Original Research Article

INFLUENCE OF HORMONAL TREATMENTS ON STEM CUTTINGS THROUGH MULTIPLICATION IN THERAPEUTICALLY WORTHWHILE PLANT *TINOSPORA CORDIFOLIA*

Abstract

India is a leader in the usage of the ancient ayurveda system and is blessed with a vast richness of medicinal herbs. Over 68 Ayurveda formulations contain the medicine Guduchi as one of its main constituents, and need for this medication has climbed from 3000 to 6000 MT with a 9.1% yearly increase. Unfortunately, uncontrolled harvesting is causing the natural populations to decline. There is plenty of room to expand the commercial production of these plants given the enormous increase in demand. For the purpose of creating a technique for rapid multiplication, propagation by stem cuttings was examined in the current experiment. For this species, the impact of various GA3 concentrations on stem cuttings during 30 DAP and 45 DAP was investigated using the randomized block design (RBD) with four replications. The 200 ppm GA3-treated stem cuttings of *T. cordifolia* (T1) showed considerable rooting (85.75%).The highest shoot lengths at 45 days after planting were measured for treatments T1 (average 106.15 cm). Maximum root numbers were measured for treatments T1 (6.25cm) and treatment T1 had slightly longer roots (17.42 cm), but there were no statistical differences in mean values. Throughout the course of the experiment, *T. cordifolia* cuttings' average lowest and maximum growth rates ranged from 4.33 cm/day to 7.1 cm/day. The results of this study showed that the optimal propagation approach for T. cordifolia for commercial multiplication is influence of hormonal treatments on stem cuttings.

Keywords: *Tinospora cordifolia*, Cuttings, propagation, GA3

Introduction

India is a leader in the usage of the ancient ayurveda system and is blessed with a vast richness of medicinal herbs. *Tinospora cordifolia* (Willd.) Miersis amembers of the Menispermaceae family and go by a variety of popular names, including "Guduchi," "Gulvel," and "Giloy." The plant in this genus, particularly their stem, provide a wide range of health advantages that have been mentioned in ancient texts and conventional medical practises.This plant speciesdeciduous climbing shrub may be found all over, especially in tropical regions, and can be found in India climbing to an altitude of 300 m as well as in some regions of Sri Lanka, Bangladesh, and China (Mittal et al. 2014). A desire to balance the crisis has been made possible by the rapid loss of natural resources of medicinal plants in general, and in particular giloy, together with an exponential spike in demand (Sinha and Sharma, 2015). For collectors, forests are currently their main supply of plant material unprocessed drugs.

The resource of this important medicinal plant has recently been recklessly depleted due to widespread, unrestricted anthropogenic exploitation, insufficient natural regeneration, rising demand from the pharmaceutical industry, conflicting with constrained cultivation, and insufficient efforts to replace the plant stock (Veeraiah and Reddy, 2012). Increased availability with correct farming techniques to easily and sustainably fulfil the rising demand is needed. Meeting the huge demand in the ayurvedic pharmaceutical sectors is only achievable by providing enough high-quality planting material at a viable cost for agricultural purposes. The medicine *Tinospora* has a wide range of therapeutic action, which contributes to its rising demand on both the domestic and global markets(Singh *et al*., 2004).

The National Medicinal Plant Board (NMPB) of India has lately undertaken a concentrated effort to resolve these problems and selected this significant species for mass multiplication due to Tinospora's significance in India (Handique, 2014). The aforementioned medicine ranks 29th in terms of volume utilised for the creation of various Ayurveda formulations based on market demand. *T. cordifolia* demand increased annually by 9.1% and ranged from 2000 to 5000 MT (NMPB, 2012). In this context, many techniques are seen as necessary to promote the commercial production of this species (Handique, 2014). The primary issues in giloy are the extremely low viability of seeds, poor seed set, and poor seed germination (Mittal *et al.* 2014).Thus, propagation by cuttings is a simple and affordable process. Similar to this, semi-hard wood cutting was the most popular cutting style for commercial multiplication (Yogeshwar*et al.* 2010). It is evident that the demand for the narcotic "Guduchi" cannot be satisfied entirely by wild sources today, hence increased and targeted agricultural activities are essential. For the *Tinosporacordifolia* plant, it is crucial to develop reproduction and agricultural techniques in order to close the supply-demand imbalance. Nevertheless, there is no scientific method for growing or propagating the *T.cordifolia*. So, the goal of the current study was to standardise the propagation of this plant using cutting for mass multiplication.

