



Research Article

VEGETATIVE PROPAGATION OF *Terminalia arjuna* (Roxb.) WT. & ARN. BY STEM CUTTINGS UNDER MIST

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Abstract- The effect IBA and NAA concentrations were examined to mass multiply plus trees of *Terminalia arjuna* (Roxb.) by vegetative propagation via rooting of stem cuttings. The experiment was conducted in a completely randomized design (CRD) with three replications. One-year old leafless branch cuttings were taken from selected superior phenotypes from the surrounding environs of Raipur, Chhattisgarh. Cuttings were treated with 0, 500, 1000, 1500 and 2000 mg L⁻¹ concentrations of Indole 3- Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) and planted in poly bags kept under a phyto-environmentally controlled mist chamber. A limited rooting was achieved in untreated cuttings (control), while rooting of cuttings of significantly increased with an increase in concentration of IBA and NAA. Among two auxins tested, IBA was most effective in inducing rooting, sprouting and associated traits. Out of different concentrations, 2000 mg L⁻¹ IBA concentration was found to be best and achieved over 75% rooting in cuttings. It also triggered more number of roots, higher root length, shoot proliferation, maximum shoot and root biomass. This paper discusses the role of auxins in influencing rooting of stem cuttings. This has a practical implication for the development of protocol for asexual propagation and establishing clonal plantations of *Terminalia arjuna* for promoting tassar cultivation.

Keywords- Auxins, Clonal multiplication, Multipurpose tree, Plus trees, Tassar silk.

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Introduction

Terminalia arjuna (Roxb.) a member of Combretaceae family popularly known as *Arjun* is an economically important multipurpose tree widely distributed throughout tropical and subtropical peninsular region of India. It is a large evergreen tree with spreading crown with drooping branches mostly found near moist tracts along the streams, river banks and occasionally in moist and dry tropical forest areas. All most all parts of tree like leaves, bark, flowers, fruits, wood and roots are useful for household, medicinal and industrial purposes [1]. The bark or leaf decoction or infusion is considered antibacterial, antimutagenic, hypolipidemic, antioxidant and hypocholesterolaemic and anti-inflammatory effects [3]. A fruit decoction is also taken as a tonic. The crushed leaves are externally applied to wounds, sores, acne and ulcers. Timber is used for making carts, agricultural implements, boat building, mine props etc. It is suitable for making of plywood of second grade and for tea chests. The wood has high calorific value, makes excellent firewood, and produces good quality charcoal for producer gas plants.

The arjuna is one among the primary host plants for rearing *Antheraea mylitta* D. insect, which produces commercial important Tassar silk primarily grown in the states of Jharkhand, Chhattisgarh, Odisha, West Bengal, Telangana, Madhya Pradesh and Maharashtra in India [9,21]. It is a backbone for tribal development because about 1.25 lakh tribal families are associated with tassar culture in the country [21]. Government of India is further promoting increase production of Kosa-Silk to provide livelihoods for people residing in rural areas and this could be possible through clonal plantation of superior phenotypes of Arjun having high protein and nutritive values and use of high yielding eco races of *Antheraea* sp. Commercial exploitation of this plant is hampered by the shortage of superior planting stock, primarily due to the difficulties experienced in propagating species using the conventional method of multiplication by seeds due to poor seed

viability, inadequate germination and lower survival under field conditions [19]. Moreover, *Terminalia arjuna* is a cross pollinated species exhibit a wide variability in terms of growth and foliage quality. Selection of novel genotypes for silkworm rearing and development of rapid methods of multiplication are necessary for commercial exploitation.

Vegetative techniques are used as indispensable tool for mass multiplication of superior phenotypes/genotypes and producing true to type uniform plants [14]. Rooting of stem cuttings is easiest and economical methods of vegetative propagation usually exploited in many tree species. Only a limited success was achieved in rooting of stem cuttings of *Terminalias* and concluded as difficult to root genus [6,7]. Rooting of cuttings will be affected by age of the ortet, season and exogenous application of root promoting hormones. It is essential to understand the critical factors in influencing the rooting [26, 10, 8]. Therefore, an attempt was made in the present study to develop an efficient, economically viable and reproducible vegetative propagation protocol through stem cuttings for commercial propagation of selected phenotypes of *T. arjuna*.

