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Leaf Propagation in Guava (*Psidium guajava* L.)- An Unique Approach for Producing Quality and Nematode-free Planting Materials

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ABSTRACT: Guava is the fourth most important fruit crop grown in India. In the existing commercial propagation method of ground layering/stooling, root knot the nematode is transferred through soil media along with planting materials and it is becoming the serious threat for guava orchards. The production of nematode free planting materials without carrying from the infested soil from mother plants is very essential for the sustainable production of guava. A study was undertaken to develop a new propagation method for producing nematode free planting materials in guava. The fourth mature leaf from the shoot tipin guava cv. Lucknow-49, Allahabad Safeda and Arka Kiran were collected in the early morning hours during the month of November 2021. The leaves were washed with running water followed by quick dip of the petiole portion in 1% Bavistin followed by dipping in 500, 1,000 and 1,500 ppm of Indole-3-Butyric Acid (IBA) for one and two minutes. The treated guava leaves were then planted in 50 cavity protrays containing well decomposed cocopeat (pH7) mixed with *Pseudomonas fluorescens* and *Trichoderma viride* and placed in a small polytunnel under shade netat a temperature of 28-30°C and relative humidity of 75±5% were maintained which is congenial for rooting. The leaves of Lucknow-49 rooted when treated with 1,500 ppm IBA dipped for 1 and 2 minutes with rooting percentage of 70% and 72% respectively. These rooted leaves were further treated with benzyl adenine at different concentration (100, 200, 300, 400 and 500 ppm) for better shoot formation. Rooted leaf treated with 300 ppm BA for one minute recorded better result as it took less time for shoot formation (28.40 days) and survival percentage of 80% was recorded.

Keywords: Guava, leaf, propagation, IBA, BA, shade net and polytunnel.

INTRODUCTION

Guava (*Psidium guajava* L.) also known as "Apple of tropics" is one of the most common and popular fruits grown in tropical and sub-tropical regions of India. Owing to its luscious, wider adaptability, prolific bearing and high remunerative in nature it is popular across the world and also due to its availability round the year and reasonable price it is called as Poor man's apple (Das *et al*., 1995; Brijesh *et al.,* 2014).

In guava, various drawbacks are reported in conventional methods of propagation (air-layering, stooling, ground layering). Seed propagated plants have various disadvantages such as a long juvenile phase, lack of true-to-type progeny, genetic heterogeneity, segregation and recombination of characters (Martínez-De-Lara *et al*., 2004; Soni *et al.,* 2016). In asexual method of propagation namely stooling, ground layering, budding, grafting, stem cutting, air layering and inarching are still not commercially feasible because of various disadvantages. In ground layering and stooling, there are more possibilities that soil media might carry nematodes along with the planting materials. When these planting materials are used, it becomes very difficult to manage nematode as it rapidly spreads and leads to heavy loss in production (Poornima *et al.,* 2016). In case of budding (Gupta and Mehrotra (1985, Kaundal *et al.,* 1987), air layering (Sharma *et al.,* 1978; Manna *et al.,* 2001) and inarching (Mukherjee and Majumder 1983) it has been reported that all these methods are time-consuming, wax and wane in success percentage, laborious, expensive, absence of tap root system and uneconomical (Soni *et al.,* 2016; Singh *et al.,* 2019). Stem cutting is an effortless method but due to guava stem being hard to root this is also not suitable therefore leaves can be an option for propagation. As root formation in the leaf is

a key step in fruit crops' vegetative propagation. The mechanism of root development could be divided into three stages: root induction, root initiation and root protrusion and all these stages are regulated through auxins. Owing to natural auxin synthesis in leaves and stem tip leading to more chances for root formation in leaf (Ljung *et al.,* 2001). Hence, the objective of this experiment was framed in such a way to develop a new commercial method of clonal multiplication in guava.

MATERIALS AND METHODS

The research was carried out at Horticultural College and Research Institute, TNAU, Coimbatore, Tamil Nadu during 2020-21 in a Factorial Completely Randomized Design with five replications (10 leaves per replication) and two factors (F1: Varieties and F2: Plant growth regulators at different concentration).

Mother block for each variety (Lucknow 49, Allahabad Safeda and Arka Kiran) are maintained within the premises of nursery. Mother block is one of the most important inputs which decides the fate of production efficiency of fruit orchard. They are planted at a closer spacing of 2x1m in order to accommodate more number of plants and to get continuous supply of propagation material. These plants are severely pruned once in a year in the month of February to keep them in vegetative phase to produce enough shoots for propagation purposes. They are maintained rigorously so as to keep the plants healthy and free of diseases and insect pests.

Leaves for the purpose of this experiment were collected from the mother block mentioned above. 50 leaves from guava cv. Lucknow-49, Allahabad Safeda and Arka Kiran were collected and used in this study.

