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New technique for adventitious rooting and clonal propagation of *Piper longum* L. (pippali) through leaf cuttings

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A simple but unique protocol was developed for root production and clonal propagation of *Piper longum* L. (pippali), an important medicinal plant of India. Roots and shoots were induced in apical and basal petiolar halves of leaf using auxins. Average root number, root length and survival of rooted leaf cuttings were not significantly affected by type of auxin and leaf cuttings. Highest number of roots (13.40), root length (9.65 cm), rooting behaviour (91.69%) and survival of rooted cuttings (83.33%) were recorded in petiolar leaf cuttings treated with indole 3-butyric acid (IBA)/naphthalene acetic acid (NAA) (1000 ppm each). The petiolar leaf cuttings treated with IBA/NAA showed significantly higher percentage of shooting (83.33). The shoot number (2.0 per cutting) was also significantly highest in IBA/NAA treated leaf cuttings. *Pippali* can be regenerated via leaf-cuttings, either without hormone treatment, or for better results by using low concentrations of IBA or combination of IBA and NAA hormones. Production of planting material using leaf can substitute traditional propagules such as sucker, vine cutting, etc.

Key words: Auxins, indole 3-butyric acid (IBA), medicinal plants, naphthalene acetic acid (NAA), vegetative propagation.

INTRODUCTION

Piper longum L. (long pepper, pippali), an unisexual perennial climber with woody roots belonging to the family piperaceae is distributed throughout India. Almost all parts of it, namely roots, stems and fruits are medicinally important and used especially in the treatment of diseases of respiratory tract like bronchitis, asthma, cough, etc (Sivarajan and Balachandran, 1994). The principal pharmacological constituents are piperine and piplartine. The crude extract of *P. longum* contains 3-8% of piperine (James, 1999).

Collection of roots from wild habitats and deforestation has made this plant species a threatened taxon. As the plants are extracted from its natural habitat for use in drug formulation, the species has become very rare in the forests of Kerala (Nair, 2000). Conventionally, *P. longum* is propagated through seeds, suckers or cuttings or by layering of mature branches at the beginning of rainy season. Although, conventional propagation is beset with problems of poor seed viability, low percentage of germination and scanty or delayed rooting of vegetative cuttings. Therefore, there is a need to explore alternate propagation methods (Sarasan et al., 1993).

Vegetative propagation through leaf cutting can be a suitable way to develop plants economically and in a controlled manner. The advantage of this type of propagation is that, with this technique plants can be raised throughout the year and the mother plant is less disturbed unlike the stem cutting method. Several reports are available regarding vegetative propagation of *P. longum*. Vegetative propagation by the application of root

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Table 1. Experimental layout and allocation of treatments for rooting in leaf-cuttings of *P. longum.*

Leaf cutting	Treatment				
	T_0: Control (without auxins)	T1: IBA (1000 ppm)	T2: NAA (1000 ppm)	T3: IBA (1000 ppm) + NAA (1000 ppm)	Total
Apical halves	30	30	30	30	120
Petiolar halves	30	30	30	30	120
Total	60	60	60	60	240

promoting substances in stem cutting has been studied (Bhat et al., 1995). Soniya et al. (2002) has also reported micro propagation of *P. longum.* However, regeneration of adventitious roots and shoots from the leaf cuttings of *P. longum* at *in vivo* condition has not been attempted. As root of *P. longum* have great medicinal value this study aimed at inducing growth of root. This could be an effective and less expensive way for production of root from leaves of *P. longum*.

MATERIALS AND METHODS

This experiment was carried out during July-September, 2009 at the institutional nursery of Regional Plant Resource Centre, Bhubaneswar, Odisha, India. Leaves were obtained from stock plants grown under shade-net house. Semi-mature and fresh leaves having 5 prominent veins were used for rooting and shooting experiment. A total of 240 leaf-cuttings (both apical and petiolar halves) were prepared out of 120 mature leaves by transverse cutting in the middle of the leaf blade. Two types of common rooting hormones viz. indole 3-butyric acid (IBA) and naphthalene acetic acid (NAA), all at four levels were used as per details given:T_0- Control (without auxins); T_1 - IBA (1000 ppm); T_2- NAA (1000 ppm); T_3- IBA (1000 ppm) + NAA (1000 ppm).

A total of 60 leaf-cuttings were treated with each type of auxin treatment in 3 replications (10 cuttings per replication) (Table 1). The rooting hormones were applied following quick dip method (Basak et al., 2000). The treated cuttings were put (at 45 deg angle) in rooting bed filled with sand:soil mixture (1:1) at the rate of 10 cuttings per block (representing one replication).

