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On vegetative propagation through stem cuttings in medicinally lucrative *Tinospora* species

Rakshe Abhijeet and Digambar Mokat

Abstract
Tinospora cordifolia (Willd.) Miers and *Tinospora sinensis* (Lour.) Merr. belong to family Menispermaceae and known by different common names such as '*Guduchi*', '*Gulvel*' and *'Giloy*'. The genus has the plethora of health benefits described in ancient scripts and traditional systems of medicine especially its stem. The drug *Guduchi* is the major ingredients of about 68 *Ayurvedic* formulations and demand of this drug has been increased up to 2000 to 5000 MT with 9.1% annual growth rate. Nevertheless, natural populations of both the species are dwindling due to indiscriminate harvesting. The huge surge in demand provides ample scope for scaling up the commercial cultivation of these plants. In the present investigation, propagation through stem cuttings was studied for developing protocol pertaining to mass multiplication. Effect of different concentrations of IBA on stem cuttings after 30 DAP and 45 DAP was studied using design RBD with four replications for both these species. The stem cuttings of *T. cordifolia* treated with 100 ppm IBA (T_1) exhibited significant rooting percentage i.e. 83.75±3.75^a. However, in *T. sinensis*, 63.75±2.39^a percent rooting was recorded in treatment control (T_0) after 45 DAP. The maximum and minimum shoot lengths were recorded in treatment T_1 (104.15±7.84 cm.) and T_0 (48.159±15.58 cm.) respectively and the maximum numbers of roots (4.25±0.25) and root length (15.42±1.09 cm) was slightly higher in treatment T_1 but significant differences in mean value were not recorded. The average minimum and maximum growth speed were reported 2.33 cm/day to 5.1 cm/day respectively in *T. cordifolia* cuttings during the present investigation. In *T. sinensis*, the maximum rooting percentage was recorded in control T_0 (63.75±2.39) followed by treatment T_1 (56.25±8.26). Increased rooting (16.25%) was observed in 30 DAP to 45 DAP. The number of sprouted shoots was higher in control T_0 (1.72±0.11). The maximum number of roots was recorded in control T_0 (5.5±0.48a). The fresh root biomass (0.85±0.00 g.) and dry root biomass (0.29±0.00 g.) were found significant in control T_0 (Table 4).

Keywords: *Tinospora cordifolia*, *Tinospora sinensis*, propagation

Introduction
The man's quest for exploration his natural surrounding for drugs has old tradition and history (Kelly, 2009, Rakshe and Mokat, 2016) [1, 2]. The Indian classic *Ayurveda* describes a vivid and detailed account of the medicinal knowledge and practices which were in vogue about 2500 years ago. The therapeutic properties of plants have been used for treating plethora of diseases under this oldest yet surviving branch of medicine. The global acceptance of our traditional system is gaining prominence thereby registering the steep rise in demand for various plants with medical properties. India is bestowed with enormous biodiversity of astonishing medicinal plants having applications and reference not only to *Ayurveda* but also for Unani, Siddha and Homeopathy (Pal and Shukla, 2003) [3]. About 47,513 plant species included 18,117 flowering plants comprising of more than 6,198 medicinal plants have been documented in India (ENVIS, 2016, Arisdason and Lakshminarasimhan, 2016, Kavita *et al.* 2016) [4, 5, 6]. The Plant *Tinospora* is one of the most important plant drug popularly known as *'Guduchi* or *Amrita*' and has wide-ranging bioactive constituents. It has been verified medicinally important plant by the traditional system as well as modern systems of medicine. Nevertheless, this plant not received considerable scientific attention and more investigation especially its propagation urgently needed. The drug *Tinospora* is the major ingredients in about 68 *Ayurvedic* formulations like '*Amritharishtam', 'Amrithadienna', 'Amrithadichoornam', 'Dhanvantaram tailam', 'Cheriya rasnadi Kashayam', 'Valiya marmagulika',* etc. (Sereena and Remashree, 2014) [7]. The innumerable properties of *Tinospora* drug are described in ancient scripts of *Ayurveda,* like *Rasayana, Agnideepana, Prameha, Jwarhara, Krimihara, Tridoshshamaka, Dahnashaka,* etc. and also confirmed its scientific validity through modern-day research (Upadhyay *et al*. 2010) [8]. The *T. cordifolia* and *T. sinensis* are both have been recommended in traditional systems of medicine from prehistoric times. *T. sinensis* is being used almost in the same way in place of the drug derived from *T. cordifolia* (Srinivasan *et al*. 2008) [9].

