AIR LAYERING IN GUAVA AS INFLUENCED BY GROWTH REGULATORS AND AZOSPIRILLUM

Thesis submitted to the
University of Agricultural Sciences, Dharwad
in partial fulfillment of the requirements for the
Degree of

MASTER OF SCIENCE (AGRICULTURE)

IN

HORTICULTURE

By

SANJEEV KUMAR

DEPARTMENT OF HORTICULTURE
COLLEGE OF AGRICULTURE, DHARWAD
UNIVERSITY OF AGRICULTURAL SCIENCES,
DHARWAD – 580 005

JUNE, 2011

ADVISORY COMMITTEE

DHARWAD **(S. G. ANGADI)**

JUNE, 2011 **MAJOR ADVISOR**

Approved by :

Chairman : _____
(S. G. ANGADI)

Members : **1.** _____
(J. C. MATHAD)

2. _____
(U. V. MUMMIGATTI)

3. _____
(C. R. PATIL)

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDIX

I. INTRODUCTION

The guava (*Psidium guajava* L.) is one of the hardy fruit crops being cultivated throughout Karnataka state. It is native of tropical America and is widely distributed throughout the tropical and sub tropical regions of the world. Guava is fourth most important fruit in area and production after mango, banana and citrus in India. Guava shares 3.3 per cent of area and 3.3 per cent of production of total fruit crop grown all over India. Guava is 5th in productivity among different fruit crops grown in India. It is being cultivated in India on 2.04 lakh hectares area with an annual production of 22.70 lakh tonnes (Salaria and Salaria, 2010). Uttar Pradesh leads in area and production while Karnataka leads in productivity (21.6 t/ha) of guava.

Uttar Pradesh is the highest guava producing state, accounting for about half of the total area of guava in the country. Allahabad has the reputation of growing the best guava of the world. The other important guava growing states are Karnataka, Bihar, Madhya Pradesh and Maharashtra. In Karnataka, Allahabad safeda, sardar (Lucknow-49) and red fleshed are the important grown varieties of guava.

Guava is considered as “common man’s apple” and ‘the apple of tropics’ because of its availability for a longer time during the year at very moderate price. The major components of guava fruits are vitamin ‘C’ (250 mg/100 g fresh fruits), carbohydrates (13%) and minerals (calcium 29 mg, phosphorus 10 mg and iron 0.5 mg/100 mg fresh fruits). It is a very rich and cheap source of vitamin ‘C’ as it contains 4-6 times more vitamin ‘C’ than citrus fruit. Guava fruits are rich in pectin content, hence are extensively used in preparation of jelly. Besides, its diabetic value, the fruit also is used in preparing jelly, cheese, butter, paste, juice, juice concentrate, powder, canned slice/shell, nectar, puree and ice cream.

Guava being a hardy crop is grown in variety of soils and climatic conditions. However, the suited are red loams, medium black and other well drained soils.

The guava plants can be propagated by several ways such as seed, cuttings, air layers, grafting *etc*. The seed propagation was wide spread earlier is now restricted to raising of rootstock material. The vegetative propagations by air layering are becoming more and more popular on account of their cheaper cost and easy method. They also have better success obtained. However, greater deal of variation in per cent success is observed in air layering. One of the causes for variation has been observed to be the age of shoots/trees used in air layering. Although germplasm trees are known to give higher success, use of such trees in commercial nursery is rather limited. Most of the nurserymen use mature trees which are also used for commercial fruit production obviously the percentage of success is lower in air layers in such trees on account of exhaustion by crop load. It is possible that if the trees, especially the mature germplasm trees are exclusively used for air-layering or making cuttings, the percentage of success would not only be higher but also quicker which goes a long way in making available genuine planting material (air-layering) in abundance to meet the ever increasing demand.

The rooting ability of air layered shoots is decided by several factors that vary with the crops, cultivar and biochemical constituents of the clone (*viz*., carbohydrates, nitrogen, sugars, starch, phenols, auxins levels *etc*.) and the climatic conditions prevelling in the season (*viz*., temperature, relative humidity, rain fall *etc*.) of layering. All these factors should be at optimum level to attain better rooting of a guava layers.

Kunal Kumar and Syamal (2005) observed that the highest number of primary roots was produced using IBA 3,000 ppm treatment followed by NAA 2,000 ppm. High number of roots was recorded with IBA + NAA (1:1) at 2,000 ppm each. Etiolation along with exogenous application of auxin had stimulating effect on producing longer roots. The longer primary roots of 11.30 cm were obtained with 3,000 ppm of IBA followed by NAA at 2,000 ppm, each produced 9.17 cm long primary roots. Etiolation along with auxins treatment had marked influence on rooting of air layers. Use of IBA 3,000 ppm had maximum success of 93.34 per cent followed by NAA 1,000 ppm with a success of 86.68 per cent.

Azospirillum is nitrogen fixing bacterium that lives in symbiotic (associative) relationship in the rhizosphere of several tropical crops. It stimulates plant growth through nitrogen fixation and production of growth promoting substances like auxins, gibberellins and cytokinin. It is estimated that almost 10 to 15 % of the required nitrogen can be met by *Azospirillum* (Tanuja and Purohit, 2008).