Rooting/shooting was recorded after 60 days in respect of the following parameters:

i. Percentage of rooted leaf-cuttings (rooting %)
ii. Root number per cutting
iii. Root length per cutting
iv. Percentage of shoot leaf-cuttings (shooting %)
v. Shoot number per cutting
vi. Survivability of shoot cuttings (survival %)

The data were subjected to analysis of variance followed by Student-Newman-Keuls test using GraphPad Prism (Ver,5,0). All the percentile values were converted into angular transformation for analysis.

RESULTS

Percentage of rooting

The type of auxins applied and leaf cuttings used had no relationship with number of roots developing out of plant ($p>0.05$) (Tables 2 and 3). The percentage of leaf-cuttings producing root was impacted by all four treatments (T_0- Control, T_1 - IBA (1000 ppm), T_2- NAA (1000 ppm), T_3- IBA (1000 ppm) + NAA (1000 ppm). Petiolar leaf-cuttings treated with T3 developed maximum roots (91.69%). Although there was variation in the rooting proficiency in relation to treatments, all leaf cuttings nevertheless developed roots in sand : soil mixture (1:1). But the rooting percentage varied from 70% (in apical leaf cuttings with control) to 91.69% (in petiolar leaf-cuttings with T3).

Root number per leaf-cutting

Average root number was not significantly ($p>0.05$) affected by auxin treatments in leaf-cuttings (both apical and petiolar halves) (Tables 2 and 3). The highest number of roots (13.40) was obtained in leaf cuttings treated with T3 (IBA 1000 ppm). Root number varied from 8.57 (in apical leaf cuttings with control) to 13.40 (in petiolar leaf cuttings treated with T3) (Figure 1).

Effect on length of root

The effect of auxin and leaf parts used (apical and petiolar halves) had no significant ($p>0.05$) relationship on the root length per cutting. Longest roots (9.65 cm) were obtained from leaf-cuttings treated with T3. The average length of roots ranged from 6.18 cm (in apical leaf cuttings with control) to 9.65 cm (in petiolar leaf-cuttings with T3 (Figure 1).

Shooting percentage

The variation in shooting out of leaf cutting was significantly influenced ($p<0.05$) by the type and dosage of rooting hormones (Tables 2 and 3). Petiolar leaf-cuttings treated with T3 exhibited highest percentage of shooted cuttings (83.33%). As seen in the case of rooting, shoots are also formed in leaf cutting treated with auxins irrespective of concentrations. Percentage of shoots in the treated leaf-cuttings ranged from 60% (in apical leaf cuttings with control) to 83.33% (in petiolar leaf-cuttings with T3) (Figure 1).

Table 2. Performance of adventitious rooting and multiple shoots regeneration from the leaf cutting of *Piper longum* as influenced by plant growth regulators.

Parameter/Type of leaf-cuttings	Treatment			
	T_0: (Control)	**T1: (IBA 1000 ppm)**	**T2: (NAA 1000 ppm)**	**T3: (IBA 1000 ppm + NAA 1000 ppm)**
Rooting (%)				
Apical leaf cutting	**70 ± 1.93**	80.24 ± 1.92	78.29 ± 2.52	87.65 ± 2.6
Petiolar leaf cutting	73.33 ± 1.92	81.47 ± 0.98	75.55 ± 4.05	**91.69 ± 2.25**
Root number				
Apical leaf cutting	**8.57 ± 0.43**	11 ± 0.12	11.03 ± 0.12	12.11 ± 0.56
Petiolar leaf cutting	8.73 ± 0.36	11.67 ± 0.52	11.21 ± 0.56	**13.4 ± 0.47**
Root length				
Apical leaf cutting	**6.18 ± 0.48**	8.79 ± 0.53	8.78 ± 0.56	9.1 ± 0.25
Petiolar leaf cutting	6.87 ± 0.43	8.6 ± 0.49	8.23 ± 0.69	**9.65 ± 0.51**
Shooting (%)				
Apical leaf cutting	**60 ± 1.92**	69.99 ± 1.93	63.33 ± 1.92	73.33 ± 1.92
Petiolar leaf cutting	63.33 ± 1.92	76.67 ± 3.85	70 ± 1.93	**83.3 ± 1.93**
shoot number				
apical leaf cutting	1.4 ± 0.06	1.4 ± 0.1	1.5 ± 0.12	1.7 ± 0.06
petiolar leaf cutting	1.6 ± 0.24	1.5 ± 0.15	1.8 ± 0.17	2 ± 0.12
Survival (%)				
Apical leaf cutting	**50.01 ± 3.86**	73.33 ± 1.92	73.33 ± 3.85	80 ± 1.92
Petiolar leaf cutting	53.33 ± 6.94	80 ± 5.17	76.66 ± 1.92	**83.33 ± 2.59**

