



Mini cutting technique: an easy and cost-effective way of *Tinospora cordifolia* multiplication

PARMESHWAR LAL SARAN¹, RIDDHIBEN PATEL², RAM PRASNNA MEENA³, KULDEEPSINGH A. KALARIYA⁴ and RAVISH CHOUDHARY⁵

ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat 387 310,

Received: 15 March 2018; Accepted: 25 October 2018

ABSTRACT

The experiment was carried out to determine the commercially viable technique for multiplication of *Tinospora cordifolia* (Willd.) Miers ex Hook. F and Thoms (*giloy*). In the open field condition using the variable numbers of nodes per cutting were investigated and observed that three and four nodes cuttings were significantly superior in growth and survival parameters but involved more planting/stock materials. Therefore, to reduce the planting stocks, cuttings with one node with and without stem part were evaluated. Single bud cuttings with both side wood (stem part) showed significantly higher growth and survival percent in comparison to only bud without stem portion and bud with one side stem wood. Among all the tested technique, four buds' technique was nearly found superior in growth parameters but not significant with three buds, which was proven for higher survival percent. However, single bud cutting technique involved minimum cost and economically proven superior for one-ha plantation as compare to available traditional techniques. The present study indicated that for *T. cordifolia*, mini cuttings (bud with both side stem wood) techniques is the best propagation method for commercial multiplication.

Key words: Economics, Food reserve, Mini cutting, Semi-hard wood cutting, *Tinospora cordifolia*.

India is bestowed with enormous biodiversity of medicinal plants and front runner to use in traditional ayurvedic system. *Tinospora cordifolia* (Willd.) Miers ex Hook. F and Thoms (*giloy*) belonging to the family Menispermaceae, is a glabrous, deciduous, climbing shrub. The shrub found throughout, especially in tropical parts ascending to an altitude of 300 m in India and also in certain parts of Srilanka, Bangladesh and China (Mittal *et al.* 2014). Rapid depletion of natural resources of medicinal plants in general and particularly *giloy* accompanied with exponential rise in demand has paved way for quest to balance the critical situation (Sinha and Sharma 2015). An easy and sustainable way to meet the growing demand is to increase the availability through proper cultivation practices. This is possible only through supply of sufficient quality planting material at cheaper way for cultivation purpose and to meet out high demand in ayurvedic pharmaceutical industries.

Viability of seeds is very less, poor seed set and germination of seeds are the main problems in *giloy* (Mittal *et al.* 2014, 2017). Therefore, propagation through cuttings is an easy and less expensive method. Similarly,

most common cutting type for commercial multiplication was semi hard wood cutting (Somashekhhar and Sharma 2002). In the traditional methods usually, a division of the stem of a plant with 20-25 cm long cuttings containing 3-4 buds and plant materials obtained from tender shoots and branches of the current year's growth is used as semi hard wood cutting. In this technique more stem part required, planting, weeding and transplanting are very tough, costly and time-consuming. Similarly, the plant multiplied through tissue culture needs a high cost involvement (Mittal *et al.* 2017), ex-plants from same nodal portion (Gururaj *et al.* 2007), long gestation period and poor field transfer. With this background keeping in view, the current study was undertaken to identify the cost-effective multiplication method with fast multiplication ratio.

MATERIALS AND METHODS

The study was conducted on the experimental fields of the Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat (India) during two consecutive harvesting seasons in the years 2016 and 2017. The experimental farm is located at 22°35'N and 72°55'E at an altitude of about 45.1 m above mean sea level.

Plants were collected from Directorate *giloy* repository during 2nd week of March, 2016 for preparation of cuttings. In mini cutting (single bud) technique, three stem cuttings were tried, viz. (1) four centimetres both sides of bud, (2)

¹(e mail: plsdehradun@gmail.com); ²(e mail: patelriddhi20295@gmail.com); ³(e mail: rpm.agri@gmail.com); ⁴(e mail: kuldeep_ka@yahoo.co.in), ICAR-DMAPR, Boriavi, Anana, Gujrat. ⁵(e mail: ravianu1110@gmail.com), ICAR-IARI, New Delhi 110012.