The plant is designated in *Ayurvedic Rasayanas* to promote longevity, anti-stress, as an adaptogen, improve or building up immunity system and body resistance against diverse types of infections (Upadhyay *et al*. 2010, Jain *et al*. 2010, Choudhary *et al*. 2013) [8, 10, 11].

In India, there are about 10,000 *Ayurvedic* drug manufacturing companies exit out of which about 750 are functional in Maharashtra (Wankhade *et al*. 2013) [12]. Further, it may be noted that there are currently about 2,50,000 registered medical practitioners under *Ayurvedic* system while about 700,000 of the modern medicine system (Ganesan *et al*. 2016) [13] which utilizes several plants as raw material obtained from the wild and cultivated source of plant origin. The *Tinospora* drug has a wide therapeutic activity therefore, it has ever-increasing demand in local as well as international market. Considering the importance of *Tinospora* in India, the National Medicinal Plant Board (NMPB) recently launched a concerted effort to address these concerns and prioritized this important species for mass multiplication (Handique, 2014) [14]. On the basis of market demand the said drug rank 29th in their volume utilization for the preparation of different Ayurvedic formulations. The demand of *T. cordifolia* ranged from 2000 to 5000 MT with annual growth registered at 9.1% (NMPB, 2012)[15]. In this context, different methods are considered as inevitable to promote commercial level cultivation of this species (Handique, 2014) [14]. Presently, forest areas are the major source of raw drug for collectors. In recent days the large scale, unrestricted anthropogenic exploitation, inadequate natural regeneration, increasing demand by the pharmaceutical industry tangled with constricted cultivation and inadequate efforts for its replacement resulted into the indiscriminate depletion of wild stock of this valuable medicinal plant (Bapat *et al*. 2008, Veeraiah and Reddy, 2012) [16, 17]. It is cleared that the demand of '*Guduchi*' drug obviously cannot be complete from wild sources any more and more focused efforts pertaining to farming are crucial. To fulfill the supply-demand gap it is essential to develop propagation and agro technique for the *Tinospora* species. However, there is complete lack of scientific approach on propagation and cultivation of *Tinospora* drug. Hence, the present study was carried out to standardize the techniques for mass multiplication.

Material and methods

a) Collection of plant materials and details of experimental site

The samples of *T. cordifolia* and *T. sinensis* (especially stem) were collected from Savitribai Phule Pune University, Pune campus and College of Forestry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli respectively during April-May 2015. The taxonomic authentication was carried out from Botanical Survey of India (BSI), Pune (MH). The voucher specimen deposited to BSI, Western Circle, Pune and received authentication reference numbers BSI/WRC/Cert./2015/AR01 and No. BSI/WRC/Cert./2015/AR 02 for *T. cordifolia* and *T. sinensis* respectively. The field experiments were conducted in medicinal plant garden that has been established in the university campus of Savitribai Phule Pune University, Pune, Maharashtra (Latitude 18° 33' 22. 5" N, Longitude 73° 49' 17. 5"E). The garden soil used for the experiment was analyzed for common agronomical parameters namely soil pH (7.3), electrical conductivity (0.30 mmhos/cm).

b) Preparation of stem cuttings

The healthy, thumb size of about 15-20 cm long cuttings having 2-3 nodes were prepared by taking the horizontal cut at the apex and slanting cut at the base. The cuttings were specifically prepared by considering position and direction of cup-like nodes (Photo plate).

c) Treatments to stem cuttings

The cuttings thus prepared were treated with 1% (w/v) Bavistin solution (Carbendazim powder), a broad spectrum fungicide, for 10 min. Further distal part of cuttings (2-3 cm) was deep into different concentration of IBA solutions for half an hour as described below; *viz* control (T_0), 100 ppm (T_1), 200 ppm (T_2), 300 ppm (T_3), 400 ppm (T_4) and 500 ppm (T_5); in order to find out rapid and efficient pretreatment for propagation. Cuttings without any treatments considered as control.