MATERIALS AND METHODS

Collection

Stem samples of one year old *T. cordifolia*(Fig.1) were gathered in January 2022 from the campus of Tamil Nadu Agricultural University in Coimbatore. The field studies were carried out at a medicinal plant garden that was constructed on the grounds of the medical school (18° 33' 22.5" N, 73° 49' 17.5" E).

Preparation

By making a vertical cut at the top and a sloping downward cut at the base, healthy cuttings with 2-3 nodes and a length of 10-15 cm were created (fig.2). The positioning and orientation of the cup-like nodes was deliberately taken into account when creating the cuts.

Figure 1. Selected plant for cuttings

Figure 2. cuttings with 2-3 nodes and a length of 10-15 cm

Treatments

The clippings were then treated for five minutes with a 1% (w/v) solution of the broad-spectrum fungicide bavistin for reduce the infection of pathogens. To determine a quick and effective pretreatment for growth, cuttings' farther distal parts (2–3 cm) were deep-soaked in GA3 solutions at various concentrations for 30 minutes as detailed below: control (T0), 200 ppm (T1), 400 ppm (T2), 600 ppm (T3), 800 ppm (T4), and 1000 ppm (T5). Cuttings only without being dipped were another control (T6).

Plantation of cuttings and data collection

Cuttings were placed vertically in a sunken bed of 5 x 20 feet filled with nursery polythene bags containing soil material measuring 6 x 9" in size. One treated cutting were inserted into each nursery bags at a depth of three to four centimetres and kept in shade net. To prevent water from evaporating before planting, the top section of each cut was coated with a single coating of wax. Each nursery bag included one stem. For each treatment, 20 cuttings were employed in four random blocks. Eighty cuttings in total were assessed for each treatment (control (T0), 200 ppm (T1), 400 ppm (T2), 600 ppm (T3), 800 ppm (T4), and

1000 ppm (T5)). The treated cutting was kept in medicinal department nursery under controlled condition of 80% humidity and 20-23^{0}c temperature. For the duration of the trial, common organic procedures including watering, weeding, disease, and pest management were carried out.The plants were given meticulous organic maintenance. Observations were made for both species at 30 and 45 days following planting. The morphological information, which included the amount of new shoots produced, stem length, leaf, roots, and root-to-shoot ratio, as well as the thickness of the stems, petiole length, leaf length, and leaf breadth, was noted. The Gupta *et al*.(1998) approach was used to calculate the biomass yield.

Data analysis using statistics

RBD (Randomized Block Design) was used to conduct the experiment with four blocks and 10 replicates for each treatment (n = 204). Analysis of variance (ANOVA) was used to evaluate the data in order to find significant mean differences. Using the statistical analysis programme SPSS 16.0, the means that differed substantially were examined utilizing Duncan's (1955) multi-ranges test (DMRT) at 5% probability value. The mean and standard error were used to describe data variability.

Results

a) GA3's impact on 30-day-old *T. cordifolia* stems

The greatest rooting percent (69.50) was seen in *T. cordifolia* stem cuttings treated with 200 ppm GA3 (T1), followed by T2 (48.25). Treatment T4 had the larger number of sprouting shoots (3.45). Treatment T1 reached the longest shoot length possible (30.92cm), however there were no measurable variations in mean shoot length. Moreover, treatment T1 had a larger sprouting shoot diameter (4.54mm). Treatment T1 produced new shoots with more leaves, longer petioles, wider leaves, and longer leaves overall, but there were no significantdifference any of these metrics (Table 1).

Table 1. Effect of pre-treatment *T. cordifolia* stem cuttings with varying concentrations of GA3 on eight parameters on growth at 30 DAP

Parameters	Treatments					
	T 0	**T 1**	**T 2**	**T 3**	**T 4**	**T 5**
RP	39.5±3.23^{b}	69.5±5.95^{a}	48.25±3.75^{b}	44.5±1.44^{b}	37.00±3.54^{b}	44.50±2.50^{b}
NS	3.16±0.05^{b}	3.25±0.09^{ab}	3.20±0.08^{ab}	3.40±0.08^{ab}	3.45±0.05^{a}	3.25±0.09^{ab}
SL	14.31±7.08^{a}	30.92±5.20^{a}	22.77±4.60^{a}	25.55±7.74^{a}	25.27±2.71^{a}	19.85±3.29^{a}
DS	4.09±0.22^{ab}	4.54±0.14^{a}	4.02±0.16^{ab}	4.03±0.07^{ab}	4.06±0.02^{b}	4.16±0.45^{ab}
NL	5.30±0.51^{a}	5.85±0.15^{a}	5.00±0.24^{a}	5.50±0.50^{a}	5.45±0.17^{a}	5.10±0.17^{a}