Fig 1 Types of mini cuttings (a. half centimetre both sides of bud; b. four-centimetres one side of bud and; c. four centimetres both sides of bud) and their response to growth and survival per cent.

four centimetres one side of bud and, (3) half centimetre both sides of bud (Fig 1). The survival percentage and growth parameters were recorded by using standard methods (Aminah *et al.* 2015).

A separate experiment was conducted during last week of July, 2016 for comparison of mini cutting technique with traditional technique (Semi-hard wood cutting). The extracted cuttings were trimmed and planted in the soil at 40° angle. In case of mini cutting technique, the propagules were planted 1-1.5 cm deep in the nursery beds horizontally and covered with grass mulch. These cuttings were planted in the layout with a spacing of 10 cm × 10 cm. The layout and beds were prepared as per standard nursery practices. Four variables cutting types; one bud, two buds, three buds and four buds, and three different cuttings thickness; thick (5.88 mm²), medium (4.21 mm²) and thin (2.77 mm²) were taken in our experiment. These four blocks were partitioned into three plots with properly randomized treatment allocation. The observations were recorded on the randomly chosen ten plants from each plot. The mean value for a single trait was retrieved from the three replications. The observations included number of branches, length of branches (cm), number of leaves per plant, diameter of branches (mm), plant weight (kg), leaf area (cm²), number of roots per cutting, length of root (cm), diameter of root (mm) and survival (%) were observed during the 2nd week of September for both the seasons. The days taken to sprout of cuttings were measured by counting survived cuttings up to 30 days

duration from transplanting.

Four different type of cuttings, viz. one bud, two buds, three buds and four buds were planted in separate experiment during 2nd week of September, 2016 randomly in our experiment to work out economics of the treatments. On an average, survival (%) was observed during the 2nd week of September by counting the survived cuttings in each plot. Number of cuttings required per hectare was calculated on the basis of row to row and plant to plant recommended spacing (3 × 3 m). The weight of cuttings (kg/ha) observed according to the recommended cuttings per ha and average weight of cuttings under different cutting types. The equivalent cost involved per plant was calculated by using survival percent and rate per plant was decided on the basis of plants retained to farmers by our Directorate (₹ 15/plant).

The statistical analysis of the data was performed using standard statistical procedures. The analysis of variance was done in randomized block design by using statistical software SAS 9.2 (SAS 2008) in all three experiments. The results were presented at 5% level of significance ($P = 0.05$). The critical difference (CD) values were calculated to compare the various treatment means.

RESULTS AND DISCUSSION

The traditional multiplication methods of *giloy* involves more human force, more space for nursery raising and more stem wood in multiplication. To resolve this problem, propagation carried out through semi hard wood stem with mini cuttings (single node) like in sugar cane and cuttings planted in horizontal position (Fig 1). The extracted cuttings were trimmed and planted at 1-1.5 cm depth in the nursery beds horizontally and covered with grass mulch. By this technique multiplication is very easy, economic, easy to perform intercultural operation (weeding), require less space in nursery, minimum disturbed by animal and high survival percentage during field and polytube transfer.

Morphological variation for growth traits and survival percent were observed among different types of mini cutting (Table 1 and Fig 1). Cutting of bud with both side wood showed significantly higher number of branches (2.33), length of branch (187 cm), number of leaves (64), diameter of branch (3.69 mm), plant weight (0.09 kg), leaf area (23.68 cm²), number of roots (2.33), length of root (35 cm) and diameter of root (4.32 mm) as compared to only bud and bud with one side wood. The survival percent was also

Table 1 Effect of mini cutting types on growth and survival per cent of *T. cordifolia*

Mini cutting type	No. of branch per cutting	Length of branch (cm)	No. of leaves	Diameter of branch (mm)	Plant weight (Kg)	Leaf area (cm ²)	No. of roots	Length of root (cm)	Diameter of root (mm ²)	Survival (%)
Only bud	1.33 ^b	156.50 ^b	24.00 ^c	2.69 ^c	0.05 ^b	21.07 ^{ab}	1.00 ^b	14.00 ^c	3.45 ^b	15.14 ^c
Bud with one side wood	1.33 ^b	138.00 ^c	32.33 ^b	3.44 ^b	0.05 ^b	17.05 ^b	1.00 ^b	25.00 ^b	3.27 ^c	28.85 ^b
Bud with both side wood	2.33 ^a	187.00 ^a	64.00 ^a	3.69 ^a	0.09 ^a	23.68 ^a	2.33 ^a	35.00 ^a	4.32 ^a	67.68 ^a