Govind and Pandey (1985) have found that pepper cuttings inoculated with *Azospirillum* spp. had higher rooted cuttings, length of sprout, more number of fully opened leaves. According to them the bacteria apart from producing root hormone (IAA) also synthesised gibberellic acid which had enhanced vegetative growth.

Keeping these points in view, the present study was undertaken with the fallowing specific objectives:

i. To study the effect of growth regulators and microbial inoculants at different concentrations on rooting of guava air layers.
ii. To study the effect of *Azospirillum* on growth and vigour of air layers in guava.
iii. To find out bio - chemical parameters involved in rooting of guava air layers.
iv. To find out best combination of growth regulators and microbial inoculants for increasing survival percentage of guava air layers in nursery.

II. REVIEW OF LITERATURE

The special significance of vegetative propagation in the maintenance of true-to-type plants is well recognised in horticulture crops. In the recent years many of the horticulturally important plant species which are difficult-to-root are made to root easily by use of root inducing chemicals and microbial inoculant. Such chemicals and microbial inoculant have become so popular that they find their use even in easy-to-root plants in order to get faster and denser rooting.

Commercially, guava is propagated by air-layering with the varying degree of success. Moreover, the process of rooting in the absence of root promoting treatments is known to be slow.

The relevant literature on propagation by air-layering in guava as well as in other related horticultural crops are briefly reviewed under different headings as mentioned below.

2.1 Role of growth regulators in rooting

Bhandary and Kololgi (1960) stated that Indole Butyric Acid (IBA) and Naphthelene Acetic Acid (NAA) in combination were good for early rooting of guava air-layers (variety Lucknow-49) during February to August period and reported that, 10,000 ppm and 15,000 ppm concentrations were optimum to induce rooting in shortest period of five to six weeks. It was also reported that 20,000 ppm had toxic effect while 5,000 ppm was found to be sub-optimal. Mixtures of IBA and NAA each at concentrations of 5,000 ppm gave highest number of primary, secondary and tertiary roots. Bhujbal (1972) reported 86.60 per cent rooting during July in guava variety Lucknow-49 air-layers when treated with IBA at 3,000 ppm. Similar results were also obtained in guava by Patel *et al.* (1989).

Anonymous (1961) has pointed out that June was found to be the best month for air-layering in guava and the growth substances used were an equal mixture of NAA and IBA in talc, at 10,000 ppm which was found to be the best concentration tested. The rainy season (August-September) proved more favourable compared to spring. Sixty eight per cent of the layers done during rainy season showed callus development and root initiation within a month compared to 30 to 40 per cent in spring (Ahamed, 1964).

Application of two per cent of IBA or NAA in lanolin paste singly or in combination induced better rooting in guava (Singh and Singh, 1970). Verma *et al.* (1970), observed a strong synergistic effect of IBA and NAA combination at 7,500 ppm in mango, guava, citrus and marcots.

Sadhu *et al.* (1972) revealed that, P-hydroxybenzoic acid alone showed a small increase in the rooting of guava layers but in combination with IBA it showed a fourfold increase in the number of roots. While, another synergist, salicylic acid, on the other hand inhibited rooting singly and in combination with IBA.

Nanda (1975) reported that the air-layers of guava cv. 'Sardar' gave low percentage of rooting without growth regulator IBA.

According to Sharma *et al*. (1978) guava shoots treated with 100 and 200 ppm of IBA took 35 and 31 days respectively for root initiation. While in untreated shoots the root initiation was observed after 45 days. Early and higher rooting was observed with layers done during July compared with layers done during winter months.

Venkatesh (1983) found that pre girdling and etiolation with IBA treatment recorded the maximum percentage of rooting in guava. The average root number and length of roots were the highest in shoots that were pre girdled, etiolated and treated with IBA.

Sharma *et al.* (1991) reported that layers treated with 10,000 ppm IBA resulted in better rooting in guava as compared to other concentrations of IBA and NAA. The callus formation, percentage success, root parameters such as number, length, diameter and fresh weight of primary as well as secondary roots were higher in layers treated with 10,000 ppm IBA as compared to NAA at 10,000 ppm.

The air layers were treated with sucrose (5 or 10 %) with or without coconut milk, IBA (4000 and 8000 ppm) or 4000 ppm IBA + 4000 ppm IAA (Ram Chandra and Sheo Govingd, 1993). The highest number of primary and secondary roots and the maximum primary root diameter were obtained with the IBA + IAA treatment. Greatest primary root length was obtained with 5 per cent sucrose treatment.

Mahabir Singh *et al*. (1995) investigated on the effect of IAA, IBA, NAA, IBA + NAA + IBA or NAA + IBA + NAA (2500, 5000 or 10000 ppm) and white or black polythene wrappers on rooting, growth of air layers of guava cv. Allahabad Safeda. The best plant growth regulator treatment for air layering found was IBA + NAA in most respects and this treatment was on par with IAA + IBA + NAA. Rooting and growth was found to increase with increasing concentration of plant growth regulators.

Mishra and Singh (1995) opined that multiple air-layering is feasible for increasing the efficiency of guava propagation.

