

Original Research Article

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Effect of Plant Growth Regulators on Rooting Behavior of Stem Cuttings of *Terminalia chebula* (Retz.)

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ABSTRACT

The effect of IBA and IAA (Auxins) concentrations were examined to mass multiply plus trees of *Terminalia chebula* (Retz.) through 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 SHUATS, Allahabad. Cuttings treated with 0, 500, 1000, 1500 and 2000ppm concentrations of Indole 3-Butyric acid (IBA) and Indole 3- Acetic acid (IAA) were planted in poly bags kept under a phyto-environmentally controlled mist chamber. A limited rooting was recorded in untreated cuttings (control), while rooting of cuttings of significantly increased with an increase in concentrations of IBA and IAA. Among two auxins tested, IBA was most effective in inducing rooting, sprouting and associated traits. Out of different concentrations, 2000ppm IBA concentration was found best and achieved over 70% 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 growth regulators in influencing rooting of stem cutting and has practical implication for the development of protocol for asexual propagation and establishing clonal plantations of *Terminalia chebula* for promoting, increasing greenery and supply of timber.

Keywords

Auxins, Clonal multiplication, Multipurpose tree, Plus trees

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Introduction

Terminalia chebula (Retz.) a member of combretaceae family popularly known as chebulic myrobylan is an economically important multipurpose tree widely distributed throughout South East Asia including India, Srilanka, Bhutan, Nepal, Bangladesh, Mynmar etc. In India it is found in the Sub Himalayan region from Ravi eastwards to West Bengal and Assam, Tamil nadu, common in Madras, Mysore and southern part of South India.

(Gupta *et al.*, 2003) 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, Ayurvedic and industrial purposes. *Terminalia chebula* (Retz.) which exhibited a number of medicinal activities due to the presence of a large number of different types of phytoconstituents. The fruit of the tree

possesses diverse health benefits and has been used as traditional medicine for household remedy against various human ailments since antiquity (Nayagam and Varghese, 2015; Rangsiwong *et al.*, 2009). *T. chebula* has been extensively used in Ayurveda, Unani and Homoeopathic medicine and has become a cynosure of modern medicine. The powder of *T. chebula* fruits has been used in chronic diarrhea. It is used in nervous weakness, nervous irritability. It promotes the receiving power of five senses. It is adjuvant in hemorrhages due to its astringent nature and good for chronic cough, chorizo, sore throat as well as asthma. Also it is useful in renal calculi, dysurea, retention of urine and skin disorders with discharges like allergies, urticaria and other erythematous disorders (Bhardwaj *et al.*, 1993; Swamy *et al.*, 2002).

The fruits of *T. chebula* are rich in tannins (about 32%-34%) and its content varies with geographical distribution (Aslokar *et al.*, 1992; Williamson, 2002). The tannins of *T. chebula* are of pyrogallol (hydrolysable) type. A group of researchers found 14 components of hydrolysable tannins (gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl- β -D-glucose, 1,6-di-o-galloyl-D-glucose, casuarinin, 3,4,6-tri-o-galloyl-D-glucose, terchebulin) from *T. chebula* fruits (Singh, 2001). Other constituents include phenolics such as chebulinic acid, ellagic acid and anthraquinones. Some of the other minor constituents were polyphenols such as corilagin, galloyl glucose, punicalagin, terflavin A, maslinic acid (Husen and Pal, 2006). Besides, fructose, amino acids, succinic acid, betasitosterol, resin and purgative principle of anthraquinone are also present (Choudhary *et al.*, 2015; Gehlot *et al.*, 2014). Flavonol, glycosides, triterpenoids, coumarin conjugated with gallic acids called chebulin as well as other phenolic compounds were also

isolated (Husen and Pal, 2006; Nanda *et al.*, 1973; Yoganarasimhan, 2000). Twelve fatty acids were isolated from *T. chebula* of which palmitic acid, linoleic acid and oleic acid were main constituents (Vander Krieken *et al.*, 1993). The observed health benefits may be credited to the presence of the various phytochemicals like polyphenols, terpenes, anthocyanins, flavonoids, alkaloids and glycosides. It has immense economic importance in various industries viz., pharmaceutical, timber, paper, soap, match, food, fodder and fuel (Kumar *et al.*, 2017). The wood has high calorific value, makes excellent firewood, and produces good quality charcoal for producer gas plants. 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 (Tubtimdee and Shotipruk, 2011). Moreover, *Terminalia chebula* is a cross pollinated species exhibit a wide variability in terms of growth and foliage quality. Vegetative techniques are used as indispensable tool for mass multiplication of superior phenotypes/genotypes and producing true to type uniform plants (Thakur *et al.*, 2008). 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 *Terminalia* species and concluded as difficult to root genus (Leakey *et al.*, 1982; Mewar and Naithani, 2016). 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 (Siddiqui and Hussain, 2007; Singh, 2001; Zhang *et al.*, 1997). 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. chebula*.

