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Vegetative Propagation of *Piper sarmentosum* Roxb. - A Medicinally Important Species

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Abstract

Piper sarmentosum Roxb. of the botanical family Piperaceae is distributed in South East Asian countries, including Andaman and Nicobar Islands of India. The species is used in a number of medicinal preparations in various parts of the world. Recent studies have shown the potential of this species as antimicrobial, antioxidant, anti-tuberculosis and anti-spasmodial agent. However, the species has not received much attention on its cultivation aspects. The present study deals with standardizing vegetative propagation technique in *P. sarmentosum* for further utilization. Effect of single, double and triple node stem cuttings along with pre-treatments of indole-3 butyric acid (0, 500 and 1,000 mg/L) was studied. Further, effect of retention or removal of leaves from stem cutting was also studied on rooting success. Results revealed that stem cuttings with two nodes were optimum for propagation. Use of 1,000 mg/L IBA as pre-treatment helped in improving sprouting percentage and further plant growth. Also, removal of leaves on the cuttings, though did not enhance rooting percentage was beneficial for improving the plant growth. To our knowledge, the present study is the first report on the vegetative propagation of this medicinally important species.

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Introduction

Piper is one of the important genera of Piperaceae family with a number of species being valued in flavor, fragrance and medicinal industries worldwide.[1] Among them, economically important species are cultivated while others are largely found in the backyards or in wild habitats. Andaman and Nicobar Islands (ANI) are situated in the Bay of Bengal comprising of about 572 tropical islands and islets.[1] These islands are known for rich diversity of flora and fauna with high degree of endemism.[2] In ANI, the genus is represented by seven species *viz*. *P.*

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nigrum L., *P. betle* L.*, P. longum* L.*, P. ribesioides* Wall, *P. sarmentosum* Roxb., *P. pedicellosum* Wall. ex. DC and *P. miniatum* Bl.[2]. Considerable variability has been noticed for *Piper* species in the islands and hence, detailed intra-specific studies have been envisaged for the identification of desirable genotypes.[1,3,4]

P. sarmentosum is a small and spreading species that was reported from Mount Harriet Hills, South Andaman of ANI during 2004 and is also found growing in Northeastern India, Malaysia and China.[5] The species is being used by the local Bengali people for curing cough and cold apart from consumption as a leafy vegetable.[4] The species has high therapeutic potential as it is known to possess antioxidant, antibacterial, anti-tuberculosis and anti-spasmodial activities.[4,6-7] A drug named '*Pikutbenjakul*' was formulated from the roots of *P. sarmentosum* and is commonly used against diarrheal disease.[8] However, except for a report dealing with optimization of potting medium, no reports are available on standardization of crop production techniques for this species.[9]

Considering the potential of this species, efforts have been initiated at authors' institute to collect and characterize the intra-specific diversity occurring in different islands of ANI. In order to promote cultivation of medicinal plants, availability of disease free planting material is a pre-requisite.[10] Propagation using stem cuttings has been reported as successful and efficient method of propagation in many *Piper* species.[11-13] However, many factors *viz*. length/ type of cutting, pre-treatment, curing treatments, substrate/medium, growing environment *etc*. are known to affect the success. The present study was an attempt to know the effect of length of cutting, auxin pretreatment and leaf retention on propagation success in *P. sarmentosum*.

Materials and Methods

The present study was conducted at Division of Horticulture and Forestry, ICAR- Central Island Agricultural Research Institute, Port Blair, Andaman and Nicobar Islands, India during 2016. Experiment was carried out in a naturally ventilated polyhouse and following values of weather parameters were observed during the study period: mean max temperature of 30.4°C, mean minimum temperature of 24.8°C, mean relative humidity of 86.4%. Cuttings of *P. sarmentosum* were collected from germplasm maintained in polyhouse and were immediately transferred to a container with water. Bases of the cuttings were dipped in fungicide solution (Carbendazim, 0.5%, w/v) for ten minutes to eliminate any chance contaminants.

Experiment 1: Effect of Length of Stem Cutting and Pre-Treatments

Stem with single (SNC), double (DNC) and triple node (TNC) cuttings were excised from healthy plants using sharp secateurs. These cuttings were used for studying the rooting response with or without auxin as pretreatment. The cut ends of stems were given quick dip treatment (20 s) with water and indole-3 butyric acid (IBA) solution (500 mg/L and 1,000 mg/L) as pretreatment. Such treated cuttings were then planted in polybags filled with soil and farm yard manure (1:1, v/v) as a substrate. In order to reduce the transpiration losses from the cuttings, leaf lamina was reduced to half before planting. Experiment was laid out in factorial design with four replications (40 cuttings in each treatment).