Patel and Pasaliya (1995) investigated on the effect of IBA, NAA / IAA at 3000, 6000 / 9000 ppm applied immediately after ringing or 10 or 20 days later on rooting in air-layered shoots of guava cv. L- 49. It was observed that NAA at 9000 ppm applied immediately after ringing gave the highest number of primary and secondary roots and heaviest root fresh and dry weights.

A study was conducted to investigate the effect of different concentrations of IBA and NAA on the propagation of Feijoa cv. Nikitskii by stooling (Pandey, 1996). Rooting and survival percentage were the highest (71.96 and 75.83%, respectively) with the application of 1000 ppm IBA.

Patel *et al.* (1996) reported that IAA + IBA promoted per cent rooting, number of roots, length of roots. IBA alone was better than IAA. The best concentration of plant growth regulators for promoting rooting and growth of guava air-layers was 3000 ppm.

Kamleshkar Singh and Jain (1996) concluded that in guava (cv. Allahabad safeda), the highest (78.75%) per cent of rooting, number of primary and secondary roots, length of the longest root were obtained when the etiolated shoots were treated with IBA @ 6000 ppm during the month of July.

Singh *et al*. (1996) reported the best rooting (97.80%) in stool-layers of Sardar guava when treated with 7,500 ppm IBA.

Singh and Singh (1996) reported that 5000 ppm of IBA produced the maximum per cent of rooted air layers (74.99) over all other levels of IBA in guava (*Psidium Guajava* L.) cv. Allahabad surkha.

Application of auxins with 1000 ppm of 1, 2, 5 acid during different months of layering were found to be significantly effective in rooting of air layers in inducing rooting of air layers in guava. Air layers prepared during June month treated with 10,000 ppm of IBA + 1000 ppm of 1, 2, 4 acid gave maximum rooting percentage (90.00), better root characters (Chandrappa and Nache Gowda, 1998) .

Bhagat *et al*. (1999) reported that, application of 1500, 3000 or 4500 ppm IBA, 1500, 3000 or 4500 ppm NAA or IBA + NAA (1500 + 1500, 3000 + 3000 ppm or 4500 + 4500 ppm IBA) and inserted in a soil: sand: cattle manure (6:1:3) medium. Roots were visible within 45-60 days of ground layering. Treatment with 4500 ppm IBA resulted in the highest rooting (91.67%) and survival (85%) percentage.

Athani *et al.* (2001) revealed that significantly higher values for rooting percentage (90%) in guava, number of roots (18.23) and length of longest roots (9.56 cm) were observed in T_5 (30 days advance girdling + etiolation + IBA 5000ppm), while the values for these parameters were minimum (20%, 6.25 and 4.87cm), respectively in T_1 (control).

The results revealed that use of IBA was beneficial in enhancing the callus formation, number, length and diameter of both primary and secondary roots of air-layered guava twigs. Plant growth regulator IBA at 20,000 ppm was found optimum for better rooting success and was significantly superior to 5,000, 10,000 and 15,000 ppm (Mahabir Singh, 2001).

Albany *et al.* (2004) found that the application of NAA and IBA at 5000 mg per kg each and a mixture of both (2000 and 1000 mg / kg, respectively) using river peat as substrate. In another experiment, 5000 mg NAA per kg were applied and two substrates: river peat and mixture of river peat + phenolic foam (3:1 v/v) were evaluated. The mixture of river peat + phenolic foam media favoured a bigger percentage of rooted layering (92.07%) in guava. Based on the percentage of rooted air layers, number and longitude of primary roots it was concluded that NAA 5000 mg/kg was best simulative for the formulation of river peat + phenolic foam.

The results of Ghosh and Ranjan (2005) revealed that air layering conducted in September, October and November months the resulted in higher rooting success of (85%) guava.

Kunal Kumar and Syamal (2005) observed that the highest number of primary roots was produced by IBA at 3000 ppm treatment followed by NAA at 2000 ppm, high number of roots was recorded with IBA + NAA (1:1) at 2000 ppm. Etiolation along exogenous application of auxin had stimulating affect on producing longer roots. The longer primary roots of 11.30 cm were obtained with 3000 ppm of IBA followed by NAA at 2000 ppm, each produced 9.17 cm long primary roots. Etiolation along with auxins treatment had marked influence on rooting of guava air-layers. IBA at 3000 ppm had maximum success of 93.34 per cent followed by NAA at 1000 ppm maximum success of (86.68%).

Animesh and Bikash (2006) investigated on the effect of IBA at 1000 ppm and 2000 ppm wrappers and date of guava air-layering (30 May to 30 August, at 15 day intervals). Air layers prepared during June and July showed maximum rooting success, number of primary and secondary roots in new alluvial zone.

Abnave *et al.* (2007) found that application of IBA induced more primary roots (29 and 27 respectively). High concentrations that are 5000 ppm of both the chemicals were more effective in producing more number of secondary roots, i.e., 71 and 64 respectively. The diameter of shoot after rooting was significantly increased by higher concentration of IBA (1.85 cm) and NAA (1.81 cm) than control (1.31 cm). Higher concentration of NAA i.e., 5000 ppm significantly produced longer primary (8.7 cm) and secondary (11.7 cm) roots and increased survival percentage (78%).

The combination of IBA with rooting media helped in producing maximum number of primary roots (18.57), secondary roots (23.91), leaves (14.36) and length of shoots (5.31 cm) on 60 days air-layering in guava (Prabhar Singh *et al.,* 2007).