Means with the same letter (superscript) in the columns are not showing significantly different ($P=0.05$) – (Duncan Multiple Range Test).

significantly higher in bud with both side wood (67.68%) as compared to only bud (15.14%) and bud with one side wood (28.85%). Similarly, more number of roots were reported in 22.5 cm length cuttings as compared to 1.3 cm cuttings in *T. crispa* (Aminah *et al.* 2015). It may be due to higher amount of reserve food materials in the longer size cuttings of patchouli (Jadhav *et al.* 2003).

In this experiment traditional technique was compared with a new mini cutting technique (single bud with both side wood) for growth traits and survival of cuttings (Table 2 and Fig 2). The interaction effect of number of buds per cutting and cutting thickness on growth traits and survival per cent was assessed but found non-significant, therefore, number of buds compared with growth traits and survival per cent using DMRT test. The number of branches and leaf area were observed higher in four buds (2.33 and 42.44 cm²), respectively. The length of branches was observed higher in three bud medium cutting thickness (176.69 cm) and lower in single bud thick cutting (92.33 cm). Variation in number of leaves/branch was higher in two bud medium cutting (38.00) and lower in single bud thick cutting (12.33). Diameter of branch was higher in two buds with thick cutting (2.59 mm) and lower in single bud medium cutting (1.47 mm) while plant weight was observed higher in single bud thin cutting (0.13 kg) and lower in two buds and three buds thin cutting (0.05 kg). Maximum leaf area was observed in four bud thin cutting (42.44 cm²) and lowest in single bud thin cutting (18.63 cm²). Maximum number of roots per cutting was observed in four bud' smedium type cutting (15.67), length of roots was maximum in three buds and two buds thick type cuttings (3.33 cm in both), whereas diameter of root in two buds with medium thickness (2.51 mm). Over all the cuttings, survival percent played major role, which was maximum (81.42%) in three buds thin type cuttings, while minimum in single bud thick type cuttings (59.88%). The maximum number of roots and survival per cent was

reported in 22.5 cm length cuttings as compared to 7.5 cm and 15 cm length cuttings in *T. crispa* under Malaysian conditions (Aminah *et al.* 2015).

The number of buds and cutting length had positive relationship with sprouting of cuttings (Fig 3). The earliest sprouting was observed in four buds (18.67 days) followed by three buds (19.67 days) while, in one bud, it was delayed by 29.66 days. More number of buds or longer cutting lengths



Fig 2 Comparative performance of cutting techniques on growth parameters.

Table 2 Effect of number of nodes on growth and survival per cent in *T. cordifolia*