d) Plantation of cuttings and data collection

Vegetative propagation assessments were tested in the sunken bed of size 5 x 20 feet containing nursery polythene bags having size 6 x 9" filled with soil medium. The treated cuttings of both the species were planted at 3-4 cm depth in nursery bags. Before plantation, an upper end of each cutting was sealed by the single layer of wax to reduce water evaporation. Single cutting was put into each nursery bag. Twenty cuttings were used for each experimental treatment. Total eighty cuttings were evaluated under each treatment. Standard organic practices such as watering, weed control, disease and pest control were implemented during the entire study period. The care was taken to maintain the plants exclusively organically. The observations recorded after 30 DAP and 45 DAP (Days after plantation) for both the species. The total numbers of days taken for initial sprouting and rooting of cuttings were recorded. The morphological data such as the number of shoots, shoot length, number of leaves, number of roots, root length, root-shoot ratio, the diameter of the stem, petiole length, leaf length and leaf width were recorded. The yield of biomass was determined by the method described by Gupta *et al*. (1998) [18].

e) Statistical analysis of data

The experiment was carried out following RBD (Randomized Block Design) with four replicates (n =4). Data were analyzed by Analysis of variance (ANOVA) to detect the significant difference between means. The means differing significantly were compared using Duncan's (1955) [19] multiple range test (DMRT) at the 5% probability level using the software SPSS 16.0. Variability in data was expressed as a mean± standard error.

Results

i) Effect of IBA on *T. cordifolia* stem cuttings 30 DAP

The stem cuttings of *T. cordifolia* treated with 100 ppm IBA (T_1) exhibited highest rooting percentage (67.50±5.95) followed by treatment T_2 (46.25±3.75). First sprouting was recorded 11 DAP. Nevertheless, the number of the sprouted shoots was higher in treatment T_4 (1.45±0.05). The maximum shoot length was attained by treatment T_1 (28.92±5.20 cm.) but significant differences in mean shoot length were not recorded. The diameter of the sprouted shoot was also higher in treatment T_1 (2.54±0.14 mm.). The number of leaves, petiole length, leaf width as well as leaf length was slightly higher in treatment T_1 but significant differences in the mean value of all these parameters were not recorded (Table 1).

ii)Effect of IBA on *T. cordifolia* stem cuttings 45 DAP

The maximum rooting observed in *T. cordifolia* stem cuttings treated with 100 ppm IBA (T_1) i.e. 83.75±3.75. Enhanced

rooting (16.25%) was reported in 30 DAP to 45 DAP. The maximum and minimum shoot lengths were recorded in treatment T_1 (104.15±7.84 cm.) and T_0 (48.159±15.58 cm.) respectively. Shoot length mean value of treatment T_2, T_3, and T_5 was found significant at 95% confidence intervals.

The maximum numbers of roots (4.25±0.25) and root length (15.42±1.09 cm) was slightly higher in treatment T_1 but significant differences in mean value were not recorded. The diameter of a sprouted shoot was also higher in treatment T_1 (2.71±0.11 mm). Maximum fresh shoot biomass recorded in T_1 (14.57±0.30 g.) followed by T_3 (14.05±0.30 g.). The fresh root biomass, total fresh biomass, dry shoot biomass, dry root biomass and total dry biomass was also higher in treatment T_1 (Table 2). The average minimum and maximum growth speed were reported to be 2.33 cm/day to 5.1 cm/day respectively in *T. cordifolia* cuttings during the present investigation.

iii) Effect of IBA pre-treatment on *T. sinensis* stem cuttings 30 DAP

The effects of IBA on growth parameters of *T. sinensis* propagated through stem cuttings did not reveal any significant difference 30 DAPS. The maximum rooting was recorded in control i.e. T_0 (47.5±1.44). First sprouting was observed 10 DAP. The maximum numbers of sprouted shoots were observed in treatment T_0 (1.72±0.11). Nevertheless, treatment T_0 (3.17±0.05 mm.) and T_1 (3.33±0.09 mm.) were found significant for increasing diameter of a sprouted shoot (Table 3).

iv) Effect of IBA pre-treatment on *T. sinensis* stem cuttings 45 DAP

The maximum rooting percentage was recorded in control T0 (63.75±2.39) followed by treatment T_1 (56.25±8.26). Increased rooting (16.25%) was observed in 30 DAP to 45 DAP. The number of sprouted shoots was higher in control T_0 (1.72±0.11). The maximum number of roots was recorded in control T_0 (5.5±0.48a). The maximum diameter of the newly sprouted shoot was reported in control T_0 (3.66±0.16 mm.), followed by T_1 (3.91±0.05 mm.) and T_5 (3.68±0.46 mm) respectively. The maximum numbers of leaves were recorded in treatment T_3 (10.5±1.00), while leaf width was higher in control T_0 (12.73±0.17). The fresh root biomass (0.85±0.00 g.) and dry root biomass (0.29 ±0.00 g.) were found significant in control T_0 (Table 4).