Lal *et al*. (2007) reported that, application of IBA (7500 ppm) gave maximum rooting percentage (96.67%), average number of roots per shoots (46.93) and average root length (8.45 cm) in guava.

Related species in guava family

Hore and Sen (1991) revealed that highest percentage rooting was obtained with the 1000 ppm P-hydroxybenzoic acid (PHB) + 5000 ppm IBA treatment in August (98.36%) followed by the 1000 ppm ethrel + 5000 ppm NAA treatment in June (97.45%).

The highest number of roots was obtained with the 1000 ppm ferulic acid (FA) + 2500 ppm NAA treatment in August and June (21.42 and 17.42, respectively). Survival of air-layer was greatest in both June and August (98.36 and 94.38%, respectively) with the 1000 ppm FA + 2500 ppm NAA treatment. Rooting success was greatest with air layers of rose apple made in August but survival was highest with air-layers made in June.

Application of P-hydroxybenzoic acid (PHB) at 1000 ppm + IBA at 5000 ppm in July resulted in the highest percentage rooting (98.3%) in (*Syzygium Javanic*) air layers. Percentage rooting was also higher in layers treated with ferulic acid (FA) at 1000 ppm + NAA at 10,000 ppm in June and layers treated with ferulic acid (FA) at 1000 ppm + IBA at 2500 ppm. In April (91.48 and 88.36%) (Hore and Sen, 1993).

Singh (2006) revealed that Jamun can be propagated by air-layering with instant ringing of shoots and application of root promoters percentage and their survival under field conditions were highest under the influence of instant ringing and application of IBA at 10,000 ppm. Main and secondary roots and length of main longest root per rooted layers were significantly higher in instant ringed layers treated with IBA or NAA at 10,000 ppm. Control produced fewer and shorter roots.

Gowda *et al.* (2006a) reported that layers treated with 5000 mg/lit IBA in combination with 1000 mg/lit 1, 2, 4 acid recorded maximum rooting of 66.5 per cent rose apple during August and it was lowest in untreated control (0.0) during October.

Other fruit crops

Litchi

Success in litchi air layering depends on the temperature and relative humidity of the atmosphere, leaf area and activity of root producing tissue of the air layered shoots. Some of the plant factors influencing root formation in air layering are, food supply, the hormone or auxin supply and activity of root producing tissue at the upper end of the girdled surface. Adequate supply of carbohydrates and certain growth regulators in minute quantities are also necessary for root production (Bhullar, 1962).

Yee (1972) Litchi can be air layered all round the year in Hawaii although they are usually layered in spring before active growth commences. In Florida, air layering is done in June, under prevailing conditions of warm temperatures, high humidity and daily rain (Storey, 1973).

Sharfuddin and Husain (1973) reported that air layered shoots of litchi showed 100 per cent rooting when treated with IBA at 500 ppm compared with 22 per cent in the control. Kadman (1985) reported that 90 to 100 per cent rooting in litchi within two to three months when treated with 250 ppm IBA compared with 32.8 per cent in control.

Syamal and Singh (1993) found that high percentage of rooting and survival of air-layers of litchi have been successfully achieved by improved method of exogenous application of IBA at 300 ppm after 60 days of etiolation. Highest success of 82.96 and followed by 77.48 per cent in IBA at 30 days of etiolation, average number of primary roots, length of primary roots, number of secondary roots and final survival percentage were recorded with IBA at 300 ppm.

Rahman *et al*. (2000) reported that maximum number of roots per plant (9.94), root length (10.94 cm), number of leaves per plants (10.55) were recorded in litchi air-layers treated with 2500 ppm of IBA.

The highest rooting percentage (94.93%) and number of roots (52.00 per layer) were obtained with 5000 ppm NAA + 200 ppm -hydroxybenzoic acid (PHB). In general, root length was improved by the application of 5000 ppm singly or in combination with 200 ppm PHB or 5000 ppm IBA (Sinha and Ray, 2002).

Duarte and Sachini (2003) reported that air layering on Brewster Lychee (*Litchi sinensis* Sonn) in different seasons with and without IBA at 3000 ppm. Air layers with mature terminal leaves and 5000 ppm IBA were superior to those with immature leaves. Rooting was 80.0, 91.6, 100.0 and 91.6 per cent in June, August, September and November respectively.

Air layering with IBA treatment was superior over other treatments, recording two fold rooting percentages. The number of roots per air layer was 3 or less without IBA, and between 5 and 8 when coupled with IBA treatment.

The greatest number of root per plant (32.97), root length (9.59 cm), root diameter (1.77 mm), shoot length (29.27 cm), shoot diameter (4.48 mm) and number of leaves (15.63) in litchi when treated with 2500 ppm of IBA (Jan *et al.,* 2003).

Namita Boro *et al*. (2006) observed that, IBA at 1000 ppm significantly increased plant height first, second and third order roots per layer, length of longest roots, mean root length, mean thickness, diameter of first order roots. Maximum values on various shoot and root parameters were noted in 1000 ppm IBA and root trainer treatment combination.

According to Smarsi *et al.* (2008) the best results were obtained using plant max and IBA between 2.166 and 2.430 and 2.250 mg/lit also gave good results in the development of air layers of litchi.