Material and methods

The experiment was conducted in Experimental Research Farm of Indira Gandhi Agricultural University, Raipur Chhattisgarh state, located at 21° 12' N latitude and 81° 36' E longitudes. The altitude of the study area is about 295 meters above mean sea level.

Preparation of cuttings

One year old branch cuttings of *Terminalia arjuna* were collected from selected mature superior phenotypes during February-March. The survey was conducted

and 25-30 year old superior phenotypes were selected with good qualities of leaves, stem and bark compared to normal population. The leafless cuttings about 15±2.5 cm length and 1-2 cm in diameter having 4-5 buds were taken from the selected phenotypes. Cuttings were treated with 0, 500, 1000, 1500 and 2000 mg L⁻¹ concentrations of Indole 3- Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA). The basal cut ends up to 2.5-cm of cuttings was dipped in IBA and NAA solutions for 24 hours duration.

Planting of cuttings in mist chamber and design of experiment

The cuttings were planted immediately after treatment with IBA and NAA in the polythene bags (10 x 6 inches) filled with rooting medium of Sand, FYM and Soil in equal proportions. One-third length of the cuttings was inserted in the rooting medium and arranged in phyto-environmentally controlled mist propagation unit according to Completely Randomized Design (CRD). The basal ends of the cuttings were dipped in 0.2% Bavistin solution just before planting to prevent attack of pathogens. Each treatment had 30 cuttings. In all 300 (10 treatments (5 IBA and NAA) x 3 replications x 10 cuttings per treatment) cuttings of each species were planted in factorial completely randomized design. Three factor nested effect factorial experiment in completely randomized design with growth hormone as treatment factor, concentration as nested factor into treatment factor and species as third factor was employed.

Data collection and Statistical Analysis

The observations on rooting, sprouting, callusing, root number, root length, number of shoots, root biomass and shoot biomass were recorded at 50 days after planting of cuttings. Data on rooting %, callusing % and sprouting % did not follow normal distribution; therefore, arc sine transformation was employed. Similarly, data on root number and leaf number were also transformed using square root transformation method. The transformed data was subjected to analysis of variance as suggested by the statistical methods [25]. The significance of treatments was tested using F test, and treatment means were separated using Least Significant Difference (LSD) at p<0.05 levels [25].

Results and Discussion

Although, a limited rooting (13%) was achieved in untreated cuttings, but the application of auxins (IBA and NAA) significantly ($P < 0.05$) increased rooting in stem cuttings from 20% to 76%. Lower concentrations of IBA and NAA (500 mg L⁻¹) were also least effective and statistically at par with untreated cuttings. There is

an overwhelming evidence that auxins promote rooting, which can be either naturally occurring within the plant (endogenous) or applied to the plant (exogenous) during vegetative propagation [26, 10, 8, 17]. Synthetic auxins like IBA and NAA are most commonly used to promote root development in clonal propagation. Auxin promotes the starch hydrolysis and the mobilization of sugars and nutrients at the base of the cuttings during the regeneration of adventitious roots [18].

The present study revealed that IBA treatments were more efficient than NAA in inducing rooting of stem cuttings. Further, the rooting percent steadily increased with an increase in concentration of auxins from 500 mg L⁻¹ to 2000 mg L⁻¹. These results are consistent and corroborated with the findings of earlier workers, who demonstrated that IBA was most effective auxin in triggering rooting in stem cuttings than IAA and NAA [27, 16, 11, 10]. IBA was quite strong auxin, while NAA is readily destroyed [15]. IBA may also enhance rooting via increased internal-free IBA, or may synergistically modify the action of IAA or the endogenous synthesis of IAA; IBA can enhance tissue sensitivity for IAA and increase rooting [27]. The effectiveness of higher concentrations of IBA and NAA on rooting (%) in this study confirm that high auxin requirement to compensate the low endogenous levels of auxin in mature cuttings especially in difficult to root species. *Terminalia arjuna* is difficult to root species [5, 7], as such it need higher concentrations of auxins for triggering rooting in stem cuttings.