Materials and Methods

The experiment was carried out in the mist chamber of Forest Nursery, College of Forestry, Allahabad, SHUATS during 2017-18. The altitude of the study area is about 98 meters above mean sea level. The cuttings of *Terminalia chebula* were prepared from selected superior genotypes from the surrounding environs of Allahabad. Plus trees were selected on the basis of their phenotypically superior stems and crown characteristics and stature in the stand. One year old branch cuttings were taken from 15±5 year- old selected trees during January. The leafless cuttings about 15±2.5 cm length and 1-2 cm in diameter having 4-5 buds were taken. The sharp Secateurs were used for preparation of cuttings. Cuttings were immediately placed in 10⁰C water to prevent desiccation. Mature cuttings were brought to laboratory and treated with 0, 500, 1000, 1500 and 2000 mg L⁻¹ concentrations of Indole 3-Butyric Acid (IBA) and Indole -3- Acetic Acid (IAA). The different IBA and IAA concentrations were prepared by dissolving the appropriate amount of IBA and IAA in 5-10 ml of methanol and volume gradually made up to 1000 ml with distilled water. The care was taken to prevent the precipitation of IBA and IAA during the process of dilution. The IBA and IAA solutions were transferred separately in to ten containers for giving treatments. The cuttings were divided in to nine groups and each group contained 27 cuttings, which were dipped in these nine solutions. The basal cut ends up to 2.5-cm of cuttings was dipped in following concentrations of IBA and IAA for 24 hours duration. The upper cut ends of treated cuttings were sealed with inert paraffin wax to

avoid desiccation through surface loss of water during rooting and also prevent fungal attack during continuous misting. The cuttings were planted immediately after treatment with IBA and IAA in the polythene bags filled with rooting medium. One-third length of the cuttings was inserted in the rooting medium and poly bag were arranged 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. The observations on rooting, sprouting, root number, root length; root biomass and shoot biomass were recorded at 90 days after planting of cuttings. Data on rooting %, 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 (Snedecor and Cochran, 1989). The significance of treatments was tested using F test.

Results and Discussion

Although, a limited rooting (10%) was achieved in untreated cuttings, but the application of auxins (IBA and IAA) significantly ($P < 0.05$) increased rooting in stem cuttings from 12% to 62%. Lower concentrations of IBA and IAA (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 (Kumar *et al.*, 2009; Siddiqui and Hussain, 2007; Singh, 2001). Synthetic auxins like IBA, IAA 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 (Khare, 2007).

The present study revealed that IBA treatments were more efficient than IAA 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 (CSIR, 2002; Jayaramkumar, 2006; Singh, 2001; Varier, 2002). IBA was quite strong auxin, while IAA is readily destroyed (Leopold, 1995). 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 (CSIR, 2002). The effectiveness of higher concentrations of IBA and IAA 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 chebula* is difficult to root species (Leakey *et al.*, 1982), as such it need higher concentrations of auxins for triggering rooting

in stem cuttings. The auxin treatments significantly increased Rooting (%), number of roots, root length, sprouting (Table 1). 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 IAA in *Terminalia* spp, a difficult to root species, whereas the lower concentration ended with callusing and little differentiation in to roots. The number of roots increased from 6.06 to 15.95 per cutting and root length from 1.87 to 8.99 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 (Luna and Kumar, 2006), 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 IAA enhanced root proliferation and as well as root number in many species (Zhang *et al.*, 1997; Kumar *et al.*, 2009). 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.

