

# A Conservation Approach on Auxins Impact in Shoot Growth of *Tinospora* species.

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**Abstract:** A study was carried on *Tinospora crispa* and *Tinospora sinensis* stem cuttings by using two nodal stem lengths. The stem cuttings were treated with different auxin concentrations and controller (without hormone) was taken. Stem cuttings of the plant has potential to develop new individuals like parental plants. The length of mature stems around 10-15 cm long were slantly cut and placed in the soil mixture (soil + vermicompost) in equal ratios 1:1 to produce new shoots & roots of stem cuttings. The effect of different auxin concentrations on stem cuttings after 40-60 days was recorded. Treatment of maximum shoot lengths of *Tinospora crispa* were noted at 500ppm NAA i.e.,  $88.12 \pm 17.10$ , whereas the *Tinospora sinensis* was noted at 400ppm NAA i.e.,  $79.70 \pm 2.80$  respectively.

**Key words:** *Tinospora sinensis*, *Tinospora crispa*, Stem cuttings, Auxin concentrations.

## 1. INTRODUCTION:

*Tinospora species* belongs to the family of Menispermaceae.

The *T. Crispa* *Tinospora cordifolia* is also known by many synonyms, including *Menispermum crispum*. These two species: *Tinospora tuberculata* and *Tinospora rumphii*, and other local names like Makabuhai, Andawali, Putarwali, Kattukkodi, People from all areas: Baraphet, Boraphet, and Wan Kab Hoi Yai (Asif Iqbal et al., 2012). The plant *Tinospora* is one of the most important plant drug properly known as 'Guduchi or Amrita' and its shows a wide range of bioactive properties. This plant verifies as medicinal plant by traditional systems as well as modern systems of medicines (**Rakshe Abhijeet and Digambar Mokat., 2018**). The drug *Tinospora* is the major ingredients in about 68 ayurvedic formulations like Amritharishtam', 'Amrithadienna', 'Amrithadichoornam', 'Dhanvantaram tailam', 'Cheriyarasnadi Kashayam', 'Valiya marmagulika', etc. (Sereena and Remashree, 2014). Found in tropical and subtropical India, this plant is a woody climber with glossy green leaves in primary rainforests or mixed deciduous forests across the Philippines, as well as in the other tropical regions of Asia, with elevations ranging from 1,000 to 3,000 m. Belief in abilities (Dweck et al. 2012). It has been grown in Thailand, Sri Lanka, and India as a medicinal plant (Umi Kalsom et al. 1999).

This recent research has shown that the health benefits of *Tinospora crispa* include antioxidant properties, as well as anti-proliferative activity on human cancer cell lines (Zulkhairi et al. 2008). Supplementation of the *T. crispa* extract was shown to be able to delay or slow the formation of atherosclerotic plaque due to dietary cholesterol (Zulkhairi et al. 2009). According to its future usage, this species' demand is growing on a regular basis. To examine the relationship between hormone levels and cutting length on the shooting of *T. crispa* cuttings, the current experiment was performed. Some significant variables influencing the rooting potential of cuttings are hormone levels and the length of the cuttings (Hartmann et al. 1990).

*Tinospora sinensis* is also named as *Tinospora* in English, Giloy in Hindi, Amruta in Sanskrit, and Thippatega in Telugu. It is found almost throughout all of India and other southeast Asian countries. *T. sinensis* is known to be a climber with a 1-2 cm thick stem. Deciduous vines 15-25 m long with large succulent climbing shrubs growing on them. When young, lenticels are

typically 4 fids in number and they are fleshy. Over time, they become membranous and glabrous and have long aerial roots. Under these circumstances, using various techniques is seen as unavoidable to raise the commercial level of this species. (Handique, 2014). Presently, forest areas are the major source of raw drugs 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 as a consequence of indiscriminate as a consequence of wild stock of this valuable medicinal plant (Bapat *et al.* 2008, Veeraiah and Reddy, 2012) It is cleared that the demand for the 'Guduchi' drug obviously cannot be complete from wild sources any more and more focused efforts about 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 a complete lack of a scientific approach to the propagation and cultivation of the *Tinospora* drug. According to its future usage, this species' demand is growing on a regular basis. To investigate the impact of hormone and cutting length on the shooting of *T.crispa* and *T.sinensis* cuttings, the current experiment was carried out. Some significant variables influencing the rooting potential of cuttings are hormone levels and the length of the cuttings (Hartmann *et al.* 1990).