Pomegranate

The growth regulators such as IAA, IBA and NAA significantly influenced the root formation. Roots appeared in shortest time in air layers treated with 10,000 ppm of IBA. The maximum number of roots with higher root length and diameter was obtained with 10,000 ppm of IBA during August (Suryanarayana and Venkateshwar Rao, 1984).

Hegde and Sulikeri (1989) reported that highest rooting (93%) was observed with 1500 ppm of IBA and lowest (69%) with control. The highest percentage of rooting was also associated with the highest number of roots (8.27 primary and 51.65 secondary roots per layer) and highest cumulative root length of primary roots was observed with IBA at 1000 ppm (52.66 cm per layer).

Hore and Sen (1997) studied that maximum rooting success of 96.75 and 88.12 per cent could be achieved in ringed + etiolation (R + E) shoots respectively with P-hydroxybenzoic acid (PHB) at 1000 ppm + IBA 5000 ppm as against 54.56 and 50.28 per cent rooting in untreated ringed + etiolation (R + E) and ringed + non etiolation (R + NE) controls.

Patil *et al*. (2004) reported that maximum shoot length (38.4 cm), rot length (8.25 cm) highest number of root emergence (42.25), highest shoot number (7.0) and maximum root dry weight were obtained under PSB treatment, followed by PSB + Azospirillum.

Sapota

In case of Kalipatti variety of sapota air layers combination of IBA and NAA in equal proportions gave earlier rooting of the various concentrations tried, 20,000 ppm gave earlier rooting and better root formation with highest percentage of success (Chinnappa and Kololgi, 1961). In similar study, Sulladmath and Kololgi (1969) found that IBA and NAA at 10,000 ppm mixed in equal parts was optimum.

Gowda *et al*. (2006b) the maximum rooting percentage (76%) of khirni air layers was recorded with 5000 mg/lit IBA and the minimum rooting percentage (36%) was recorded in the control shoots. Application of IBA alone resulted in better rooting compared to an IBA + NAA combination. Khirni air layered shoots treated with 10,000 ppm mg/lit IBA recorded the maximum number of primary (9.2), secondary (49.4) and tertiary (34.4) roots.

Jackfruit

Barholia (1995) investigated the effect of IAA, IBA and NAA at 5000 ppm, 10,000 ppm or 15,000 ppm respectively applied to air layers of jackfruit. The highest percentage rooting (92.5%), length of primary root (29.7 cm) and numbers of primary roots (29.00) were obtained with IBA at 15,000 ppm.

According to Alila *et al*. (2000) application of growth regulators had perceptible influence on the number of roots per layer and IBA at 5000 ppm and 10,000 ppm produced the highest number of roots per layer.

Treatment with 5000 ppm IBA produced the longest roots while there was no distinct variation of mean root length with different concentrations of growth regulators. Application of IBA induced earlier emergence of roots than other treatments.

Application of IBA + NAA at 5000 ppm produced the highest number of rooted layers (90%), primary and secondary roots per layer (19.00 and 46.00, respectively), length of primary (11.39 cm) and secondary roots (8.27 cm). The lowest primary and secondary root diameters (0.10 and 0.06 cm, respectively) were observed in IBA + NAA at 5000 ppm treatments (Sengupta and Thakur, 2001).

Pawan Kumar *et al*. (2004) studied the effect of different concentrations (2500, 5000 and 7000 ppm) of IBA, NAA and their combinations on the rooting of jackfruit through air-layering. All the treatments were significantly superior to the untreated control. However, IBA + NAA at 5000 ppm and IBA alone at 5000 ppm enhanced root formation and quality of rooting better than the other treatments. IBA had more pronounced effect than NAA.

Singh and Singh (2004) studied the effect of IBA and NAA, at 2,500, 5000, 7,500 and 10,000 ppm and their combination (1:1) on the air-layers of jackfruit. The combination of IBA + NAA at 5000 ppm each showed the best effect on the air layers of Jackfruit. IBA alone at 5000 ppm improved root initiation of the air layers. Rooting under the growth regulator treatments was superior compared to the control.

Citrus

Application of 5000 ppm IBA + 200 ppm P- hydroxybenzoic acid (PHB) promoted 100 per cent rooting 45 days after layering (Dutta, 2000).

Bael

Bid and Mukherjee (1969) obtained more number of primary and secondary roots in mango by treating the air-layers with IBA and NAA in combination with 5,000 ppm concentration. This was attributed to the possible synergistic effect of IBA and NAA in combination.

Rajan and Sant Ram (1985) studied the changes in root promoting co-factors and inhibitors during different stages of root development in mango air-layers. They observed that endogenous rooting co-factor activity of aqueous fraction increased conspicuously at callusing and root emergence stages, while the rooting inhibitor level decreased.

Mukherjee *et al.* (1986) observed that the rooting of air-layers of Bael (*Aegle marmelos corre*) has been successfully induced by applying IBA 10,000 ppm in the etiolated shoots. Maximum rooting (80.90%) and maximum survival were obtained by treating the etiolated shoots with IBA 10,000 ppm.