PL	5.71 ± 1.16^{a}	7.58 ± 0.22^{a}	6.38 ± 0.77^{a}	7.36 ± 0.76^{a}	7.57 ± 0.45^{a}	6.98 ± 0.58^{a}
LL	6.49 ± 1.59^{a}	8.52 ± 0.44^{a}	8.04 ± 0.79^{a}	8.30 ± 1.04^{a}	8.85 ± 0.36^{a}	7.10 ± 0.48^{a}
LW	5.97 ± 1.33^{a}	7.99 ± 0.44^{a}	7.27 ± 0.73^{a}	7.46 ± 1.15^{a}	7.49 ± 0.42^{a}	6.06 ± 0.50^{a}

(RP- Rootingpercentage, NS-NumberofShoots, SL- ShootLength(cm.), DS-Diameterofstem(mm.)(Sproutedshoot), NL- Numberofleaves, PL- Petiolelength(cm.), LL-Leaflength(cm.), LW- Leafwidth(cm.).The numbers in this table represent the mean and standard error (SE) based on four replications. At the 5% level, there is no discernible difference between the means that are followed by identical letters within rows (DMRT).

b) GA3's impact on 45-day-old *T. cordifolia* stems

The highest rooting rate, or 85.75%, was seen in *T. cordifolia* stems supplied with 200 ppm GA3 (T1). At 30 DAP to 45 DAP, there were current study of enhanced rooting (18.25%). Although treatment T1 had somewhat more roots overall (6.25 cm) and longer roots (17.42cm), there were no observable variations in mean values that were statistically significant.106.15cm and 50.159 cm were the greatest and shortest shoot lengths measured in treatments T1 and T0, respectively. At 97% confidence intervals, the mean value of shoot length for treatments T2, T3, and T5 was shown to be significant.A sprouting shoot's diameter was likewise larger in treatment T1 (4.71mm). T1 reported the highest fresh shoot biomass (16.57 g), followed by T3 (16.05g). Treatment T1 also had larger levels of new root biomass, maximum fresh biomass, shoot dry biomass, root dry biomass, and dry matter biomass (Table 2). During the current experiment, *T. cordifolia* cuttings' average lowest and highest growth rates were found to be 4.33 cm/day and 7.1 cm/day, respectively.

Table 2. *T. cordifolia* Pre-treatment with GA3 has an impact on stem cuttings at 45DAP.

Parameters	Pre-treatments					
	T 0	**T 1**	**T 2**	**T 3**	**T 4**	**T 5**
RP	49.5 ± 3.23^{b}	85.75 ± 3.75^{a}	57 ± 4.08^{b}	54.5 ± 3.23^{b}	48.25 ± 3.15^{b}	57.00 ± 7.36^{b}
NS	3.16 ± 0.05^{b}	3.30 ± 0.12^{ab}	3.30 ± 0.12^{ab}	3.60 ± 0.14^{a}	3.50 ± 0.05^{a}	3.30 ± 0.05^{ab}
SLt	50.16 ± 15.58^{b}	106.15 ± 7.84^{a}	86.7 ± 7.91^{a}	105.35 ± 11.23^{a}	81.6 ± 5.41^{ab}	92.63 ± 14.33^{a}
DS	5.75 ± 0.48^{a}	6.25 ± 0.25^{a}	5.75 ± 0.48^{a}	5.50 ± 0.29^{a}	6.00 ± 0.41^{a}	6.00 ± 0.41^{a}
NL	15.02 ± 0.68^{a}	17.42 ± 1.09^{a}	16.75 ± 1.40^{a}	15.72 ± 1.19^{a}	14.02 ± 1.20^{a}	16.82 ± 0.98^{a}
PL	2.43 ± 0.17^{a}	2.15 ± 0.02^{b}	2.17 ± 0.02^{b}	2.14 ± 0.02^{b}	2.15 ± 0.02^{b}	2.18 ± 0.04^{b}
LL	4.03 ± 0.21^{b}	4.71 ± 0.11^{a}	4.09 ± 0.17^{b}	4.06 ± 0.06^{b}	4.29 ± 0.06^{ab}	4.39 ± 0.08^{ab}
LW	10.75 ± 0.89^{a}	14.35 ± 1.15^{a}	8.8 ± 0.35^{a}	14.10 ± 1.12^{a}	10.35 ± 0.45^{a}	10.8 ± 0.77^{a}
RP	9.33 ± 1.76^{b}	13.74 ± 1.130^{a}	12.09 ± 0.60^{ab}	13.23 ± 0.56^{a}	12.66 ± 0.36^{a}	12.46 ± 0.28^{a}