Experiment 2: Effect of Leaf Retention

To study the effect of leaf retention on propagation success, DNC were excised from the mother plants and treated as described earlier. All the leaves on the cuttings were retained or removed depending on the treatment before treating with commercial powder formulation (Humiroot). Treated cuttings were planted in polybags filled with substrate as detailed above. In each treatment, 50 cuttings were maintained with five replications.

Observations and Data Analysis

Observations were recorded after 60 days of planting in different variables *viz*. rooting (%), number of roots per cuttings, root length (cm), number of leaves and dry matter content (%). Data was subjected to analysis of variance using Web Agri Statistical Package (WASP v. 2.0, ICAR-CCARI, Ela, India).

Results

Effect of Length of Cutting and Pre-Treatments

In case of length of cutting, SNC was found to be poorest for all the studied variables (Fig. 1, Table 1). In terms of rooting percentage, maximum success (84.2%) was observed with DNC, which did not differ

statistically from TNC. Rooting was as low as 35.8%, when single nodes were used for propagation. Numbers of roots per cutting and mean root length were high in DNC (16.0 and 20.0 cm), which remained statistically similar with TNC (14.2 and 17.9 cm). Length of cutting had a marked influence with respect to production of leaves, in which DNC favoured maximum mean leaf production (5.4) followed by triple node cuttings. Dry matter content also increased subsequently from 14.3 to 18.7% with increase in number of nodes in the cutting (Table 1).

Amongst the auxin pretreatments, no significant difference was noticed between rooting in control (69.2%) and 1,000 mg/L of IBA (70.8%). Also, the reasons were not clear about lower rooting (53.3%) at 500 mg/L of IBA treatment. Maximum mean number of roots (14.3) was observed in cuttings dipped in water, which remained statistically similar with higher concentration of IBA. Mean number of leaves per plant (5.1 to 3.6) and corresponding dry matter accumulation (17.8 to 15.7%) decreased with sequential increase in auxin concentration, from 0 mg/L to 1,000 mg/L.

Interaction effect for rooting percentage was significant, and its mean values ranged between 27.5 and 92.5%. Arc sin transformation of data revealed maximum rooting (76.3%), when TNC were dipped in 1,000 mg/L IBA and this treatment did not differ statistically from DNC + 1,000 mg/L IBA (75.9%) and DNC + water (72.3%) combinations. In general,

Table 1: Effect of length of cutting, pre-treatments and their interactions on growth parameters in *Piper sarmentosum* after 60 days of treatment

Variable	Rooting (%)	No. of roots	Root length (cm)	Collar thickness (mm)	No. of leaves	Dry matter (%)
Length of cutting						
Single node (C_1)	35.8 (36.0)	7.8	10.1	4.7	2.8	14.3
Double node (C_2)	84.2 (69.1)	16.0	20.0	4.4	5.4	17.3
Triple node (C_3)	73.3 (61.8)	14.2	17.9	7.9	4.4	18.7
lsd (5%)	9.91	1.93	2.36	0.62	0.60	1.87
F-test	*	*	*	*	*	*
Pretreatment						
Water (P_1)	69.2 (58.2)	14.3	19.4	5.6	5.1	17.8
IBA 500 mg/L (P_2)	53.3 (47.2)	11.3	14.1	4.9	3.9	16.7
IBA 1000 mg/L (P_3)	70.8 (61.5)	12.4	14.4	6.4	3.6	15.7
lsd (5%)	9.91	1.93	2.36	0.62	0.60	1.87
F-test	*	*	*	*	*	*
Interaction effect						
C_1P_1	50.0 (44.9)	11.3	18.5	5.0	4.0	14.5
C_1P_2	27.5 (30.9)	6.7	6.5	3.6	2.4	14.8
C_1P_3	30.0 (32.3)	5.4	5.4	5.3	2.0	13.6
C_2P_1	87.5 (72.3)	15.5	21.0	3.8	5.9	18.2
C_2P_2	72.5 (59.0)	13.7	19.0	3.6	5.2	16.4
C_2P_3	92.5 (75.9)	18.9	19.9	5.7	5.2	17.3
C_3P_1	70.0 (57.5)	16.2	18.8	8.0	5.3	20.8
C_3P_2	60.0 (51.6)	13.5	16.8	7.7	4.1	18.9
C_3P_3	90.0 (76.3)	12.9	18.1	8.1	3.7	16.3
lsd (5%)	17.17	3.35	4.09	1.09	1.04	3.25
F-test	*	*	*	*	*	*

*: Significant at 5% level of significance using least significant difference. Values in the parentheses are arc sin transformed values.

treatments involving SNC were rated inferior for all the parameters, except for root length in which one treatment (C_1P_1) remained statistically similar with C_2P_1. Wide variations existed for number of roots produced per cutting. Maximum roots were produced in DNC treated with 1,000 mg/L of IBA (18.9) and it did not differ from roots produced with TNC + water (16.2) combination.

