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Studies on novel method of propagation in guava through leaves

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ABSTRACT

In the present study, an attempt at the propagation of Lucknow 49 guava was made during 2020-21 through leaves. The tender just matured leaves (3rd and 4th leaves from the shoot tips) were collected. The petiole portion of the leaves was dipped in 1,000 and 2,000 ppm of Indole-3-butyric acid (IBA) for 1 and 2 minutes; after that, the treated guava leaves were planted in 50 cavities (4.5 cm top diameter, 3.2 cm bottom diameter, 4 cm depth, 50 ml capacity) protrays containing well-decomposed cocopeat (pH-6.7, EC- 0.5 mS/cm, nitrogen - 0.36 %, phosphorus - 0.75 %, potassium - 1.13 %) mixed with *Pseudomonas fluorescens* and *Trichoderma viride* and kept under 50 % shade net. The leaf age and concentration of IBA significantly affected the rooting, shoot formation, and survival of plants propagated through the leaf. Just matured leaves dipped in 2,000 ppm IBA for 1 minute (T11) were found best for rooting (35.17 days), rooting percentage (78.67%), root length (21.67 cm), number of roots/leaf (34.83), time taken for shoot formation (65.33 days), shoot length (12.77 cm) and survival percentage (80.56) of leaf propagated plants. If guava leaf multiplied plants are similar in growth and yield to other vegetatively propagated plants, leaf propagation would be a novel, easy, innovative, and best method of mass multiplication to escape from guava root-knot nematode caused by *Meloidogyne enterolobii*.

Keywords: *Psidium guajava* L., IBA, leaf propagation, root knot nematode

INTRODUCTION

Guava is the fourth most important fruit crop grown in India after Mango, Banana and Citrus. In India, guava is cultivated in an area of 2.87 lakh hectares with an annual production of 43.04 lakh tonnes (National Horticulture Board Database, 2019-20). The wider adaptability and prolific bearing nature make it as a highly remunerative crop with good management practices.

Seed propagation is not preferred due to the long juvenile phase, genetic heterogeneity and variability in fruit yield. There is a great demand for true-to-type guava planting materials (Amit *et al*., 1; Kumar and Syamal, 3, Mamta *et al*., 7; Manna *et al*., 8; Sharma *et al*., 13). It is commercially propagated by ground layering/stooling. But in asexual methods of propagation, soil media carry the nematode along with planting materials. The root knot nematode, *Meloidogyne enterolobii* has become the serious threat for guava orchards (Poornima *et al*., 10) in many guava growing regions of India which spreads rapidly through guava nurseries. Hence, the production of nematode free planting materials is very essential for the sustainable production of guava. For grafting and budding, the suitable nematode resistant rootstocks are essential. Stem cutting is an easy, quick and economical method of propagation. Since guava stems are hard to root, leaves may be another option for propagation. In this situation, air layering, stem cutting and leaf propagation may be highly useful to prevent the spread of nematodes from planting materials, if standardized protocol is available. Keeping in view, the present study was conducted to standardize the technique of nematode free multiplication of guava plants through leaves with the use of root promoting substance i.e. Indole-3-butyric acid.

MATERIALS AND METHODS

The present experiment was conducted at ICAR-Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Tindivanam, Villupuram district, Tamil Nadu during 2020-21, in a Completely Randomized Design, and replicated thrice. The leaves from guava cv. Lucknow 49 were collected during November, and 150 leaf samples per treatment were taken for the study. The tender, just matured leaves (3rd leaf from shoot tip) and matured leaves (4th leaf from shoot tip) of guava 49 were collected in the morning hours, and petiole portion of the leaves were dipped in 1,000 and 2,000 ppm of Indole -butyric- acid (IBA) for 1 and 2 minutes, respectively. After dipping, the leaves were planted in 50 cavity (4.5 cm top diameter, 3.2 cm bottom diameter, 4 cm depth, 50 ml capacity) protrays containing well decomposed cocopeat (pH-6.7, EC- 0.5 mS/cm, nitrogen - 0.36 %, phosphorus - 0. 75 %, potassium - 1.13 %) mixed with *Pseudomonas fluorescens* and *Trichoderma viride,* and kept under 50 % shade net. Watering was done daily using a rose can to keep the