The 4th mature leaves were collected from the shoot tip of current season growth during morning hours which were then washed under running water followed by a quick dip at 1% Bavistin solution prior to planting. The petiole portion of leaves was dipped in 500, 1,000 and 1,500 ppm of Indole butyric acid (IBA) solutions for 1 and 2 minutes. 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 (pH7) mixed with *Pseudomonas fluorescens* & *Trichoderma viride*, and kept in a small polytunnel under shade net. Leaves were irrigated alternately using as prayer and frequent inspection was done to check for any kind of deformity. From the 30th day of planting, root formation was observations on root formation in leaf petiole were recorded. The rooted leaves were then dipped in 100, 200, 300, 400 and 500 ppm of benzyl adenine (BA) solution. The treated rooted leaves were transferred into polybags containing a potting mixture of red soil, sand and farmyard manure (2:1:1). Irrigation was done by sprayer when it is required.

For root formation on leaves, data observed were number of days taken for rooting, rooting percentage and number of roots per leaf and root length (cm). For shoot formation, data observed were number of days taken for shoot formation, shoot formation (%), shoot length (cm) and survival percentage (%).

Factor 1

V_1: Lucknow 49
V_2: Allahabad Safeda
V_3: Arka Kiran

Factor 2

G_1: 500 ppm IBA
G_2: 1,000 ppm IBA
G_3: 1,500 ppm IBA

RESULTS AND DISCUSSION

Guava is a crop that is conventionally propagated by stooling but due to compromise in the quality of planting material as well as nematode infection, leaf propagation which has not been commonly used in fruit crops was given a trial in this experiment in order to get a perception on its success rate that can be a novel technique for producing a good quality planting materials as observed by Neelavathi *et al.,* 2021. The present experiment was carried out at a shade net where the optimum temperature (28-30^{o}C) and humidity (75±5%) required for successful rooting of the leaves were maintained by making a tunnel inside the shade net using polyethylene sheet of 200 microns (Fig.1)without which required humidity was not possible to maintain under only a shade net. Rymbai and Satyanarayana Reddy (2010) also reported that climate and media plays a crucial role in rooting. Irrigation water also plays an important role in the success of this experiment as the salt content in the water of Tamil Nadu is high which leads to the burning of leaves. Therefore, Siruvani water (The world's 2nd tastiest water) was used for irrigating the leaves on an alternate basis with the help of a sprayer as the leaves are very brittle at the initial stage of propagation. In order to maintain the humidity of the experimental site, water was sprayed on the ground as well as the wall of the shade net. The success of the propagated leaves was being judged by visual appearance. The change in colour of the midrib and vein to yellow was observed after 15 days of planting (Fig. 2). For this experiment, 4th matured leaf from shoot tip from three different varieties were taken and treated with different concentrations of IBA. Out of these three varieties taken for the study, only Lucknow 49 treated with 1,500 ppm of IBA (dipped for 1 and 2 minutes) rooted in 33.80 days and 32.60 days respectively and the rest of the leaves dried (Table 1), which may be due to the varietal differences, lower concentrations of exogenous IBA and the presence of auxin inhibitor biochemical compounds (Lomax *et al.* 1995) and also due to the lack of endogenous auxin synthesis. Treatment duration highly stimulates cambial activity thereby resulting in the mobilization of reserve food material to the site from the leaf to the petiole through the midrib and veins that enhance earlier root formation (Shahzad *et al.,* 2019). IBA is a non-toxic auxin (Hartmann *et al.*, 2002)

and effective in encouraging the rooting of a large number of plant species (Teklehaimanot *et al*., 1996).

Higher rooting percentage was observed in T_7 (72%) followed by T_6 (70%). Root length and number of roots per leaf were measured at 30th and 60th day of planting and has been found to differ due to the treatment duration as well the maturity stage of leaf *i,e.,* 4th mature leaves from shoot tip. Survival percentage recorded the highest in T_7 (82.15%) followed by T_6 (79.38%). Interaction between auxin and cytokinin plays an important role in root and shoot regeneration. The fourth matured was leaf dipped in 1500 ppm IBA for 2 minutes (T_7) recorded highest root length (19.08 cm) and number of roots per leaf (31.58) on 60th day after planting (Table 2, Fig. 3). Similar result was observed in grape cutting at higher concentrations (Shahzad *et al.,* 2019). A tremendous increase in the root length and number of roots per leaf was observed after 30th day of planting as the rooted leaves were carefully uprooted from protrays and treated with BA for initiation of shoot formation. The rooted leaves were treated with 200 and 300 ppm of BA dipped for one minute. Cytokinin such as BA increases biosynthesis of nucleic acids and mitotic activity in apices of buds those responsible for shoot formation (Chvojka 1964). Higher concentration of BA (300 ppm) was found to produce better result in terms of time taken for shoot formation (28.40 days after treatment), shoot formation percentage (83.33%), number of shoot per rooted leaves (4) and survival percentage (80%) (Table 3). Exogenous application of BA promotes shoot regeneration (Cornejo-Martin *et al.,* 1979). Similar studies were conducted using cytokinin in *Rudbeckia laciniata,Ruta graveolens*, *Gratiola officinalis* which plays an important role in shoot formation (Custers 1986). The ratio of auxin-cytokinin I s an important factor to be considered for root and shoot formation as the leaves were initially treated with IBA which were further treated with BA on 30th day of planting might be the reason for its interaction in formation of successful root and shoot propagated through guava leaf. Exogenous application of BA promoted shoot formation but this action appeared to depend on the presence of other regulators in the medium and also on the plant species used (Cornejo-Martin *et al.,* 1979; Van Aartrijk *et al.,* 1985).