The auxin treatments significantly increased callusing (%), number of roots, root length, sprouting and number of leaves [Table-1]. Unlike rooting (%), the callusing was higher in lower concentrations (26% in NAA 500 mg L⁻¹) and decreased further with an increase in concentration of IBA. The lower concentrations of auxins and untreated cuttings might allow the cuttings to differentiate to form callus but restricted further differentiation of the same into roots as the endogenous levels of auxins might not be sufficient enough for regeneration of roots. It was demonstrated that a high sucrose to low auxin ratio leads the production of excessive phloem, while high auxin to low sucrose ratio stimulates the differentiation of cambial derivatives into xylem [18]. With the exogenous application of adequate IBA levels, the callus further differentiated into xylem leading to the production of roots. In conclusion, the vascular differentiation of cells and formation of roots was taken directly with the use of higher concentrations of IBA and NAA in *Terminalia* spp, a difficult to root species, whereas the lower concentration ended with callusing and little differentiation in to roots.

Table-1 Effect of different concentrations of IBA and NAA on rooting of stem cuttings of *Terminalia arjuna*

Treatment	Rooting (%)	Callusing (%)	Root number	Root length/ cutting (cm)	Sprouting (%)	Leaves/ cutting
Control (water)	13.3 (21.1)	3.33 (23.33)	4.83	5.5 (2.45)	20.0 (26.6)	2.8 (1.8)
IBA 500 mg L ⁻¹	26.7 (31.0)	26.67 (13.33)	5.47	7.6 (2.84)	36.7 (37.2)	4.1 (2.2)
IBA 1000 mg L ⁻¹	40.0 (39.1)	10.00 (16.67)	5.85	10.3 (3.29)	50.0 (45.0)	4.4 (2.2)
IBA 1500 mg L ⁻¹	60.0 (50.9)	23.33 (13.33)	6.37	15.0 (3.92)	60.0 (50.9)	7.6 (2.8)
IBA 2000 mg L ⁻¹	76.7 (61.2)	10.00 (6.14)	7.93	19.3 (4.45)	76.7 (61.2)	13.6 (3.7)
NAA 500 mg L ⁻¹	20.0 (26.6)	28.78 (31.00)	4.63	5.5 (2.45)	26.7 (31.0)	3.0 (1.9)
NAA 1000 mg L ⁻¹	26.7 (31.0)	21.14 (18.43)	5.47	7.3 (2.79)	36.7 (37.2)	3.7 (2.0)
NAA 1500 mg L ⁻¹	43.3 (31.0)	23.86 (28.78)	6.40	8.8 (3.05)	46.7 (43.1)	4.5 (2.2)
NAA2000 mg L ⁻¹	53.3 (47.0)	21.14 (18.43)	7.33	10.5 (3.32)	53.3 (47.0)	4.8 (2.3)
LSD ($p \leq 0.05$ %)	14.01	8.55	0.70	0.37	8.34	0.39

The number of roots increased from 4.83 to 7.93 per cutting and root length from 5.5 to 19.3 cm as the IBA concentration increased from 0 to 2000 mg L⁻¹ concentration. The results are in conformity with the reports of earlier workers [4, 21], who stated that IBA treatments increased both rooting percent and root number in cuttings of *Ficus* spp.. Several researchers also reported that exogenous

application of auxin treatments especially IBA and NAA enhanced root proliferation and as well as root number in many species [2, 26, 17]. Increase in length of the roots might be due to an early initiation of roots at higher concentrations of IBA and therefore more utilization of the nutrients due to early formation of the roots.

The sprouting (%) and number of leaves of stem cuttings increased with an increase in concentrations of IBA and NAA. A significantly higher % of sprouting i.e. 74.18% and 57.09% were found in cuttings treated with 2000 mg L⁻¹ treatments of IBA and NAA, respectively. The lowest sprouting (%) was recorded in untreated stem cuttings (control). However, there were no significant differences observed between 500 and 1000 mg L⁻¹ treatments of IBA, 1000 and 1500 mg L⁻¹ IBA. The maximum sprouting and leaves in cuttings of *Terminalia* spp. with higher concentration of IBA and NAA treatments in the present study might be ascribed to better root growth which augmented absorption and translocation of nutrients from soil which take active part in various plant metabolic processes [22]. Rooting in *Terminalia* with high concentrations of IBA and NAA might lead to advanced bud break and maximum rooting and sprouting resulted in better shoot proliferation. Moreover, the root and shoot growth are intricately linked to endogenous levels of hormones and food materials [18].