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media moist. The root formation was observed in the leaf petiole from 35th day of planting. The rooted leaves were transferred from protrays to polybags containing potting mixture of red soil, sand and well decomposed farmyard manure (2:1:1) mixed with *Phosphobacteria*, *Potash solubilizing bacteria*, Vesicular Arbuscular Mycorrhizae (VAM) and *Trichoderma viride*. Watering was done daily using a rose can.

From the first experiment, the treatments with successful rooting on just matured leaves (3rd leaf from shoot tip) were taken for further study with 15 replications. The just matured leaves (3rd leaf from shoot tip) from guava cv. Lucknow 49 were collected during January and March in the morning hours, and dipped in 2,000 ppm of IBA for 1 and 2 minutes. After dipping, the guava leaves were planted in 50 cavity **(**4.5 cm top diameter, 3.2 cm bottom diameter, 4 cm depth, 50 ml capacity) protrays containing well decomposed cocopeat (pH-6.7, EC- 0.5 mS/cm, nitrogen - 0.36 %, phosphorus - 0.75 %, potassium - 1.13 %) mixed with *Pseudomonas fluorescens* and *Trichoderma viride* and kept under 50 % shade net (Plate 1). Watering was done daily using a rose can to keep the media moist. The data on days taken for rooting, rooting percentage, number of roots per leaf and root length were recorded at monthly interval for 2 months. After 45 days, the rooted leaves were transferred from protrays to polybags containing potting mixture of red soil, sand and well decomposed farmyard manure (2:1:1) mixed with *Phosphobacteria*, *Potash solubilizing bacteria*, VAM and *Trichoderma viride*. Watering was done daily using a rose can. The data on days taken for shoot formation, number of shoots, shoot length and survival percentage were recorded at monthly interval for 2 months. The data were subjected to statistical analysis (Panse and Sukhatme, 9).

Plate 1. Just matured Guava leaves (3rd leaf from shoot tip) dipped in 2,000 ppm IBA for 1 minute and planted in protrays containing cocopeat

RESULTS AND DISCUSSION

The maturity stages of leaves, concentration of IBA and treatment duration significantly influence the rooting (Table 1)**.** Rooting was observed in the just matured leaves (3rd leaf from shoot tip) dipped in 2,000 ppm IBA dip for 1 minute (T_{11}) and 2 minutes

Table 1. Effect of leaf maturity and concentration of IBA on rooting of guava leaves cv. Lucknow 49

S. No.	Treatments	Rooting in leaf petiole
1.	T_1 : Tender leaves	Leaves dried
2.	T_2 : Just matured leaves (3rd leaf from shoot tip)	Leaves dried
3.	T_3 : Mature leaves (4th leaf from shoot tip)	Leaves dried
4.	T_4 : Tender leaves + 1,000 ppm IBA for 1 minute	Leaves dried
5.	T_5 : Just matured leaves (3rd leaf from shoot tip) + 1,000 ppm IBA for 1 minute	Leaves dried
6.	T_6 : Mature leaves (4th leaf from shoot tip) + 1,000 ppm IBA for 1 minute	Leaves dried
7.	T_7 : Tender leaves + 1,000 ppm for 2 minutes	Leaves dried
8.	T_8 : Just matured leaves (3rd leaf from shoot tip) + 1,000 ppm for 2 minutes	Leaves dried
9.	T_9 : Mature leaves (4th leaf from shoot tip) + 1,000 ppm for 2 minutes	Leaves dried
10.	T_{10} : Tender leaves + 2,000 ppm IBA for 1 minute	Leaves dried
11.	T_{11} : Just matured leaves (3rd leaf from shoot tip) + 2,000 ppm IBA for 1 minute	Rooted
12.	T_{12} : Mature leaves (4th leaf from shoot tip) + 2,000 ppm IBA for 1 minute	Leaves dried
13.	T_{13} : Tender leaves + 2000 ppm IBA for 2 minutes	Leaves dried
14.	T_{14} : Just matured leaves (3rd leaf from shoot tip) + 2,000 ppm IBA for 2 minutes	Rooted
15.	T_{15} : Mature leaves (4th leaf from shoot tip) + 2,000 ppm IBA for 2 minutes	Leaves dried