2.2 Role of microbial inoculants

Azospirillum sp. are also known to produce auxins such as Indole-3-acetic acid from tryptophan (Tien *et al.,* 1979). Plant growth promoting substances released by *Azospirillum* isolated from rhizosphere of crop plants was reported by Harari *et al*. (1988) and Mascarua-Esparca *et al. (*1988). Zimmer and Bothe (1988) reported that cultures of *Azospirillum brasilense* sp. 7 and *Azospirillum lipoterum* sp. 59 produced IAA only in the stationary phase of growth when D-L-Tryptophan was added to the medium.

Fallik and Okon (1984) identified the growth promoting substances produced by *Azospirillum brassilense* in maize roots as indole acetic acid. Govind and Pandey (1985) found that *Azospirillum lipoferum* induced roots on one year old pepper cuttings cv. Panniyur-1. Omay *et al.* (1992) observed indole acetic acid production by *Azospirillum* sp. under in-vitro conditions.

Bar and Okon (1993) reported that the phytohormone indole acetic acid was produced by *Azospirillum* and the organism promoted the plant growth. Sateesh Kumar (1999) reported that dipping of pomegranate cuttings in *Azospirillum brasilense* gave maximum rooting percentage.

The maximum shoot length (38.4 cm), root length (8.25 cm), highest number of roots emerged (42.25) shoots emerged (7.0) and maximum dry wt. of roots (0.2g), shoots (5.5g) in pomegranate air layers were noticed in the treatment involving the inoculation with P-solubilizing bacteria (PSB) alone followed by again PSB along with *Azospirillum* in the treatments studied. The better shoot length possibly was due to the suppression of root growth by the production of higher amount of root growth promoting substances is more than the desired levels (Patil *et al*., 2004).

2.2.1 Mechanism of root induction by microbial inoculants

Fallik and Okon (1984) Inoculation of maize seedlings with 107 colony forming units (cfu) of *Azospirillum brasilense* per plant had significantly increased root surface area two weeks after and higher amount of free and bound IAA two weeks after sowing.

2.3 Biochemical factors affecting rooting

The change in the biochemical constituents brought about by the auxin treatment in air layers determine the ease with which the rooting is accomplished. The role of auxins in initiation of rooting is attributed to their capacity to enhance hydrolysis of the nutritional reserves.

2.3.1 Nutritional status of shoots

Working under Dharwad conditions, Karunakara (1997) obtained good rooting in easy to root guava cv. *Psidium guajava* L. and this was attributed to the latter having lower contents of starch, total sugar, total phenol, carbohydrate compound and nitrogen but higher C/N ratio content.

2.3.2 Role of Nitrogen and Carbohydrates

The past and recent studies have revealed that a proper ratio between carbohydrates and nitrogen determines the rooting capacity of layers. Thus, the level of nitrogen is of vital importance in determining the proper balance with carbohydrate conducive for rooting.

Basu *et al*. (1967) carried out analytical studies on callusing at root emergence stage of mango air layers treated with IBA and found that they contained relatively greater quantity of carbohydrates in root forming regions that stimulated rooting. Investigations on the propagation of sapota revealed that the invigorated shoots contained a higher soluble nitrogen and total nitrogen (Uthaiah, 1975). Telang (1981) observed a low level of nitrogen and higher C/N ratio favouring rooting by the exogenous application of auxins in seedless lime. Venkatesh (1983) reported that high sugars and carbohydrates/nitrogen ration favours better rooting in air layers of guava. Rao *et al*. (1990) noted reduction in total nitrogen content towards root initiation stage in cashew.

2.3.3 Role of phenol compounds

Phenolic compounds play an important role in regulation of growth. These compounds regulate the growth primarily affect the compounds on the decarboxylation of IAA.

Natural differences in the capacity of cuttings to regenerate may be due to the difference in the occurrence of phenolic compounds (Bose *et al*., 1972).

In most plants it has been reported that the enzyme polyphenol oxidase can introduce second – OH into the ring of a monophenol and convert into diphenol (Goodwin, 1976).

2.4 Influence of growth regulators and microbial inoculants on survivability of air-layers

The success in propagating plants by any method lies in their survivability after transplanting. Thus, knowledge about their extent of survivability is important to obtain higher success.

Bhujbal (1972) found that IBA 3,000 ppm produce 86.6 per cent rooting and 76.6 per cent survival in guava air-layers. The survival rate was also maximum (72.5 %) in guava due to pre girdled with etiolation and IBA treatment (Venkatesh, 1983).

The highest field survival of guava layers (94.67%) was obtained with the 10 per cent sucrose + coconut milk treatment closely followed by the 5 per cent sucrose treatment (94.29%), (Ram Chandra and Sheo Govind, 1993).

Saroj and Pathak (1994) found that IBA + NAA (7500 ppm) induced the best rooting in (*Psidium chinensis* and *Psidium cujavallis*) both the species. The concentration promoted establishment (100 % establishment in *Psidium chinensis* and 96.67 % in *Psidium cujavallis*). Longer roots were observed at lower concentration (2500 ppm IBA + NAA) compared with higher concentrations.

Singh *et al*. (1996) reported that etiolation stool of guava treated with IBA increased survival per cent. The maximum survival (96.30 percentage) of rooted stools was obtained in sardar guava cultivar receiving 7500 ppm IBA. The maximum survival was obtained due to high rooting potentiality of sardar guava cultivars with IBA treatments (7500 ppm).