Table 1: Effect of IBA pre-treatment on *T. cordifolia* stem cuttings 30 DAP

Parameters			Pre-treatments			
	T_0	T_1	T_2	T_3	T_4	T_5
Rooting percentage	37.50±3.23^b	67.50±5.95^a	46.25±3.75^b	42.50±1.44^b	35.00±3.54^b	42.50±2.50^b
No. of shoots	1.16±0.05^b	1.25±0.09^{ab}	1.20±0.08^{ab}	1.40±0.08^{ab}	1.45±0.05^a	1.25±0.09^{ab}
Shoot length (cm.)	12.31±7.08^a	28.92±5.20^a	20.77±4.60^a	23.55±7.74^a	23.27±2.71^a	17.85±3.29^a
Diameter of stem (mm.) (Sprouted shoot)	2.09±0.22^{ab}	2.54±0.14^a	2.02±0.16^{ab}	2.03±0.07^{ab}	2.06±0.02^b	2.16±0.45^{ab}
No. of leaves	3.30±0.51^a	3.85±0.15^a	3.00±0.24^a	3.50±0.50^a	3.45±0.17^a	3.10±0.17^a
Petiole length (cm.)	3.71±1.16^a	5.58±0.22^a	4.38±0.77^a	5.36±0.76^a	5.57±0.45^a	4.98±0.58^a
Leaf length (cm.)	4.49±1.59^a	6.52±0.44^a	6.04±0.79^a	6.30±1.04^a	6.85±0.36^a	5.10±0.48^a
Leaf width (cm.)	3.97±1.33^a	5.99±0.44^a	5.27±0.73^a	5.46±1.15^a	5.49±0.42^a	4.06±0.50^a

Values mentioned in this table are a mean ± standard error (SE) with four replications. The means followed by the same letters within rows are not significantly different at the 5% level (DMRT).

Table 2: Effect of IBA pre-treatment on *T. cordifolia* stem cuttings 45 DAP

Parameters			Pre-treatments			
	T_0	T_1	T_2	T_3	T_4	T_5
Rooting percentage	47.50±3.23^b	83.75±3.75^a	55.00±4.08^b	52.50±3.23^b	46.25±3.15^b	55.00±7.36^b
No. of shoots	1.16±0.05^b	1.3±0.12^{ab}	1.3±0.12^{ab}	1.6±0.14^a	1.5±0.05^a	1.3±0.05^{ab}
Shoot length (cm.)	48.16±15.58^b	104.15±7.84^a	84.70±7.91^a	103.35±11.23^a	79.60±5.41^{ab}	90.63±14.33^a
No. of roots	3.75±0.48^a	4.25±0.25^a	3.75±0.48^a	3.5±0.29^a	4.00±0.41^a	4.00±0.41^a
Root length (cm.)	13.02±0.68^a	15.42±1.09^a	14.75±1.40^a	13.72±1.19^a	12.02±1.20^a	14.82±0.98^a
Root-shoot ratio	0.43±0.17a	0.15±0.02^b	0.17±0.02^b	0.14±0.02^b	0.15±0.02^b	0.18±0.04^b
Diameter of stem (mm.) (Sprouted shoot)	2.03±0.21^b	2.71±0.11^a	2.09±0.17^b	2.06±0.06^b	2.29±0.06^{ab}	2.39±0.08^{ab}
No. of leaves	8.75±0.89^a	12.35±1.15^a	6.8±0.35^a	12.1±1.12^a	8.35±0.45^a	8.8±0.77^a
Petiole length (cm.)	7.33±1.76^b	11.74±1.130^a	10.09±0.60^{ab}	11.23±0.56^a	10.66±0.36^a	10.46±0.28^a
Leaf length (cm.)	7.85±1.54^b	11.97±0.23^a	11.69±0.19^a	12.00±0.11^a	12.07±0.16^a	11.41±0.14^a
Leaf width (cm.)	7.17±1.43^b	11.54±0.26^a	10.24±0.26^a	10.84±0.36^a	10.74±0.19^a	10.92±0.36^a
Fresh shoot biomass (gm.)	6.42±0.21^d	14.57±0.30^a	11.35±0.34^{ab}	14.05±0.30^a	10.50±0.23^c	11.90±0.11^b
Fresh root biomass (gm.)	0.55±0.03^b	0.73±0.02^a	0.71±0.00^a	0.59±0.01^b	0.54±0.00^b	0.42±0.01^c
Total fresh biomass (gm.)	6.97±0.24^d	15.30±0.28^a	12.06±0.34^b	14.64±0.30^a	11.03±0.23^c	12.32±0.12^b
Dry shoot biomass (gm.)	0.95±0.03^d	2.19±0.05^a	1.67±0.04^{ab}	2.11±0.07^a	1.54±0.03^c	1.75±0.02^b
Dry root biomass (gm.)	0.19±0.01^b	0.24±0.01^a	0.25±0.02^a	0.20±0.00^b	0.18±0.00^b	0.14±0.00^c
Total dry biomass (gm.)	1.13±0.05^d	2.44±0.04^a	1.93±0.03^b	2.31±0.07^a	1.72±0.03^c	1.90±0.03^b