Table 3. Statistical analysis of variance for different parameters, that is, rooting (%), shooting (%), root number and length, shoot number per leaf cutting of *P. longum.*

Parameter	Source of variation	Df	F	P
Rooting (%)	Treatment	2	0.2565	0.7765
	Type of leaf-cuttings	1	0.2466	0.6255
	No. of replications	2	0.8997	0.8997
Root Number	Treatment	2	0.04909	0.9522
	Type of leaf-cuttings	1	0.001259	0.9721
	No. of replications	2	0.1621	0.8516
Root Length	Treatment	2	0.04321	0.9578
	Type of leaf-cuttings	1	0.04364	0.8369
	No. of replications	2	0.3221	0.7287
Shooting (%)	Treatment	2	**1.155***	**0.3373**
	Type of leaf-cuttings	1	**4.575***	**0.0464**
	No. of replications	2	0.2178	0.8064
Shoot number	Treatment	2	**3.481***	**0.0527**
	Type of leaf-cuttings	1	**5.005***	**0.0382**
	No. of replications	2	0.3913	0.6818

Table 3. Contd.

Survival (%)	Treatment	2	0.4505	0.6443
	Type of leaf-cuttings	1	0.05247	0.8214
	No. of replications	2	0.2512	0.7806

*Significant at 0.05% level of probability.

Figure 1. Induction of adventitious roots and shoots in apical (upper row) and petiolar halves of leaves (lower row) of *P. longum* treated with auxin (T3, IBA/NAA at 1000 ppm each).

Shoot number per leaf-cutting

Average shoot number was significantly ($p<0.05$) affected due to various auxin treatments and varied in different leaf-cuttings (Tables 2 and 3). The highest shoot number (2.0) was obtained in leaf cuttings under the T3 treatment. Shoot number varied from 1.40 (in apical leaf cuttings with control and IBA) to 2.00 (in petiolar leaf cuttings with IBA plus NAA, that is, T3) (Figure 1).

Survival percentage in leaf cuttings

Survival percentage of rooted (and shooted) leaf-cuttings under different auxin treatments and varying doses is presented in Tables 2 and 3. Though survival percentage differed in leaf cuttings treated with different type of auxins and their combined treatments, the differential response was not significant ($p>0.05$). Petiolar leaf cuttings with T3 (IBA 1000 + NAA 1000 ppm) had the highest survival of 83.33%. Whereas the survival percentage of rooted and shooted leaf-cuttings varied from 50.01 (in apical leaf-cuttings under control) to 83.33% (petiolar leaf-cuttings with T3).

DISCUSSION

In this study, adventitious rooting and shooting were induced in leaf-cuttings of *P. longum*. Effect of growth hormones on leaf/petiolar rooting and shooting behavior was assessed under individual and combined treatment of hormones. IBA in combination with NAA was found most effective than their individual effect on rooting. These findings supported the result on adventitious rooting in stem cuttings of some mangrove species (Basak et al., 2000). In leaf cuttings, new growing points usually originate in the parenchymatous tissue closely associated with vascular tissues in the leaves. When vascular bundles of the leaf are severed but suitable growth conditions are provided new roots can be initiated but there was difficulty, in shoot regeneration. Leaf cuttings of most plants do not generate a new plant and produce only a few roots or just decay. Due to the fact

that leaf cuttings do not include an axillary bud, they can be used only for plants that are capable of forming adventitious buds (Hartmann et al., 1996). Probably, *P. longum* has the capacity to form adventitious buds in leaf-cuttings with or without been triggered by exogenous application of auxins.

In conclusion, *P. longum* can be regenerated via leaf-cuttings, either without hormone treatment, or with even better results using low concentrations of IBA or combination of IBA and NAA hormones. The non-significant differences for almost all the studied parameters in treated and control implies that *P. longum* can be propagated vegetatively at reduced cost through leaf cutting. This method, therefore, can be adopted with minimum capital to produce quality planting material.

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