No. of buds/cutting	Cutting thickness	No. of branches	Length of branch (cm)	No. of leaves per branch	Diameter of branch (mm)	Plant weight (kg)	Leaf area (cm ²)	No. of roots per cuttings	Length of root (cm)	Diameter of root (mm)	Survival (%)
Single bud	Thin	1.33 ^c	107.61 ^f	21.00 ^d	2.32 ^{abc}	0.13 ^a	18.63 ^c	13.45 ^{ab}	1.33 ^{cd}	2.40 ^{ab}	67.74 ^{bc}
	Medium	1.33 ^c	98.24 ^g	14.00 ^e	1.47 ^e	0.08 ^{bcd}	18.69 ^c	9.33 ^d	1.00 ^d	1.90 ^{bcd}	64.38 ^{cd}
	Thick	1.33 ^c	92.33 ^g	12.33 ^f	1.74 ^{de}	0.07 ^{bcd}	20.02 ^c	9.40 ^d	1.33 ^{cd}	1.50 ^d	59.88 ^d
Two Bud	Thin	1.33 ^c	130.25 ^e	25.67 ^c	2.45 ^{ab}	0.05 ^d	23.60 ^{bc}	13.49 ^{ab}	3.00 ^{ab}	1.92 ^{bcd}	77.02 ^{ab}
	Medium	1.67 ^b	153.87 ^d	38.00 ^a	2.28 ^{abc}	0.06 ^{cd}	24.86 ^{bc}	12.38 ^{bc}	2.33 ^{bc}	2.51 ^a	63.17 ^{cd}
	Thick	2.00 ^{ab}	135.91 ^e	32.67 ^b	2.59 ^a	0.07 ^{bcd}	23.53 ^{bc}	12.32 ^{bc}	3.33 ^a	1.71 ^{cd}	69.49 ^{bc}
Three Bud	Thin	2.33 ^a	160.81 ^c	21.33 ^d	1.81 ^{cde}	0.05 ^d	38.77 ^{ab}	10.70 ^{cd}	3.00 ^{ab}	1.95 ^{bcd}	81.42 ^a
	Medium	1.67 ^b	176.69 ^a	25.67 ^c	2.14 ^{abcd}	0.07 ^{bcd}	37.89 ^{ab}	13.35 ^{ab}	2.67 ^{bc}	2.05 ^{bc}	72.09 ^{abc}
	Thick	1.67 ^b	170.80 ^{ab}	26.33 ^c	2.52 ^{ab}	0.07 ^{bcd}	34.19 ^{ab}	11.62 ^{cd}	3.33 ^a	1.58 ^d	76.62 ^{ab}
Four Bud	Thin	2.33 ^a	121.31 ^{ef}	15.67 ^e	2.03 ^{bcd}	0.06 ^{cd}	42.44 ^a	10.58 ^{cd}	2.67 ^{bc}	1.84 ^{cd}	78.50 ^{ab}
	Medium	2.33 ^a	150.37 ^d	27.00 ^c	2.12 ^{abcd}	0.09 ^{bc}	37.95 ^{ab}	15.67 ^a	3.00 ^{ab}	2.40 ^{ab}	73.04 ^{abc}
	Thick	2.33 ^a	173.00 ^{ab}	32.00 ^b	2.42 ^{ab}	0.10 ^{ab}	35.58 ^{ab}	13.91 ^{ab}	3.00 ^{ab}	2.18 ^{bc}	67.85 ^{bc}

Means with the same letter (superscript) in the columns are not showing significantly different (P=0.05) – (Duncan Multiple Range Test).

Table 3 Economics of different cutting types in *T. cordifolia*

Cutting techniques	Average survival (%)	Average no. of cutting required (/ha)	Average weight of cutting (kg/ha)	Average cost involved (₹/ha)
Single buds (bud with both side wood) cutting	67.78 ^c	1607.33 ^a	6.36 ^c	24075 ^d
Two buds cutting	69.88 ^{bc}	1574.00 ^{ab}	9.73 ^b	47204 ^c
Three buds cutting	76.71 ^a	1434.00 ^c	9.44 ^b	64507 ^b
Four buds cutting	73.12 ^{ab}	1494.00 ^{bc}	10.49 ^a	88304 ^a

Means with the same letter (superscript) in the columns are not showing significantly different ($P=0.05$) – (Duncan Multiple Range Test).

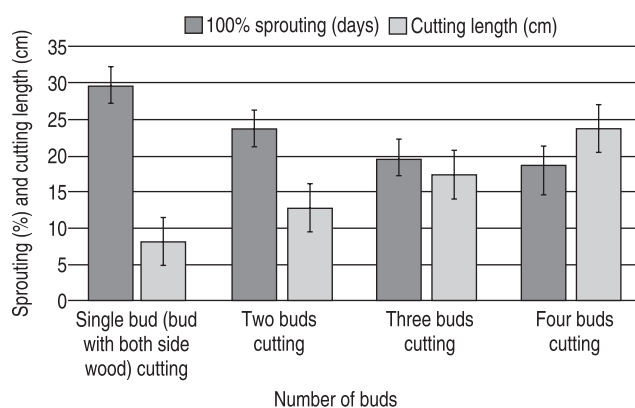


Fig 3 Relationship between number of buds and cutting length on days taken for 100% sprouting.

had positive relation with stored food in cuttings for better sprouting purpose. The enhanced efficiency is probably due to high sugar content in bigger sized shoot cuttings, and possibly due to increased mobilization of carbohydrates from starch (Guleria and Vashisht, 2014).