Values mentioned in this table are a mean ± standard error (SE) with four replications. The means followed by the same letters within rows are not significantly different at the 5% level (DMRT).

Table 3: Effect of IBA pre-treatment on *T. sinensis* stem cuttings 30 DAP

Parameters			Pre-treatments			
	T_0	T_1	T_2	T_3	T_4	T_5
Rooting percentage	47.50±1.44^a	37.50±6.29^a	37.50±7.77^a	40.00±5.40^a	35.00±4.56^a	38.75 ±3.75^a
No. of shoots	1.72±0.11^a	1.25±0.09^b	1.25±0.12^b	1.35±0.09^b	1.40±0.08^{ab}	1.40±0.14^{ab}
Shoot length (cm.)	24.96±5.52^a	26.79±6.24^a	20.47±7.22^a	23.12±10.90^a	24.21±4.07^a	16.83 ±6.61^a

Diameter of stem (mm.) (Sprouted shoot)	3.17±0.05[a]	3.33±0.09[a]	2.90±0.16[ab]	2.84±0.12[ab]	2.92±0.12[ab]	3.14±0.18[a]
No. of leaves	3.19±0.45[a]	3.45±0.38[a]	3.10±0.46[a]	3.30±0.50[a]	3.40±0.24[a]	3.25±0.43[a]
Petiole length (cm.)	3.78±0.68[a]	5.57±0.48[a]	4.03±0.98[a]	4.92±0.79[a]	5.40±0.39[a]	5.04± 0.53[a]
Leaf length (cm.)	5.55±1.04[a]	6.57±0.41[a]	5.75±1.08[a]	5.35±1.06[a]	6.53±0.62[a]	5.45±0.74[a]
Leaf width (cm.)	4.61±0.96[a]	5.72±0.35[a]	4.89±0.83[a]	4.35±1.13[a]	4.86±0.54[a]	4.22±0.69[a]

Values mentioned in this table are mean ± standard error (SE) with four replications. The means followed by the same letters within rows are not significantly different at the 5% level (DMRT).