## 2. MATERIAL METHODS

The present investigation on "Ex-SITU Conservation on *Tinospora* Species" was carried out at The College of Science and Technology, Andhra University, Visakhapatnam's Department of Botany. The details on the location study site, materials used, and techniques employed during the investigation are given below.

**Study Area:** The experiments were carried out in ex-situ conditions at the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh. It has a hot, humid weather with frequent rain. The mean year-to-year temperatures range from about 24.7- 30.6°C with the maximum month of the May and minimum month of January. The minimum temperatures range between 20-27°C.

**Preparation of plant materials:** The cuttings of *Tinospora crispa* and *Tinospora sinensis* were prepared on their height and stature in the stand and their stem and crown features are based on their phenotypically superior qualities. One-year-old branch cuttings were taken from 25±5year old selected trees during November-December and February-March. The leafless cuttings about 2-3 nodes were taken. The cuttings of the both species were planted in a nursery bags at 2-3 cm depth. The upper part of the cutting is waxed to reduce water evaporationsof each cutting placed in nursery bags. The total 24 cuttings (12 cuttings of *T.crispa* & 12 cuttings of *T.sinensis*) were taken in each hormonal treatment. Each cutting is continuously monitoring every day measuring the length of the cuttings.

**Soil compositions:** The soil composition was prepared with the combination of Sand with vermicompost in equal utilisation percentage of nutrients supplied to the cuttings and in the containers for observing better shooting growth.

### **Treatment of cuttings with hormones:**

Mature cuttings were brought to the laboratory and treated with the concentrations of hormones like IAA, IBA, NAA each with 400, 500, and 600 (mgL<sup>-1</sup>) are used with the required soil composition. The care was taken to prevent precipitation of IAA, IBA, and NAA during the process of dilution. The cuttings were divided according to hormonal concentrations of 400,

500, and 600 (mgL<sup>-1</sup>). The IAA, IBA, and NAA of different concentrated solutions were transferred separately into 24 nursery bags for giving treatments. The stem cuttings were dipped in the above concentrations of hormones for 15 min. After that the stem cuttings are propagated, explants were arranged in following order i.e., The other species were also repeated by the same combination and ratios. Total 2 species × 3 hormonal concentrations, total of nine samples (9) were taken. Along with the auxin concentration the cuttings were also been treated without control i.e., 1% fungicide for about 10-15 min.

**Results: There was a substantial variation among the length of cuts when it came to analysing variance.**

**1. Effect of the stem cuttings in IAA compositions:**

The stem cuttings of *T.crispa* treated with an IAA of 600 mgL<sup>-1</sup> have the highest shoot induction was 60% with an average number of shoots per cutting is 2.23 ± 0.05 and the average length is 42.61 ± 7.80. The lowest shoot induction was seen at 400 ppm.

The *T.sinensis* stem cuttings treated with IAA for different concentrations show the different shoot induction responses. It shows the highest at 500 mgL<sup>-1</sup> with shoot induction response of 50% with an average number of shoots per cutting 2.41 ± 0.12 and the average length of shoots 65.25 ± 2.85

**2. Effect of the stem cuttings in IBA compositions:** The maximum shooting observed in *T.crispa* stem cuttings treated with 500 mgL<sup>-1</sup> IBA which shows 80% of shoot induction response with an average number of shoots per cutting is 2.40 ± 0.16 and the average length of shoots is 69.35 ± 7.51.

The species of *T.sinensis*, the maximum shooting observed stem cuttings treated with 600 mgL<sup>-1</sup> IBA with an average number of shoots per cutting is 2.09 ± 0.17 and the average no. of shoot length is 79.62 ± 3.05 showing nearly 69% of shoot induction response.

**3. Effect of the stem cuttings in NAA compositions:** The maximum shooting observed in *T.crispa* stem cuttings treated with 400 mgL<sup>-1</sup> NAA resembles 92% of shooting response with an average number of shoots per cuttings is 2.64 ± 0.18 and the average length of shoots is 88.26 ± 13.20.

The species of *T.sinensis*, the maximum shooting observed stem cuttings treated with 500 mgL<sup>-1</sup> NAA with an average number of shoots per cuttings is 2.23 ± 2.12 and the average length of shoots is 79.12 ± 2.15 with 70% of shooting response.