(T_{14}). The complete drying of the tender and mature leaves was observed irrespective of treatment. Auxin is synthesized in young leaves and then transported downwards. Auxin moves from cell to cell in a polar fashion, with a basipetal polarity in stems (Lomax *et al*., 4). The absence of rooting in tender leaves may be due to higher auxin concentrations from natural auxin synthesis and exogenous application of IBA. The possible reason for the drying of young and mature leaves might be due to the presence of polyphenols which act as auxin transport inhibitors (Brown *et al*., 2).

Dipping of just matured leaves in IBA significantly influence the rooting, number of days for rooting, rooting percentage, root length and number of roots per leaf. The mean values of rooting from January and March planting are presented in Table 2. The just matured leaves (3rd leaf from shoot tip) dipped in 2,000 ppm IBA dip for 1 minute (T_{11}) was found best for rooting (35.17 days), rooting percentage (78.67%) in guava leaves followed by just matured leaves (3rd leaf from shoot tip) dipped 2,000 ppm IBA for 2 minutes (T_{14}). The leaves are one of the production sites of natural auxins (Wahab *et al.,* 16). The successful rooting in just matured leaves may be due to availability of sufficient auxin from accumulation of natural auxins and application of 2,000 ppm of IBA for 1 minute. Poor rooting (9.33%) in just matured leaves dipped in 2,000 ppm IBA for 2 minutes might be due to the over absorption of auxin, causing inhibitory action on rooting. Climate of the region and media plays a significant role in realizing better success rate (Rymbai and Satyanarayana Reddy, 12). Shade intensity under shade net conditions would have influenced the rooting of the guava (Manga and Jholgiker, 6).

Root length and number of roots were significantly influenced by the maturity stage of guava leaves, growth regulator concentration, treatment duration and interaction between auxins and cytokinins present in the leaves. The just matured leaves (3rd leaf from shoot tip) dipped in 2,000 ppm IBA for 1 minute (T_{11}) was found best for rooting length (21.67 cm) and number of roots (34.83) on 60th day of planting of guava leaves. The physiological state of plant parts (Luis *et al*., 5), concentration of plant growth regulators (Singh *et al*., 14), kind of auxin (Kumar and Syamal, 3), climate and media (Rymbai and Satyanarayana Reddy, 13) have a significant effect on rooting.

After 45 days, the rooted leaves were transferred from protrays to polybags containing potting mixture of red soil, sand and well decomposed farmyard manure (2:1:1) mixed with *phosphobacteria, potash solubilizing bacteria*, VAM and *Trichoderma viride* (Plate 2 & 3). The mean values of shoot formation from January and March planting are presented in Table 3. Shoot length was significantly influenced by the leaf maturity, concentration of IBA and treatment duration. The treatment T_{11} proved best for shoot formation (65.33 days) in the rooted guava leaves. Single shoot development was observed from the rooted guava leaves (Plate 3). Cytokinin is synthesized in

Table 2. Effect of IBA on rooting of just matured leaves of guava cv. Lucknow 49.

S. No.	Treatments	Time taken for rooting (days)	Rooting (%)	Root length (cm)		Number of roots/leaf	
				30th day	60th day	30th day	60th day
1.	T_{11} : Just matured leaves (3rd leaf from shoot tip) + 2,000 ppm IBA for 1 minute	35.17	78.67	4.83	21.67	9.50	34.83
2.	T_{14} : Just matured leaves (3rd leaf from shoot tip) + 2,000 ppm IBA for 2 minutes	37.33	9.33	4.33	21.33	8.83	34.50
	Mean	36.25	44.00	4.58	21.50	9.17	34.67
	$CD_{(0.05)}$	1.85	15.32	0.42	0.24	0.46	0.23

Table 3. Shoot formation from rooted leaves and survival percentage of leaf propagated plants in guava cv. Lucknow 49.