In case of root length, SNC treated with 1,000 mg/L of IBA exhibited shortest roots (5.4 cm) whereas six other treatments supported root elongation and the maximum value was recorded in DNC + water combination (21.0 cm). Leaf production per plant ranged between 2.0 (SNC + 1,000 mg/L IBA) and 5.9 (DNC + water) and the best treatment remained statistically similar with three other treatments *viz*. C_2P_2, C_2P_3 and C_3P_1. Dry matter content was high in TNC treated with water (20.8%), which did not differ significantly from that observed in DNC + water (18.2%) and TNC + 500 mg/L IBA (18.9%) combinations. Higher concentration and use of SNC adversely affected the dry matter content (13.6%). Considering the overall performance in terms of rooting percentage and growth parameters, two treatments i.e. DNC + water and DNC + 1000 mg/L IBA were rated superior. Higher number of roots produced with latter treatment could help in quicker and better field establishment and hence this could be recommended for large scale multiplication of *P. sarmentosum*.

Effect of Leaf Retention

Second experiment concerned studying the effect of leaf retention on propagation success in *P. sarmentosum* (Table 2). Retention of leaves marginally improved the rooting percentage to 76%, when compared with cuttings without leaves (70%). However, other growth parameters were significantly improved by removal of leaves during preparation of cuttings. Number of roots (13.9) and leaves (4.0) per cuttings improved with leaf removal. Mere 9.0 roots and 2.8 leaves per cutting were noticed in cuttings with retained leaves. Retention or removal of leaves did not affect root length, collar thickness and dry matter recovery percentage.

Discussion

Success of vegetative propagation is influenced by external (environmental) and internal factors of the plant. The process of root induction demands energy that is provided by the stored carbohydrates in the cuttings. Optimum length of cutting could provide required amount of carbohydrates and hormones for the rooting process, while too high or too low contents would not support it.[14] Use of one, two or three node cuttings was reported in different *Piper* species with species-dependent response. In *P. nigrum*, Singh and Singh[11] reported better rooting and plant development with DNC, when compared with longer cuttings; whereas in *P. longum*, use of TNC was found to be superior to those with lower number of nodes.[13] The present investigation revealed that DNC was found to be more appropriate in rooting and growth. Though TNC promoted root induction, growth performance was not always superior.

Use of SNC was reported to be advantageous in black pepper as it increased the availability of cuttings and thereby improved the multiplication ratio.[15] However, during present investigation, SNC was found to be unsuitable for multiplication of this species. Probably, insufficient carbohydrates in SNC and higher amount of water loss (as revealed from low dry matter) might have contributed to poor results in treatments involving single nodes. Results from the earlier reports also suggested the adverse effects of shorter cuttings on sprouting and growth in long pepper.[13]

Table 2: Effect of retention of leaves on growth parameters in *Piper sarmentosum* after 60 days of treatment

Type of cutting	Sprouting (%)	No. of roots	Root length (cm)	Collar thickness (mm)	No. of leaves	Dry matter (%)
With leaves	76.0	9.0±1.31	13.9±2.46	3.7±0.24	2.8±0.20	15.1±1.04
Without leaves	70.0	13.9±1.70	12.6±2.67	4.1±0.14	4.0±0.30	15.3±0.32

Auxins are known to support root induction activity and IBA is one of most commonly used auxins in the vegetative propagation. It improves the metabolite activity and assists the translocation of carbohydrates and other root inducing substances towards the cut ends.[12] In the present study, use of