NS	9.85±1.54[b]	13.97±0.23[a]	13.69±0.19[a]	14.00±0.11[a]	14.07±0.16[a]	13.41±0.14[a]
SL	9.17±1.43[b]	13.54±0.26[a]	12.24±0.26[a]	12.84±0.36[a]	12.74±0.19[a]	12.92±0.36[a]
FSB	8.42±0.21[d]	16.57±0.30[a]	13.35±0.34[ab]	16.05±0.30[a]	12.5±0.23[c]	13.9±0.11[b]
FRB	2.55±0.03[b]	2.73±0.02[a]	2.71±0.00[a]	2.59±0.01[b]	2.54±0.00[b]	2.42±0.01[c]
TFB	8.97±0.24[d]	17.3±0.28[a]	14.06±0.34[b]	16.64±0.30[a]	13.03±0.23[c]	14.32±0.12[b]
DSB	2.95±0.03[d]	4.19±0.05[a]	3.67±0.04[ab]	4.11±0.07[a]	3.54±0.03[c]	3.75±0.02[b]
DRB	2.19±0.01[b]	2.24±0.01[a]	2.25±0.02[a]	2.20±0.00[b]	2.18±0.00[b]	2.14±0.00[c]
TDB	3.13±0.05[d]	4.44±0.04[a]	3.93±0.03[b]	4.31±0.07[a]	3.72±0.03[c]	3.90±0.03[b]

(RP- Rootingpercentage, NS-NumberofShoots, SL- ShootLength(cm.), DS-Diameterofstem(mm.)(Sproutedshoot), NL- Numberofleaves, PL- Petiolelength(cm.), LL-Leaflength(cm.), LW- Leafwidth(cm.), FreshShootBiomass(gm.), FreshRootBiomass(gm.), TotalFreshBiomass(gm.), DryShootBiomass(gm.), DryRootBiomass(gm.), TotalDryBiomass(gm.). The numbers in this table represent the mean and standard error (SE) based on four replications. At the 5% level, there is no discernible difference between the means that are followed by identical letters within rows (DMRT).

Discussion

It has become clear that auxin has a role in a variety of physiological processes, including cell elongation and differentiation in plant tissue. Several agricultural plant species have benefited from the exogenous administration of auxin as a potent technique to promote adventitious roots (Hartmann *et al.* 1997). Some plant species induce roots because of the abundance of natural auxin (IAA), however the application of a synthetic analogue like GA3 was more successful than IAA. Its increased stability inside tissue and during storage were discovered to be the cause of its significant effect on roots and growth performance in many types of plants (Blythe et al. 2007, Ling *et al.* 2013).

In the current study, it was shown that *T. cordifolia* stem clippings supplemented with 200 ppm GA3 (T1) showed a significantly higher rooting percentage than other treatment. Within 45 days, the entire roots and sprouting process was finished. The overall findings demonstrated that although *T. cordifolia* sprouting time was significantly longer, shoot elongation was significantly improved following sprouting. The findings of the current study on *T. cordifolia*agreed with those published by Mishra *et al.* (2010). When compared to other auxins and the control, old vine clippings of *T. cordifolia* treated with 200 ppm of GA3 dramatically improved sprouting, rooting, and root length.During three months of planting, the highest plant growth (364.73 cm) and branch count (3.42) in the identical GA3 treatments were noted(Mishra *et al.,* 2010). The investigation on *T. cordifolia* macro-propagation was also conducted by Rao *et al.* (2000). According to the study, GA3 pre-treatment of *T. cordifolia* cuttings produced the best rooting responses (86%) at 200 and 300 ppm doses. *T. cordifolia*, however, had the highest rooting effectiveness (96%) without the use of growth regulator hormone, according to a researchby Warrier*et al.* (2007).

Based on recorded observationsa few recommendations are offered. In polythene bags, care was made to avoid water logging and soil compaction. It was shown that stem cuttings of *Tinospora*were sensitive to rotting when exposed to too much water. As a result, cuttings received water every 4-5 days. This discovery led to the recommendation that seedlings that were grown from seeds should be monitored for heavy Spodoptera and thrips damage to delicate leaves and stems. So, the fastest, safest, and cheapest method of proliferation was by cuttings.

Conclusion

In conclusion,*T. cordifolia*stemcuttings treated with 200 ppm GA3 showed the notable sprouting,greatest rooting percent,longest shoot length possible, and somewhat more leaves, longer petioles, wider leaves, and longer leaves, total fresh biomass, and dry biomass. According to the findings, the 200 ppm GA3-treated cutting is the best method and might be used for *T. cordifolia* reproduction on a large scale in India.

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