Table 1: Effect of IBA on rooting of 4th mature leaves of guava Cv. Lucknow 49.

Sr. No.	Treatments	Rooting in leaf petiole
1.	**T_1:** 4th Mature leaf from shoot tip (without growth regulator)	Leaves dried
2.	**T_2:** 4th Mature leaf from shoot tip + 500 ppm IBA for 1 minute	Leaves dried
3.	**T_3:** 4th Mature leaf from shoot tip + 500 ppm IBA for 2 minutes	Leaves dried
4.	**T_4:** 4th Mature leaf from shoot tip + 1,000 ppm IBA for 1 minute	Leaves dried
5.	**T_5:** 4th Mature leaf from shoot tip + 1,000 ppm IBA for 2 minutes	Leaves dried
6.	**T_6:** 4th Mature leaf from shoot tip + 1,500 ppm IBA for 1 minute	Rooted
7.	**T_7:** 4th Mature leaf from shoot tip + 1,500 ppm IBA for 2 minutes	Rooted

Table 2: Effect of IBA on rooting of 4th mature leaves of guava Cv. Lucknow 49.

Sr. No.	Treatment	Time taken for rooting (days)	Rooting %	Number of roots/leaf		Root length(cm)	
				30th day	60th day	30th day	60th day
1.	T_6: 4th mature leaves from shoot tip + 1,500 ppm IBA for 1 minute	33.80	70	9.40	31.40	4.12	20.40
2.	T_7: 4th mature leaves from shoot tip + 1,500 ppm IBA for 2 minutes	32.60	72	10.20	31.80	4.04	19.08
	Mean	33.2	71	9.8	31.60	4.08	19.74
	SEd	0.14	0.18	0.21	0.29	0.07	0.12
	CD (0.05 %)	0.29	0.36	0.42	0.59	0.14	0.25

Table 3: Effect of BA on shoot formation of rooted guava leaves.

Sr. No.	Treatment	Time taken for shoot formation (days)	Shoot formation %	Number of shoots/rooted leaf	Shoot length (cm)	Survival %
1.	T_5: Rooted leaf + 200 ppm BA for 1 minute	30.20	80.00	3.80	13.22	78.28
2.	T_8: Rooted leaf + 300 ppm BA for 1 minute	28.40	83.33	4.00	11.72	80.00
	Mean	29.30	81.66	3.90	12.47	79.14
	SEd	0.22	0.23	0.21	0.19	0.20
	CD (0.05 %)	0.46	0.49	0.45	0.41	0.43

Fig. 1. Fourth mature guava leaves from shoot tip planted in protrays and kept under polytunnel.

Fig. 2. Change of leaf midrib and vein colour to yellow after 15 days of planting.

Fig. 3. Rooting of leaf propagated guava plants

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

CONCLUSIONS

From the present study, it is concluded that fourth mature leaves from shoot tip dipped in 1,500 ppm IBA for 2 minutes (T_7) and rooted leaves dipped in 300 ppm BA for 1 minute (T_8) showed significant effect on rooting and shoot formation respectively. The highest rooting percentage of 72% was obtained from T_7 which was recorded on 30thday after planting whereas survival percentage of 80% was obtained from rooted leaves treated with T_8 which can be further improved by maintaining accurate temperature and humidity along with the good quality of irrigation water. This technique easily fulfills the quality and quantity of planting materials within a short period of time. As compared to other methods, it is simpler, less labor-intensive, true to type, early bearing, economical and free of nematodes.

FUTURE SCOPE

Further study on different treatment combinations of growth regulators and media can be taken up as they play a crucial role in increasing its success percentage. A comparison study of different propagation techniques can also be under taken. Field trials of leaf propagated plants will provide a broader knowledge of its acceptability as a commercial method of propagation for producing quality planting materials.

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