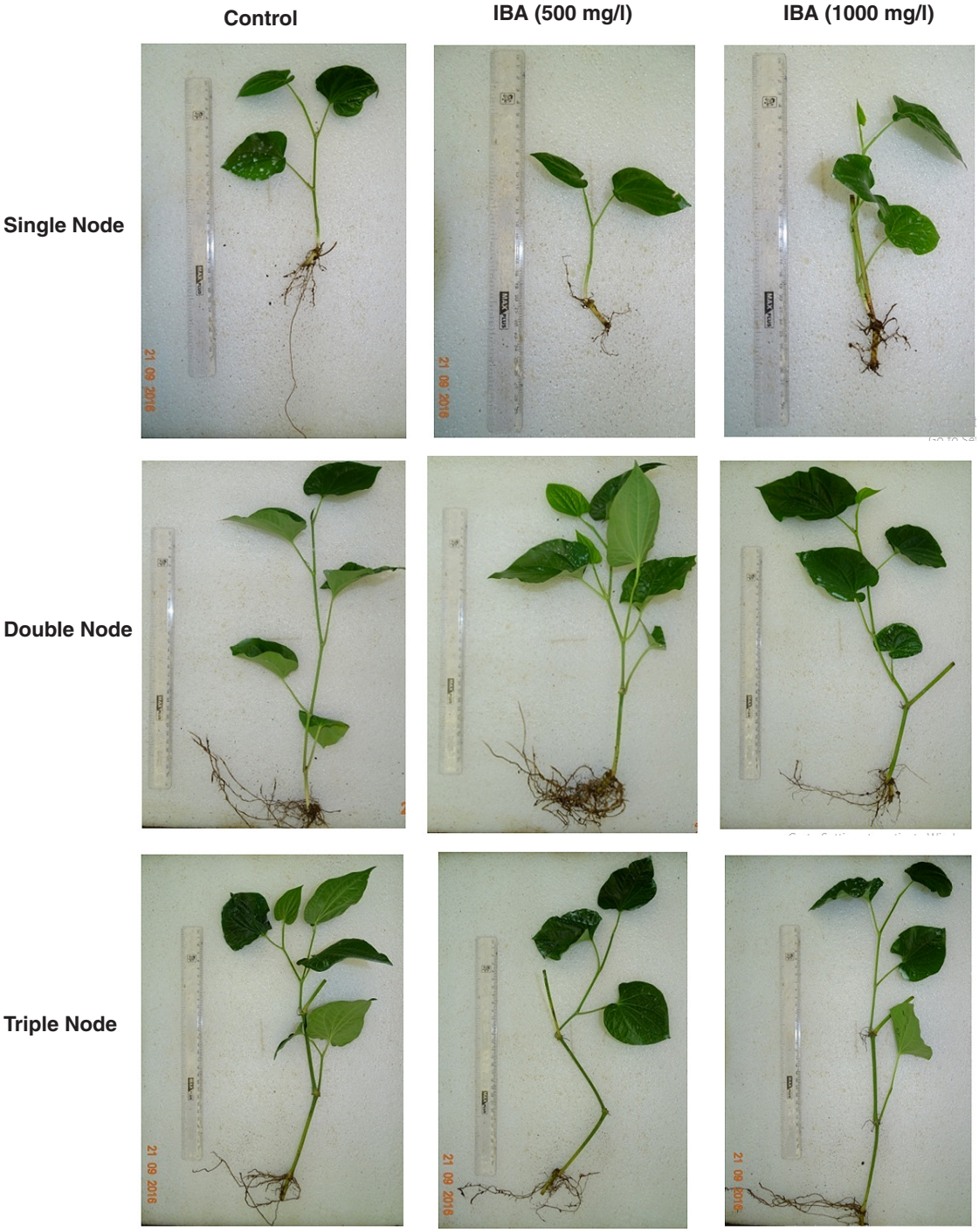

Fig. 1: Interaction effect of length of cutting (single, double and triple node cuttings) and pre-treatments (IBA 0, 500 and 1,000 mg/L) on growth parameters in *Piper sarmentosum* after 60 days of treatment

IBA did not show superior performance as a single factor but the influence was significant when coupled with DNC. Use of 1,000 mg/L IBA promoted better rooting, which is in accordance with earlier report on long pepper.[13] Appreciable rooting response in the absence of auxin noticed during present investigation could be attributed to the presence of endogenous hormones in the species. Similar results were reported in *P. longum* propagated through leaf cuttings.[16]

The practice of leaf retention has been reported to have an impact on rooting process, as the leaves in such cases serve as a source of auxins during root development.[17] However, the transpirational losses from the leaves need to be checked as excessive water loss from the cuttings may result in drying of the cuttings.[18] In the present study, removal of leaves marginally reduced the rooting percentage, but it significantly improved plant growth parameters. The response is purely species dependent, wherein cuttings with reduced number of leaves show better rooting than those with more leaves in some species (e.g. *Annona muricata*),[19] whereas in other species (e.g. *Aristolochia triangularis*) retention of leaves is mandatory for rhizogenesis to occur.[20]

Conclusion

During present study, vegetative propagation was standardized for the first time in *P. sarmentosum*, a medicinally important species. Results suggested that various factors studied *viz*. length of stem cuttings, pre-treatments and leaf retention had profound influence on root induction and growth parameters. Two node stem cuttings treated with 1,000 mg/L IBA could be recommended for large scale multiplication of this species as it helped in improving sprouting percentage and further plant growth. Removal of leaves on the cuttings was beneficial for improving the plant growth.

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Conflict of Interest

Authors declare no conflict of interest.

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