A significantly higher shoot biomass 9.93 (g) and 8.38 (g) was achieved in cuttings treated with 2000 mg L⁻¹ IBA and NAA, respectively [Fig-1]. Similarly, high root biomass of 3.47 g and 2.94 g was recorded in cuttings treated with 1500 and 2000 mg L⁻¹ IBA and NAA, respectively [Fig-2]. Increase in root-shoot biomass due to exogenous application of auxins in cuttings is not extraordinary phenomenon as several workers reported such sort of results in different species [18, 26, 23]. The high root biomass is attributed to higher number of roots and more root length in cuttings treated with auxin (IBA and NAA) at higher concentrations, whereas lower number of roots and small length of roots resulted in lower biomass in untreated and low auxin concentrations. Increase in shoot biomass is a consequence of higher sprouting and more number of leaves induced in auxin treated cuttings especially at high concentrations, whereas a very low sprouting and limited number of leaves resulted in low shoot biomass in cuttings under control and lower concentrations of auxin treatments. The present findings are in line with findings of other workers [26, 17].

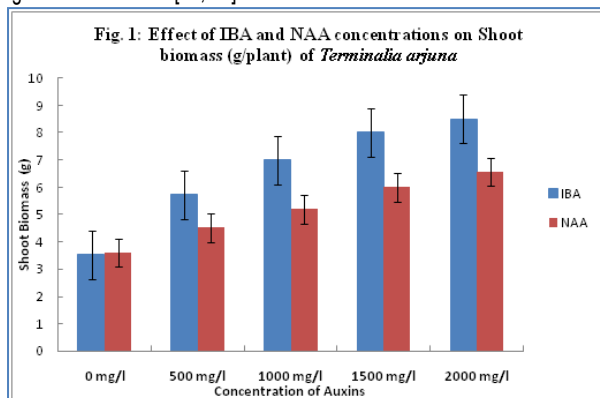


Fig-1 Effect of IBA and NAA concentrations on Shoot biomass (g/plant) of *Terminalia arjuna*

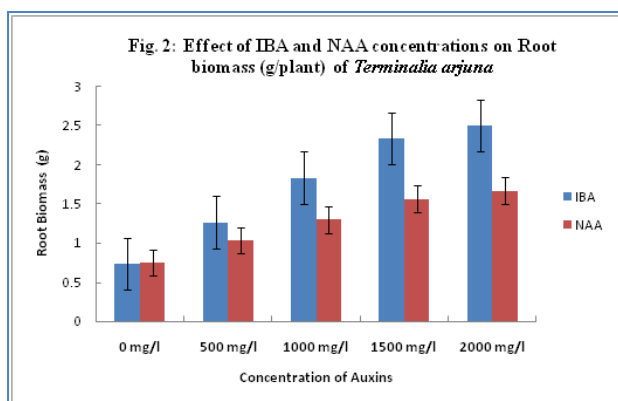


Fig-2 Effect of IBA and NAA concentrations on Root biomass (g/plant) of *Terminalia arjuna*

Conclusion

The study evolved an effective and rapid cutting propagation protocol for mass

multiplication of *Terminalia arjuna*. IBA2000mg L⁻¹ concentration is recommended to achieve maximum rooting in stem cuttings. It is also suggested to plant cuttings during spring under phyto-environmentally controlled mist chamber. If these recommendations are followed, it will be possible to produce over eighty percent of rooting in stem cuttings, which can be an asset for establishing clonal plantations of *T. arjuna* for promoting tassiar cultivation.

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Authors' contribution: The first author conducted the research experiment and collected the data. Second author prepared and revised the MS, while the third author analyzed the data and prepared the graphics.

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Conflict of Interest: None declared

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