Fig.1 Effect of IBA concentrations (500ppm, 1000ppm, 1500ppm, 2000ppm on rooting (%) of *T. chebula*



Fig.2 Effect of IAA concentrations (500ppm, 1000ppm, 1500ppm, 2000ppm on rooting (%) of *T. chebula*

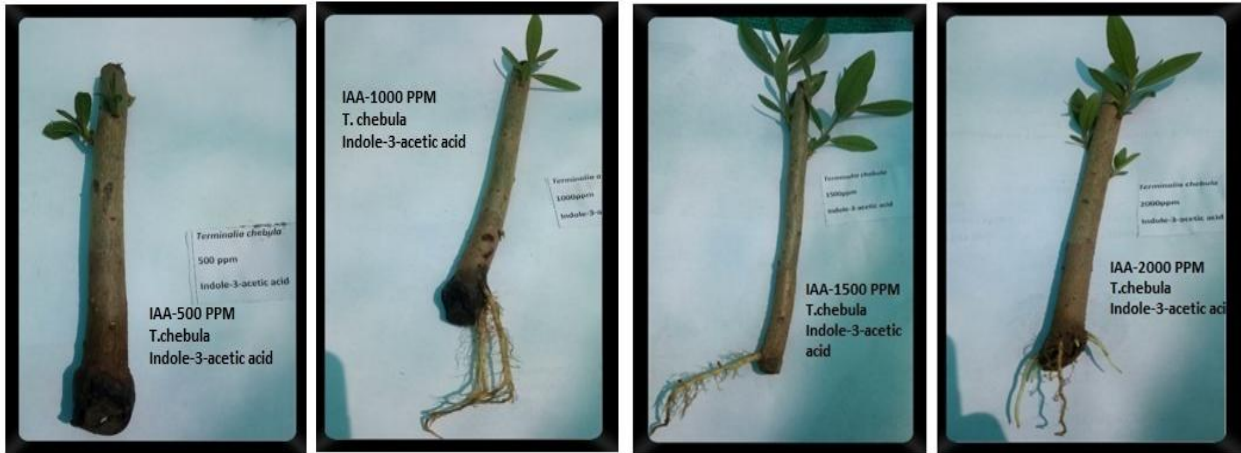


Fig.3 Effect of IBA and IAA concentrations on rooting and sprouting (%) of *T. chebula*

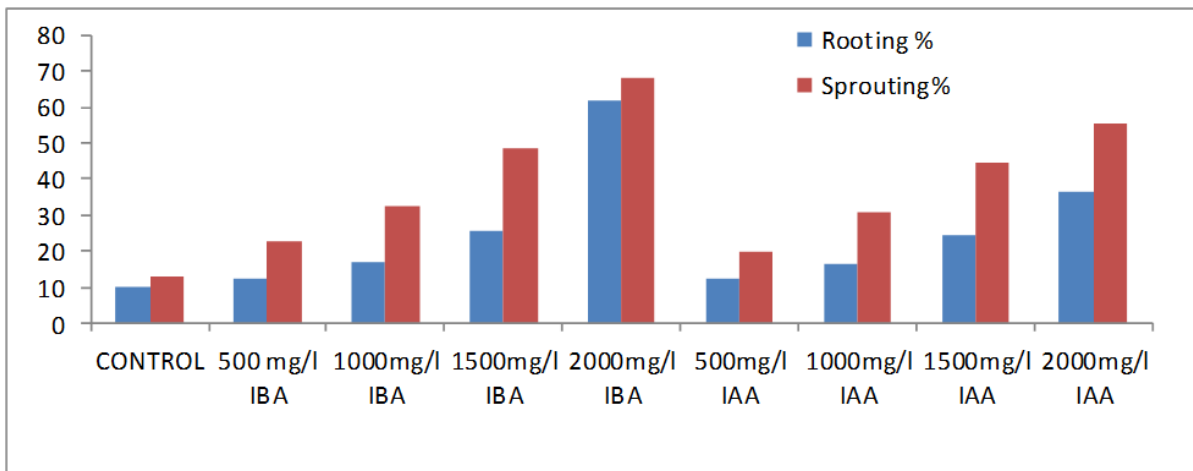


Fig.4 Effect of IBA and IAA concentrations on root biomass and shoot biomass of *T. chebula*

