm shoot length, respectively per propagule of three shoots in 4 weeks (Figure 5D and 5F). In G-2, an optimal multiplication response of 9.44 ± 0.21 shoots per propagule was obtained on 4.44 µM BAP + 0.27 µM NAA (Figure 5E).

3.3 *In vitro* rooting

In present study, three genotypes were compared for their responsiveness and ability to induce roots from *in vitro* raised shoots (Figure 4). The optimum hormonal requirement for G-1 and G-3 was 984 µM IBA which supported 80.00 ± 0.06% rooting with 4.00 ± 0.15 root number and 71.11 ± 0.07% rooting with 3.64 ± 0.14 root number, respectively (Figure 5G and 5I). Whereas G-2 showed 68.88 ± 0.07% *in vitro* rooting on 492 µM IBA (Figure 5H).

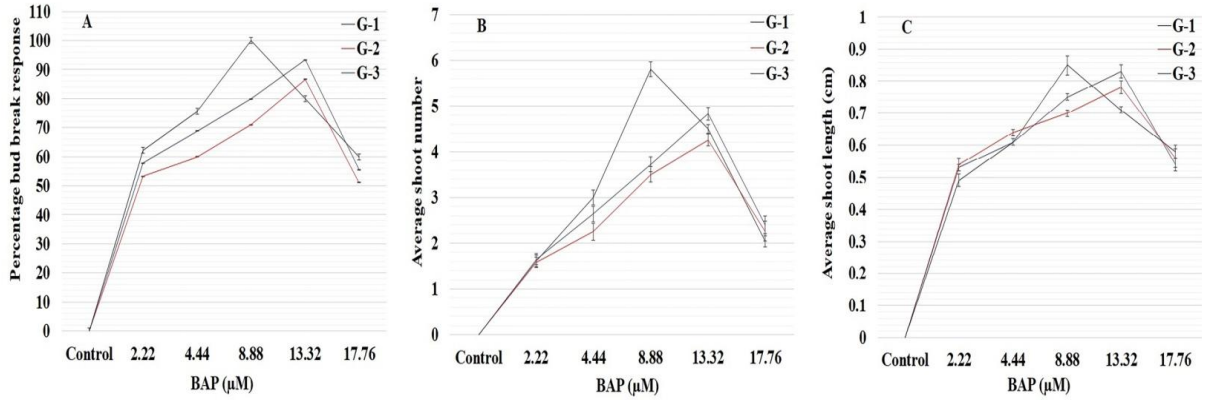

Figure 2 Effect of genotypes (G-1, G-2 & G-3) on *in vitro* shoot proliferation (A. bud break percent; B. Shoot number; C. Shoot length) in *T. arjuna*. Nodal explants cultured on MS medium supplemented with BAP + additives

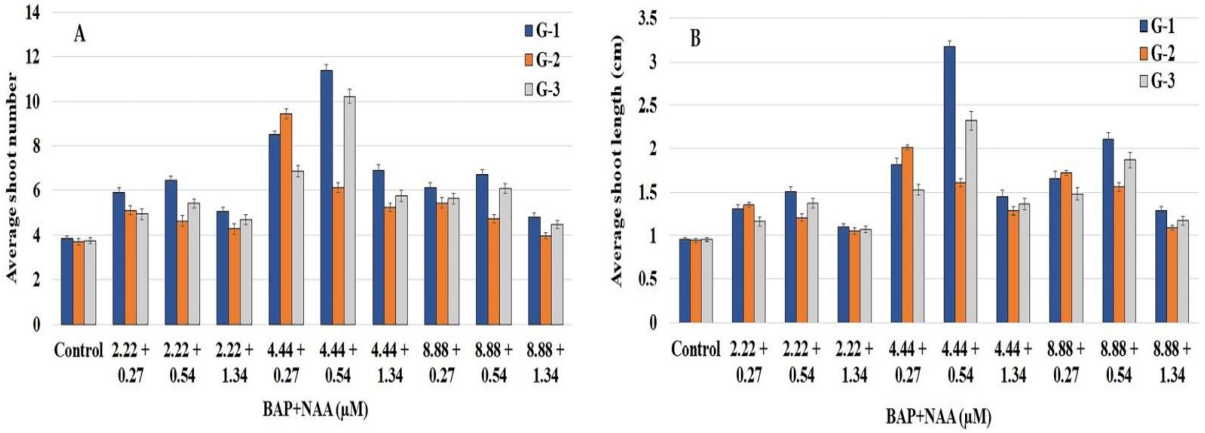

Figure 3 Effect of genotypes (G-1, G-2 & G-3) on *in vitro* shoot multiplication (A. Shoot number; B. Shoot length) of *T. arjuna*. A Propagule of three shoots were cultured on MMS medium supplemented with BAP + NAA + additives.