Auxin hormones (mg L <sup>-1</sup> )		Response (%)	Average no. of shoots per cutting (mean ± SE)	Average length of shoots (mean ± SE)
IAA	IBA			
400	-	45	2.2 ± 0.11	36.10 ± 1.23
500	-	58	2.18 ± 0.02	30.25 ± 5.41
600	-	60	2.23 ± 0.05	42.61 ± 7.80
	Control	30	2.02 ± 0.08	31.02 ± 2.61
-	400	62	2.25 ± 0.08	48.12 ± 11.01

-	500	80	2.40 ± 0.16	69.35 ± 7.51
-	600	75	2.32 ± 0.40	61.42 ± 5.32
-	-	35	1.68 ± 0.51	22.42 ± 1.35
Control				
-	-	92	2.64 ± 0.18	88.26 ± 13.20
-	400			
-	-	87	2.56 ± 0.38	88.12 ± 17.10
-	500			
-	-	80	2.50 ± 0.20	78.31 ± 10.13
-	600	42	2.02 ± 0.10	19.62 ± 0.61
Control				

Table: 1 The length of the plant cuttings at different hormonal concentrations of plant *Tinospora crispa*.

Auxin (mgL <sup>-2</sup> )	hormones		Shoot Induction Response (%)	Average no. of shoots per cutting (mean±SE)	Average length of shoots (mean±SE)
IAA	IBA				
NAA					
400	-	-	50	2.24 ± 0.21	61.52 ± 2.05
500	-	-	57	2.41 ± 0.12	65.25 ± 2.85
600	-	-	54	2.35 ± 0.08	60.11 ± 4.62
Control			55	2.19 ± 1.02	46.35 ± 3.59
-	400	-	60	2.29 ± 0.02	66.01 ± 1.24
-	500	-	53	2.12 ± 0.10	64.24 ± 2.54
-	600	-	69	2.09 ± 0.17	79.62 ± 3.05
Control			65	2.14 ± 1.25	58.18 ± 1.20
-	-	400	58	2.19 ± 1.01	70.02 ± 2.80
-	-	500	70	2.23 ± 2.12	79.12 ± 2.15
-	-	600	65	2.34 ± 1.25	68.34 ± 1.22
Control			62	2.29 ± 2.54	75.05 ± 2.35

Table: 2 The length of the plant cuttings at different hormonal concentrations of plant *Tinospora sinensis*.



A. Nursery bags



B. Stem cutting showing shooting



C. *T.sinensis* after 40 days



D. *T.sinensis* after 60 days



A. Stem cutting showing shooting



B. *T. crispa* after 40 days



C. *T. crispa* after 45-50 days



D. *T. crispa* after 60 days

### 3. DISCUSSION:

Generally, medicinal plants are grown in agroclimatic areas where economic plants are available in high altitudes. *Tinospora* species are an essential medicinal ingredient plants used in Ayurveda to serve different functions associated with inflammation, allergies, obesity, and

neurology, etc. With respect to vegetative growth, the stem cuttings method is critical for reducing the danger of species decline. This species is getting extinct gradually in its natural habitat and is rarely found in some particular regions due to its use in traditional and as well as in modern medicine.

The hormonal concentrations are involved in the different physiological processes such as cell-differentiation, cell-proliferations, cell-elongation, fruit growth and plant growth. Germination percentages and features are both affected by hormone treatments in various dosages allow regular branches and roots to form when compared to the stem cutting which auxin treatments were applied. The effect of auxin treatments on the stem cuttings of *T.crispa* and *T. sinensis* are presented in Table 1 and Table 2.

When treatments such as IAA, IBA, and NAA are applied to stem cuttings, shoot bud induction and rooting response show more variability. In present study we observed that *T.crispa* shows the highest shooting induction percentage 92 in NAA with  $400\text{mgL}^{-1}$  and shooting length was  $88.26\pm 13.20$ . In *T.sinensis* it was observed that the stem cuttings treated with NAA  $400\text{mgL}^{-1}$  showed a significant shooting percentage of 70% on treating  $500\text{mgL}^{-1}$  NAA and  $79.12\pm 2.15$  was the shooting length.

#### 4. CONCLUSIONS:

The stem cuttings of *T.crispa* treated with  $400\text{mgL}^{-1}$  NAA shows the maximum shoot length  $88.26\pm 13.20$ , whereas *T.sinensis* showed the maximum shoot length  $500\text{mgL}^{-1}$  is NAA  $79.12\pm 2.15$  respectively. The stem cutting of *T.crispa* was treated with  $400\text{mgL}^{-1}$  NAA exhibited significant shoot induction response is 92%. And however *T.sinensis*, the shoot induction response was exhibited with 70% on treating  $500\text{mgL}^{-1}$  NAA.

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