Kamleshkar Singh and Jain (1996) reported that survival of guava rooted layers (75%) was obtained when the etiolated shoots were treated with IBA at 6,000 ppm during the month of July.

Chandrappa and Nache gowda (1998) reported that guava air layers prepared during June month treated with 10,000 ppm of IBA + 1, 2, 4 acid gave maximum of 97.79 per cent survival.

The guava air-layers treated with 5000, 10.000, 15,000 or 20,000 ppm NAA or IBA before air layering. Air layers were detached after 69 days and transferred to nursery bed. Rooting success increased with increasing growth regulator concentration survival rates were highest (71.65%) with 15,000 ppm NAA and (70.29%) with 15,000 ppm IBA (Tomar *et al*., 1999).

Hore and Sen (1991) noticed the maximum survival percentage of guava air-layers in both June and August (98.36 and 94.38 %, respectively) with the 1000 ppm ferulic acid (FA) + 2500 ppm NAA treatment. Survival was highest with air layers made in June.

The maximum field establishment (100%) was observed in guava air-layers of October in red laterite zone of west Bengal followed by September with 70 per cent field establishment (Ghosh and Ranjan, 2005).

Kakon *et al.* (2005) reported that highest percentage of survivability (93.68%) was obtained from the three cut treatments. Growth characteristic like number of shoots and number of leaves were found highest in the guava layers severed by three cuts.

Lal *et al*. (2007) reported that the treatment with IBA (7500 ppm) gave maximum survival (75%) of guava air-layers after transplanting in the field.

Air layering of guava with IBA concentration of 6000 ppm with soil: sand: poultry manure rooting media produced maximum percentage (76.75%) of survival of 60 days old plants grown in poly bags. IBA at 5000 ppm and poultry manure combination was found to be the second best (73.25%) with respect to survival of air layers (Prabhar Singh *et al*., 2007).

Litchi

Sharfuddin (1983) reported that air layers detached from mother plant and pruned heavily recorded maximum (72.7%) survival percentage compared with moderate (45.4%) and light (33.6%) pruned layers. Survival of litchi layers in nursery was the highest (74.2 %), when wrapping was done after one week of ringing with 2500 ppm IBA followed by those wrapped after two weeks of ringing (Sharma *et al*., 1990). Shoots treated with 5000 ppm NAA + 200 ppm PHB exhibited 91.66 and 88.3 per cent survival percentage of transplanted layers after one month and four months, respectively (Sinha and Ray, 2002). The highest percentage plant survival (75.23%) was recorded for layers treated with 2500 ppm of IBA (Jan *et al*., 2003). Namita Boro *et al.* (2006) recorded maximum percentages of survival treated with 1000 ppm IBA.

Pomegranate

Hore and Sen (1997) the layers treated with PHB 1000 ppm + IBA 2500 ppm and FA 1000 ppm + IBA 5000 ppm recorded maximum survival percentage in R + E (94.3%) and R + NE (82.3%) shoot respectively.

Jackfruit

Barholia (1995) reported the highest survival percentage after detachment of guava air-layers (87.5%) with IBA at 15,000 ppm.

Mango

Bid and Mukherjee (1969) reported 100 per cent rooting and establishment of air-layers of Langra variety of mango which was obtained as a result of treatment of etiolation shoots with 10,000 ppm of IBA and NAA in equal parts. In Chousa variety, 98.66 per cent rooting and 90 per cent survival was obtained with etiolated shoots with IBA + NAA (1:1) at 5,000 ppm.

Chonkar and Singh (1972) concluded that IBA at 5,000 ppm was markedly more effective than NAA in promoting the rooting and establishment of mango air-layers

III. MATERIAL AND METHODS

The present investigation was conducted during 2010- 2011 in Guava germplasm block located at the Silver Jubilee Orchard (S.J.O.), Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad Karnataka State. The details of the material and methods pertaining to the present experiment are furnished in this chapter.

3.1 Experimental site

The experiment was carried out in the Silver Jubilee Orchard (S.J.O.), Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad.

3.2 Geographical location of the experimental site

The Silver Jubilee Orchard of the Department of Horticulture, College of Agriculture, University of Agriculture Sciences, Dharwad is situated in the agro climatic zone VIII which is the North transitional zone of Karnataka state. Geographically, the Main Agricultural Research Station (MARS), Dharwad is located at 15^0 26' North latitude, 75^0 07' East longitude and at an altitude of 678 meter above mean sea level.

3.3 Climate

The data on weather parameters such as rainfall (mm), mean maximum and minimum temperature (^{0}C) and relative humidity (%) recorded at Meteorological Observatory, Main Agricultural Research Station, University of Agricultural Sciences, Dharwad during the experimental year (2010-2011) and the mean of the past 60 years (1950-2010) are presented in Appendix-I.

A rainfall of 952.5 mm was received during the year 2010-2011 at Dharwad where as during experimentation it was 843.8 mm (*i.e.* from June to November). The mean maximum temperature during the year was 36.0 ^{0}C in April where as during experimentation period it was 31.2 ^{0}C in June. The mean minimum temperature both during the year and during experimentation period was 19.0 ^{0}C in June. The mean maximum relative humidity during the year 2010-2011 was 84.0 per cent in June and November both where as during experimentation it was 75 per cent in June. The mean minimum relative humidity during the year 2010-2011 was 49.0 per cent in March while during experimentation it was 75.0 per cent in June.