Table 4: Effect of IBA pre-treatment on *T. sinensis* stem cuttings 45 DAP

Characters			**Pre-treatments**			
	T0	**T1**	**T2**	**T3**	**T4**	**T5**
Rooting percentage	63.75±2.39[a]	56.25±8.26[a]	53.75±4.27[a]	53.75±5.54[a]	47.5± 4.33[a]	50 4.08[a]
No. of shoots	1.63±0.11[a]	1.30±0.12[a]	1.30±0.12[a]	1.40±0.08[a]	1.45± 0.05[a]	1.40 ± 0.14[a]
Shoot length (cm.)	91.03±3.98[a]	98.30±3.30[a]	85.45±7.37[a]	97.75±7.68[a]	83.20± 10.43[a]	94.42± 14.70[a]
No. of roots	5.50±0.48[a]	4.25±0.25[b]	4.5±0.9[b]	3.25±0.25[c]	4.75± 0.25[b]	4.5 ± 0.29[b]
Root length (cm.)	16.37±0.34[a]	18.42±3.73[a]	18.17±3.15[a]	14.50±1.36[a]	11.75± 1.45[a]	16.10± 0.81[a]
Root-shoot ratio	0.18±0.01[a]	0.19±0.05[a]	0.22±0.05[a]	0.16±0.02[a]	0.14± 0.02[a]	0.18 ± 0.03[a]
Diameter of stem (mm.) (Sprouted shoot)	3.66±0.16[a]	3.91±0.05[a]	3.19±0.17[ab]	3.24±0.10[ab]	3.08± 0.13[ab]	3.68 ± 0.46[a]
No. of leaves	8.81±0.00[b]	9.10±1.00[ab]	7.05±0.34[b]	10.5±1.00[a]	8.15± 0.51[b]	9.15 0.69[ab]
Petiole length (cm.)	11.43±0.61[a]	11.61±0.70[a]	10.93±0.58[a]	11.36±0.88[a]	10.16± 0.50[a]	10.4 ± 0.28[a]
Leaf length (cm.)	11.65±0.45[a]	11.67±0.35[a]	11.63±0.08[a]	12.08±0.33[a]	12.21± 0.34[a]	11.50± 0.36[a]
Leaf width (cm.)	12.73±0.17[a]	11.86±0.12[ab]	10.73±0.33b[c]	10.81±0.43[bc]	10.60± 0.59[c]	10.91± 0.42[bc]
Fresh shoot biomass (gm.)	20.25±1.2[a]	22.18±0.01[a]	15.77±1.71[a]	22.03±2.82[a]	18.48± 4.25[a]	16.56± 1.10[a]
Fresh root biomass (gm.)	0.85±0.00[a]	0.62±0.00[d]	0.83±0.00[a]	0.71±0.01[b]	0.65 ± 0.00[c]	0.51 ± 0.01[e]
Total fresh biomass (gm.)	21.09±1.27[a]	22.80±0.01[a]	16.60±1.72[a]	22.74±2.82[a]	19.13 ± 4.25[a]	17.07± 1.08[a]
Dry shoot biomass (gm.)	4.09±0.29[a]	3.96±0.53[a]	3.23±0.27[a]	4.42±0.56[a]	4.22 ± 1.33[a]	3.11 ± 0.01[a]
Dry root biomass (gm.)	0.29±0.00[a]	0.20±0.00[d]	0.27±0.00[b]	0.23±0.00[c]	0.24 ± 0.00[c]	0.19 ± 0.00[e]
Total dry biomass (gm.)	4.38±0.29[a]	4.16±0.53[a]	3.50±0.27[a]	4.65±0.55[a]	4.45 ± 1.33[a]	3.29 ± 0.01[a]

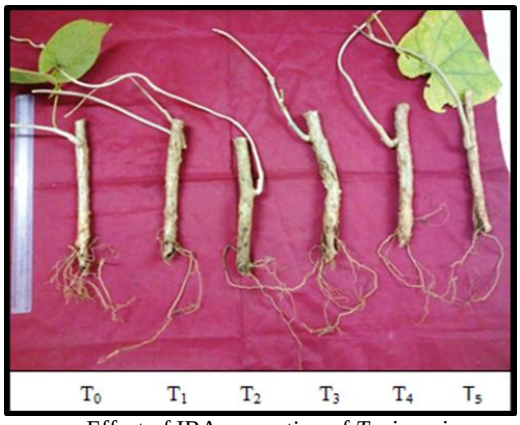
Values mentioned in this table are mean ± standard error (SE) with four replications. The means followed by the same letters within rows are not significantly different at the 5% level (DMRT).

Photo plate

Nursery bags in sunken bed | Plantation of cuttings | New sprouted shoot

Cuttings with cup shape node | *T. sinensis* 30 DAP | *T. cordifolia* 45 DAP

T. sinensis 45 DAP



Effect of IBA on rooting of *T. sinensis*

Discussion

It has been evident that the auxin has been involved in the different physiological processes such as differentiation, cell elongation in plant tissue. The exogenous application of auxin has been a powerful tool in many forestry plant species to stimulate adventitious rooting (Hartmann *et al*. 1997) [20]. The presence of natural auxin in plants (IAA) is responsible for induction of rooting in several plant species, but the application of synthetic analog like IBA was more effective than IAA. Its great influence on rooting and growth performance in different kinds of plants were found to be due to its greater stability within tissue and during storage (Blythe *et al.* 2007, Ling *et al.* 2013) [21, 22].