S. No.	Treatments	Time taken for shoot formation (days)	No. of shoots/ leaf	Shoot length (cm)	Survival (%)	Time to attain saleable size (days)
1.	T_{11} : Just matured leaves (3rd leaf from shoot tip) + 2,000 ppm IBA for 1 minute	65.33	1.00	12.77	80.56	110.45
2.	T_{14} : Just matured leaves (3rd leaf from shoot tip) + 2,000 ppm IBA for 2 minutes	63.17	1.00	11.30	50.90	120.67
	Mean	64.25	1.00	12.03	65.73	115.56
	$CD_{(0.05)}$	2.14	0.01	0.95	6.31	4.82

Plate 2. Rooting in just matured Guava leaves (3rd leaf from shoot tip) dipped in 2,000 ppm IBA for 1 minute.

Plate 4. Well developed plants from leaves in guava cv. Lucknow 49.

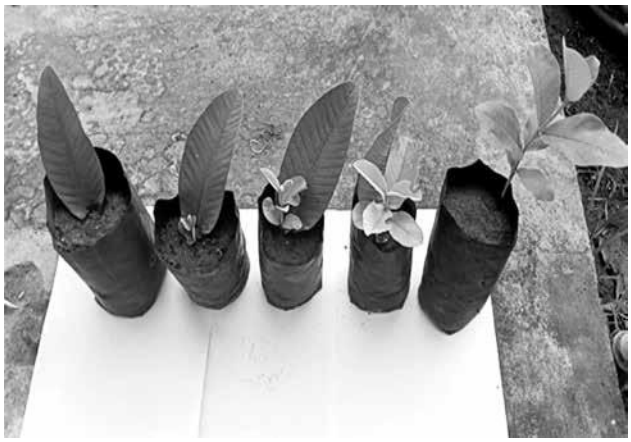

Plate 3. Different stages of leaf propagation in guava cv. Lucknow 49.

Plate 5. Leaf propagated guava cv. Lucknow 49 planted in the field.

the leaves, and regulates apical dominance (Tanaka *et al*., 15), root proliferation and shoot growth. The leaf propagated plants observed similar to seed propagated plants in appearance (Plate 4). The growth of the shoot was better in treatment, T_{11} (12.77 cm), followed by treatment, T_{14} (11.30 cm) than other treatments. The application of IBA influenced the survival of guava plantlets in stooling method of propagation under open and poly house conditions (Rymbai and Reddy, 11).

The best performing treatment for rooting and shoot formation, T_{11}-Just matured leaves (3rd leaf from shoot tip) dipped in 2,000 ppm IBA for 1 minute recorded the highest survival percentage of 80.56 (Table 2) and attained the saleable size in 110-120 days. The leaf propagated guava plants were planted in the field (Plate 5) for further study on growth, flowering and yield.

The maturity stages of guava leaves showed significant influence on rooting and shoot formation. The just matured leaves (3rd leaf from shoot tip) dipped in 2,000 ppm IBA for 1 minute showed the highest survival percentage of 80.56, and attained the saleable size in 110**.**45**-**120**.**67 days. The leaf propagated guava plants are to be tested for further growth and yield in the main field and found to perform similar to commercially propagated plants, leaf propagation will be a novel, easy, innovative and best method of propagation to prevent the spread of guava root knot nematode through planting materials and mass multiplication of new varieties and rare species.

AUTHORS' CONTRIBUTION

Conceptualization of research (RN, LP, CI), Designing of the experiments (RN), Contribution of experimental materials (PS), Execution of experiments

and data collection (RN), Analysis of data and interpretation (RN, LP, CI), Preparation of manuscript (RN, CI).

DECLARATION

The authors declare that they have no conflict of interests.

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