The success of any cutting techniques depends upon economics especially plants like *T. cordifolia*, whose stem is directly used as raw drug. The success of cutting techniques depends upon cost involved in per unit area (Table 3). The highest survival (76.71%) was observed in three buds cutting followed by four buds' cuttings (73.12%), while the lowest in single bud cutting (67.78%). Somashekhar and Sharma (2002) also reported that the 20 cm length semi hard wood cuttings with a pair of leaves retained at the tip perform better in *T. cordifolia* under Indian conditions. Average minimum number of cuttings required per hectare in three buds cutting were 1434, while maximum number in single bud cuttings were 1607.33. The maximum average weight of cuttings was observed in four buds cutting (10.49/kg/ha), while minimum in single bud cuttings (6.36/kg/ha). Similarly, the average cost of cuttings involves in four buds cutting were ₹ 88304/ha and minimum in single bud cuttings were ₹ 24075/ha. Lowest cost involvement showed that the single bud cutting technique is economically viable for end user as compares to traditional techniques. In conclusion, the average maximum growth was observed in four buds but survival of cuttings were in three buds. The differences between growth parameters were not significant in cuttings with three and four buds. Considering cost effectiveness, the single node cutting being the best technique, may be exploited for large scale multiplication of *T. cordifolia* in India.

ACKNOWLEDGMENT

The authors are grateful to the NMPB, New Delhi for providing the resources through project no. R&D/GUJ-01/2016-17-NMPB-IVA entitled "Standardization of propagation techniques and QPM production of selected medicinal plants" for conducting the experiment.

REFERENCES

- Aminah H, Ahmad F M S, Tariq M H and Hamzah M. 2015. Effect of hormone and cutting length on the rooting of *Tinospora crispa*. *International Journal of Scientific and Research Publications* 5: 1–4.
- Guleria V and Vashisht A. 2014. Rejuvenation and adventitious rooting in shoot cuttings of *T. grandis* under protected conditions in new locality of western Himalayas. *University Journal of Plant Sciences* 2: 103–6.
- Gururaj H B, Giridhar P and Ravishankar G A. 2007. Micropropagation of *Tinospora cordifolia* (Willd.) Miers ex Hook. F & Thoms. A multipurpose medicinal plant. *Current Science* 92: 23–6.
- Jadhav S G, Sadhav B B and Apte U B. 2003. Influence of growth regulators on growth and oil content of patchouli (*Pogostemon cablin* Benth.). *Indian Perfumer* 47: 287–9.
- Joshi G and Kaur R. 2016. *Tinospora cordifolia*: A phytopharmacological review. *International Journal of Pharmaceutical Sciences and Research* 7: 890–7.
- Khosa R L and Prasad S. 1971. Pharmacognostical studies on guduchi (*Tinospora cordifolia* Miers). *The Journal of Research in Indian Medicine* 6: 261–9.
- Mittal J, Sharma M M and Batra A. 2014. *Tinospora cordifolia*: A multipurpose medicinal plant- A review. *Journal of Medicinal Plants Studies* 2: 32–47.
- Mittal J, Mishra Y, Singh A, Batra A and Sharma M M. 2017. An efficient micropropagation of *Tinospora cordifolia* (Willd.) Miers ex Hook F & Thoms, A NMPB prioritized medicinal plant. *Indian Journal of Biotechnology* 16: 133–7.
- Neeraja P V, Margaret E and Amrutha V. 2013. *Tinospora cordifolia* : Multipurpose rejuvenator. *International Journal of Pharmaceutical, Chemical and Biological Sciences* 3: 233–41.
- Sinha A and Sharma H P. 2015. Micropropagation and phytochemical screening of *Tinospora cordifolia* (Willd.) Miers ex Hook F & Thoms. A medicinal plant. *International Journal of advances in Pharmacy, Biology and Chemistry* 4: 114–21.
- Somashekhar B S and Sharma M. 2002. Propagation techniques of commercially important medicinal plants; training manual. Foundation for Revitalisation of Local Health Traditions, Bengaluru.p.118.