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

Abstract

Tinospora cordifolia (Willd.) Miers are members of the Menispermaceae family and go by a variety of popular names, including "Guduchi," "Gulvel," and "Giloy." The genus, particularly its stem, provides a wide range of health advantages that have been mentioned in ancient texts and conventional medical practises. Over 68 Ayurveda formulations contain the medicine Guduchi as one of its main constituents, and need for this medication has climbed up to 3000 to 6000 MT with a 9.1% yearly increase. Unfortunately, uncontrolled harvesting is causing the natural populations of the both types 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 these specie, the impact of various GA3 concentrations on stem cuttings during 30 DAP and 45 DAP was investigated using the RBD~~design~~ with four replications. The 200 ppm GA3-treated stem cuttings of *T. cordifolia* (T1) showed a considerable rooting percentage of 85.75.The highest shoot lengths were measured for treatments T1 (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 discernible 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.

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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. The Menispermaceae family member *Tinospora cordifolia* (Willd.) Miers ex Hook. F and Thoms (giloy) is a glabrous, deciduous climbing shrub. The 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 situation 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 unprocessed drugs.

The value 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 it (Veeraiah and Reddy, 2012). Increase availability with correct farming techniques to easily and sustainably fulfil the rising demand. This is only achievable by meeting the huge demand in the ayurvedic pharmaceutical sectors by providing enough high-quality planting material at a lower 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.

The National Medicinal Plant Board (NMPB) 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. 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 Tinospora species, 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 *Tinospora* medication. So, the goal of the current study was to standardise the hormonal therapy for mass multiplication.

MATERIALS AND METHODS

Collection

The stem samples of *T. cordifolia* 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. The positioning and orientation of the cup-like nodes was deliberately taken into account when creating the cuts.

Treatments

The clippings were then treated for five minutes with a 1% (w/v) solution of the broad-spectrum fungicide Bavistin. 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 are seen as a kind of control.

Plantation of cuttings and data collection

Assessments of vegetative propagation were conducted in a sunken bed of 5 x 20 feet that included nursery polythene bags filled with soil material measuring 6 x 9" in size. Both species' treated cuttings were inserted into nursery bags at a depth of three to four centimetres. 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 clipping. For each experimental procedure, 20 cuttings were employed. Eighty cuttings in total were assessed for each treatment. For the duration of the trial, common organic procedures including watering, weeding, disease, and pest management were used.The plants were given meticulous organic maintenance. The observations were made for both species at 30 and 45 days following planting. The total days required for the initial sprouting and re-rooting of cuttings were kept track of. The morphological information, which included the amount of shoots, stem length, leaf, roots, and root-to-shot ratio, as well as the thickness of the stems, petiole length, leaf length, and leaf breadth, was noted. The Gupta et al. approach was used to calculate the biomass yield (1998).

Data analysis using statistics

RBD (Randomized Block Design) was used to conduct the experiment with four replicates (n = 4). 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) ~~just~~ 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). On 11 DAP, the first sprouting was noted. Nevertheless, treatment T4 had a 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 had somewhat more leaves, longer petioles, wider leaves, and longer leaves overall, but there were no appreciable variations in the mean values of any of these metrics (Table 1).

Table 1. *T. cordifolia* Pre-treatment with GA3 has an impact on stem cuttings at 30 DAP

Parameters	Treatments					
	T 0	T 1	T 2	T 3	T 4	T 5
RP	39.5	69.5	48.25	44.5	37.00	44.50
NS	3.16	3.25	3.20	3.40	3.45	3.25
SL	14.31	30.92	22.77	25.55	25.27	19.85
DS	4.09	4.54	4.02	4.03	4.06	4.16
NL	5.30	5.85	5.00	5.50	5.45	5.10
PL	5.71	7.58	6.38	7.36	7.57	6.98
LL	6.49	8.52	8.04	8.30	8.85	7.10
LW	5.97	7.99	7.27	7.46	7.49	6.06

Comment [D4]: Provide the standard deviation and the probability values for all the treatment groups.

(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 reports of enhanced rooting (18.25%). 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.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. 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	85.75	57	54.5	48.25	57.00
NS	3.16	3.30	3.30	3.60	3.50	3.30
SL	50.16	106.15	86.7	105.35	81.6	92.63
DS	5.75	6.25	5.75	5.50	6.00	6.00
NL	15.02	17.42	16.75	15.72	14.02	16.82
PL	2.43	2.15	2.17	2.14	2.15	2.18
LL	4.03	4.71	4.09	4.06	4.29	4.39
LW	10.75	14.35	8.8	14.10	10.35	10.8
RP	9.33	13.74	12.09	13.23	12.66	12.46
NS	9.85	13.97	13.69	14.00	14.07	13.41
SL	9.17	13.54	12.24	12.84	12.74	12.92
FSB	8.42	16.57	13.35	16.05	12.5	13.9
FRB	2.55	2.73	2.71	2.59	2.54	2.42
TFB	8.97	17.3	14.06	16.64	13.03	14.32
DSB	2.95	4.19	3.67	4.11	3.54	3.75
DRB	2.19	2.24	2.25	2.20	2.18	2.14
TDB	3.13	4.44	3.93	4.31	3.72	3.90

(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

Comment [D5]: Provide the standard deviation and the probability values for all the treatment groups.

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 considerable rooting percentage. Within 45 Minutes, the entire roots and sprouting process was finished. No other reports of rooting followed. 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* were in agreement 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. 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 research by Warrier et al. (2007). As a result, the current study deviated from the previous findings.

To obtain high-quality seedlings that lower the danger of plant damage, nursery techniques included several observations along with growing performance. Further recorded observations, findings, and a few recommendations are offered. In polythene bags, care was made to avoid water logging and soil compaction. It was shown that stem cutting of *Tinospora*were sensitive to rotting when exposed to too much water. As a result, seedlings 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 cuts.

Conclusion

The 200 ppm GA3-treated stem cutting of *T. cordifolia* showed notable sprouting, rooting percentage, total fresh biomass, and dry biomass. *Tinospora* are now unable to produce enough biomass for commercial use, which opens the door to large-scale growth with little upfront costs. For large-scale plantations, the qualified planting material (QPMs) of these plants may be easily generated year-round. The government just established the "All India Institute of Ayurveda" to promote research relevant to our ancient culture, therefore this study has major ramifications. Under the Start-up India programme, young entrepreneurs can also create commercial *Tinospora* plantations.

Comment [D6]: Conclude based on the key findings.

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