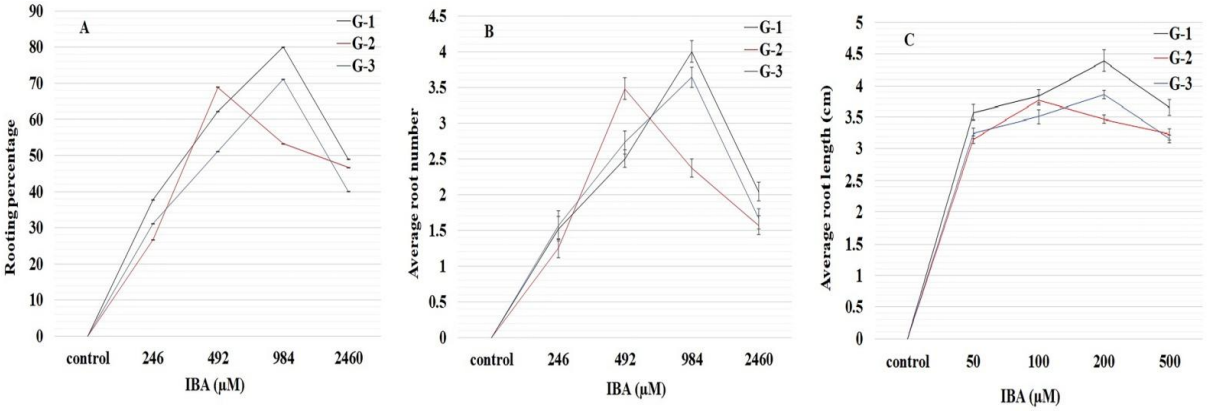

Figure 4 Effect of genotypes (G-1, G-2 & G-3) on *in vitro* rooting (A. Rooting percentage; B. Root number; C. Root length) of *T. arjuna*. Shoots were pulse treated with IBA for 10 min and then transfer on hormone-free half-strength MS medium containing 0.1% activated charcoal.

Figure 5 Effect of genotypes on different steps of micropropagation of *T. Arjuna*; (A-C) *In vitro* shoot proliferation in Genotype-1 (A), Genotype-2 (B) & Genotype- 3 (C) on MMS medium supplemented with optimal concentration of BAP; (D-F) *In vitro* shoot multiplication in Genotype-1 (D), Genotype-2 (E) & Genotype- 3 (F) on MMS medium supplemented with optimal concentration of BAP + NAA; (G-I) *In vitro* rooting: upper & lower view of *in vitro* rooting response in Genotype-1 (G), Genotype-2 (H) & Genotype- 3 (I) after pulse treatment of shoots with optimal concentration of IBA

4 Discussion and Conclusions

Studies revealed that different genotypes have a significant effect on the frequency of shoot induction and had different optimal growth regulator requirements. It may be due to the presence of different levels of the endogenous hormone in these genotypes. Similarly, Gomes et al. (2010) reported that different genotypes of Strawberry possess different levels of endogenous auxins and/or cytokinins that influence their behaviour *in vitro*. Similarly, Jain et al. (1990) also observed that genotypes of *Morus bombysis* (Schimanochi and Mizusawa) had differential growth regulator requirements for the high rate of multiple shoot induction. Cezar et al. (2015) also reported that genotype effects the shoot formation in three genotypes of *Pinus taeda.*

In plant tissue culture, it is now well known that no two genotypes of the same species give a similar response under a given set of culture conditions (Nehra et al., 1989). In the current investigation also three genotypes of *T. arjuna* were compared for *in vitro* shoot multiplication and it was found that *in vitro* shoot multiplication was at par with each other. However, ANOVA shows a significant difference among three genotypes in terms of mean shoot number and mean shoot length. Similarly, San et al. (2018) worked on micropropagation of 42 genotypes of Almond and reported a significant difference in all these genotypes in terms of the number of shoots and length of shoots. Effect of genotypes on *in vitro* shoot multiplication was also observed in *Ceratonia siliqua* (Romano et al., 2002) and *Vaccinum vitis* (Debnath, 2005), *Capparis spinosa* (Sottile et al., 2020).

In vitro rooting of the *in vitro* raised shoots is the most important step in tissue culture to develop a complete plant. Different types of morphogenetic responses such as shoot proliferation and rooting are strongly determined by the *in vitro* genotype of the explants (Bhau & Wakhlu, 2001). In the present investigation on three genotypes of *T. arjuna*, the efficacy of *in vitro* rooting was found to be variable but this difference was not significant. Genotype-1 gave maximum rooting response followed by genotype-3 and genotype-2. Feyissa et al. (2005) reported different rooting responses in five genotypes of *Hagenia abyssinica*. In the current study, the hormonal requirement for Genotype-2 was found to be slightly different from Genotype-1 and Genotype-3. Similarly, Tesfa et al. (2016) also reported the different hormonal requirements in two genotypes of *Saccharum officinarum*.

Present study concluded that different genotypes showed differential plant growth regulator requirements for their optimal growth. Thus, genotype which performed better during *in vitro* shoot proliferation, *in vitro* multiplication and *in vitro* rooting can be multiplied in large scale to meet the demand of such medicinal plant.

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Conflicts of Interest

The authors declare no conflict of interest.

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