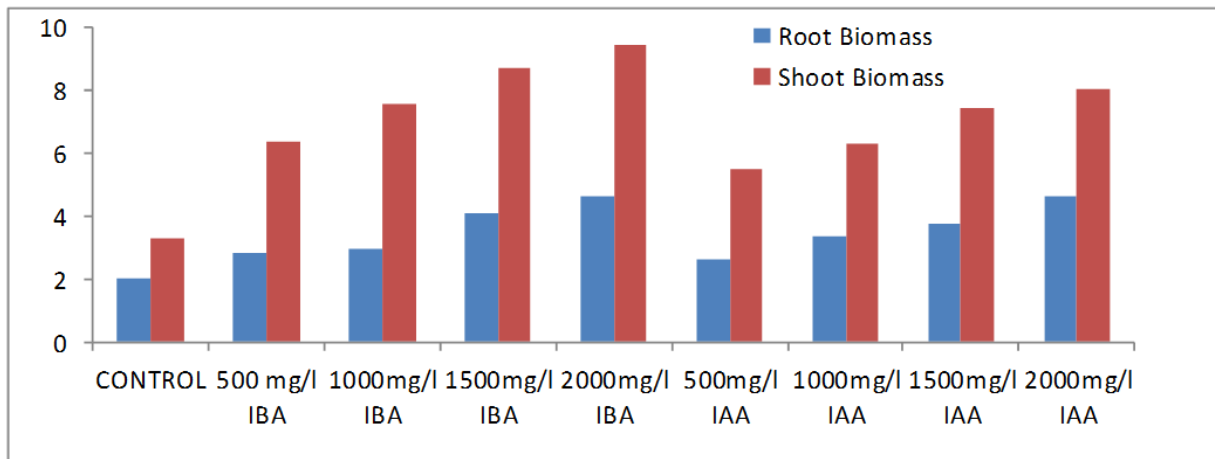


Table.1 Effect of different concentrations of IBA and IAA on rooting of stem cuttings of *Terminalia chebula*

Treatments	Rooting (%)	Root number	Root Length (cm)	Sprouting (%)	Shoot length (cm) (90 DAP)	Root Biomass (g)	Shoot Biomass (g)
Control(water)	10.01	6.06	1.87	12.80	6.09	1.98	3.26
IBA 500 mg L ⁻¹	12.66	7.38	3.32	22.79	11.79	2.82	6.34
IBA 1000 mg L ⁻¹	17.12	9.25	4.55	32.31	13.91	2.94	7.56
IBA 1500 mg L ⁻¹	25.75	12.84	7.01	48.83	17.91	4.06	8.67
IBA 2000 mg L ⁻¹	61.74	15.95	8.99	68.11	21.23	4.63	9.41
IAA 500 mg L ⁻¹	12.53	7.87	2.20	19.90	10.79	2.58	5.50
IAA 1000 mg L ⁻¹	16.35	8.14	2.71	30.71	12.80	3.35	6.30
IAA 1500 mg L ⁻¹	24.43	9.13	3.73	44.63	14.72	3.73	7.42
IAA 2000 mg L ⁻¹	36.50	10.38	6.46	55.56	17.22	4.59	8.00
F Test	S	S	S	S	S	S	S
SE(±)	1.207	0.508	0.361	0.473	0.501	0.435	0.360
CD at 0.05%	2.555	1.075	0.764	1.001	1.052	0.920	0.763

The sprouting (%) and shoot Length of stem cuttings increased with an increase in concentrations of IBA and IAA (Fig. 3 and 4). A significantly higher % of sprouting i.e. 68.11% and 55.56% were found in cuttings treated with 2000 mg L⁻¹ treatments of IBA and IAA, 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 shoot length in cuttings of *T.chebula* with higher concentration of IBA and IAA 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. Rooting in *T.chebula* with high concentrations of IBA and IAA 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. (Khare, 2007) A significantly higher shoot

biomass 9.41 (g) and 8.00 (g) was achieved in cuttings treated with 2000 mg L⁻¹ IBA and IAA, respectively (Fig. 1). Similarly, high root biomass of 4.63 g and 4.59 g was recorded in cuttings treated with 2000 mg L⁻¹ IBA and IAA, respectively (Fig. 2 and 4). 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 (Khare, 2007; Zhang *et al.*, 1997). The high root biomass is attributed to higher number of roots and more root length in cuttings treated with auxin (IBA and IAA) 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 (Zhang *et al.*, 1997; Kumar *et al.*, 2009).

The present study concludes that there were significant differences recorded between different concentrations of Auxins on rooting behavior. The study evolved an effective and rapid cutting propagation protocol for mass multiplication of *Terminalia chebula*. IBA2000mg L⁻¹ concentration is recommended to achieve maximum rooting in stem cuttings for efficient rooting during vegetative propagation in *Terminalia chebula*. It is also suggested to plant cuttings during spring season under phyto-environmentally controlled mist chamber; 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.chebula*.

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