In the present investigation, it was observed that the stem cuttings of *T. cordifolia* treated with 100 ppm IBA (T_1) exhibited significant rooting percentage. The all rooting and sprouting process were completed within 45 DAP. There after no rooting was reported. The overall results showed that, although sprouting time was considerably higher in *T. cordifolia* but shoot elongation was enhanced greatly after sprouting. The results of present investigation pertaining *T. cordifolia* corroborated well with the results reported by Mishra *et al.* (2010) [23]. Mature vine cuttings of *T. cordifolia* which were treated with 100 ppm of IBA significantly increased sprouting, rooting and root length when compared to other auxin as well as control. Maximum plant length (364.73 cm) and number of branches (3.42) were recorded in same IBA treatments after three months of the plantation. Rao *et al*. (2000) [24] also carried out the study on macro-propagation of *T. cordifolia*. The study revealed that IBA pre-treatment to the cutting of *T. cordifolia* showed the better rooting response (86%) with 200 and 300 ppm concentrations. However, a study by Warrier *et al*. (2007) [25] revealed that *T. cordifolia* best rooting performance (96%) without application of growth regulator hormone. Thus the present study showed deviation from above findings.

In *T. sinensis*, the stem cuttings treated with different concentration of IBA failed to display significant results, thereby recording better performance in untreated or control. The average minimum and maximum growth was 3.93 cm/day to 5.17 cm/day respectively during the time of investigation in *T. sinensis* which was found slightly higher than *T. cordifolia*. The initiation of the first sprout was observed on 10 DAP. Cuttings without hormone treatment found to enhance diameter of the sprouted shoot, leaf width, numbers of roots, fresh and dry root biomass. The treated cuttings of *T. sinensis* displayed poor performance. Thus it might be concluded that IBA treatment could have an inhibitory effect on initiation of rooting though it acts as a stimulant of rooting.

Sun and Bassuk (1991) [26] found that IBA inhibits shoot growth in rooted cuttings of rose with 600 mg/liter of IBA. The IBA treatment also stimulated ethylene synthesis. Auxin-induced promotion of ethylene production associated with increased ACC synthase level which was inversely correlated the bud break of cuttings. Kim and Mulkey (1997) [27] also found similar phenomena in intact primary roots of maize (*Zea mays*). Khajehpour *et al*. (2014) [28] investigated the effect of different concentrations of IBA on stem cuttings of olive (*Olea europaea* var. *manzanilla*) [30] and confirmed that cuttings treated with 3000 ppm IBA displayed maximum rooting (84.5%). However, excessive concentration of IBA tends to decrease the rooting percentage. This finding was endorsed with the present study. Ahmed *et al*. (2003) [29] also reported the total inhibitory effect of IBA on rooting of hardwood cuttings of Peach rootstocks treated with 4000 ppm IBA.

In nursery practices, some observations were taken along with growth performance to get quality seedlings which reduce the risk of damage to the plants. The additional recorded observations and results along with few suggestions are provided. The care was taken to prevent the water logging and compactness of soil in polythene bags. It was found that cuttings of both *Tinospora* species were susceptible to use of excess water resulting into decaying of stem cuttings. Therefore seedlings were watered at interval 4-5 days. On the basis of this observation, it was recommended that, 1. Need to use well-drained soil and organic farming conditions for propagation or cultivation of *T. cordifolia* and *T. sinensis.* 2. Heavy damage by Spodoptera and thrips to tender leaves and stem were observed in seedlings those have been propagated through seeds. Thus propagation through cuttings was faster, safe and inexpensive route of multiplication.

Conclusion

The stem cuttings of *T. cordifolia* treated with 100 ppm IBA exhibited significant sprouting; rooting percentage; total fresh as well as dry biomass however, in *T. sinensis*, cuttings reported similar performance without application of IBA. The cuttings of *T. sinensis* displayed poor performance to IBA treatment which may be due to it's an inhibitory effect. Both species of *Tinospora* are currently facing the dearth of biomass required for commercial utilization and therefore large-scale propagation can be taken up which indeed required low capital investment. The quality planting materials (QPMs) of these species can be easily produced throughout the year for large scale plantation. This study has significant implications as the government has recently established 'All India Institute of Ayurveda' for promoting research pertaining to our ancient tradition. Further young entrepreneurs can take up commercial plantation of *Tinospora* under start-up India scheme.

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