3.4 Experimental details

3.4.1 Source of plants for mature shoot air layering

The mature shoots used for air layering in present study were in the germplasm block maintained for plant multiplication at Silver Jubilee Orchard (S.J.O)., The air layers were prepared from single known clone of Lucknow – 49. The plants were planted at close spacing of 1.6 x 1.6 m.

The severance itself serves as pruning which help in development of forced shoots from the dormant buds of the plant. Such shoots are known to behave as mature shoots physiologically. Air layering in the present study was conducted on such forced shoots. About twenty five shoots in each plant were used for air layering.

3.4.2 Design and layout of experiment

The experiment was laid out in Randomized Block Design. There were 14 treatments consisting of growth regulators and microbial inoculants singly or in combination at different concentrations. Twenty five air layers were used each treatment which was replicated three times.

Table 1. Quantity of growth regulators, *Azospirillum* and talc powder used for different treatments

Treatment details	IBA (mg)	NAA (mg)	*Azospirillum* (g)	Talc powder (g)
T_1 - IBA, 2000 ppm	200	-	-	99.8
T_2 - IBA, 4000 ppm	400	-	-	99.6
T_3 - IBA, 6000 ppm	600	-	-	99.4
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	100	100	-	99.8
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	200	200	-	99.6
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	300	300	-	99.4
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	200	-	37.5	62.3
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	400	-	37.5	62.1
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	600	-	37.5	61.9
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	100	100	37.5	62.3
T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	200	200	37.5	62.1
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	300	300	37.5	61.9
T_{13} - *Azospirillum* 37.5 g	-	-	37.5	-
T_{14} - Control	-	-	-	-

3.4.3 Treatment details

1. T_1 – IBA, 2000 ppm
2. T_2 – IBA, 4000 ppm
3. T_3 – IBA, 6000 ppm
4. T_4 – IBA, 1000 ppm + NAA, 1000 ppm
5. T_5 – IBA, 2000 ppm + NAA, 2000 ppm
6. T_6- IBA, 3000) ppm + NAA, 3000 ppm
7. T_7 – *Azospirillum* 37.5 g + IBA, 2000 ppm
8. T_8 – *Azospirillum* 37.5 g + IBA, 4000 ppm
9. T_9 – *Azospirillum* 37.5 g + IBA, 6000 ppm
10. T_{1O} – *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm
11. T_{11} – *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm
12. T_{12} - *Azospirillum* 37.5 g + IBA,3000 ppm + NAA, 3000 ppm
13. T_{13} – *Azospirillum* 37.5 g
14. T_{14} - Control

3.4.4 Preparation of growth regulator formulations

Required quantity of growth regulators (IBA/NAA) and *Azospirillum* as per the treatment to prepare 100 g of powder formulation were weighed in an analytical balance and dissolved in 50 ml of 80 per cent alcohol. Required quantity of talc powder was weighed separately. The dissolved growth regulator/s were mixed with the weighed talc powder and made into paste by stirring. The mixture was kept for air drying over night for evaporation of alcohol. Then they were powdered and mixed with *Azospirillum* and made into paste again with water before application. The detail of the quantity of in gradients added is given in Table 1.

Lignite based microbial culture *Azospirillum* (787.5 g) was obtained from the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad., and 0.5 g of lignite based *Azospirillum* culture was more than 10^8 cells/g was used for inoculation

3.4.5 Air-layering operation

Selected mature shoots were girdled by removing 2.5 cm ring of bark in the internodal region, 25 cm below the tip of shoot. Fifteen days after girdling, the layering operation was done on 16th June, 2010. A ring of bark of 2 mm was removed from just above the upper cut, to expose the fleshy tissues for absorption of applied growth regulator and microbial inoculants from formulations. Girdled portion was moistened with distilled water and formulations of growth regulator and microbial inoculants were applied with a soft camel-hair brush to the upper cut surface and also 2 cm portion stem above the cut. The exposed region was immediately covered with a ball of moist, chopped sphagnum moss without disturbing the applied growth regulator and microbial inoculants. The moss was covered with polythene tubing of 250 gauge thickness and both the ends were secured firmly using gunny thread. In case of control, however, only talc powder (carrier) was applied (Plate 1a and 1b).

3.4.6 After care of air-layers

The layers were kept under constant observation to prevent from any mechanical damage and loss of moisture. During July – August period the occurrence of showers made the weather favourable for the retention of moisture in sphagnum moss.

3.4.7 Observations recorded

All the air-layers were detached from mother plants two and half months after air layering. The detached air layers were dipped in water and the sphagnum moss adhering to the roots was removed carefully using forceps to avoid damage to the roots.

The fallowing observations were recorded in the experiment.

1. Number of days taken for emergence of roots
2. Number of air layers rooted
3. Mean number of primary roots per air layer

Plate 1 a. General view of the gene of the gene bank of guava cv. L - 49

Plate lb. Separation of air layers from mother plant

4. Mean length of primary roots per air layer
5. Mean length of longest primary root per air layer
6. Number of side shoots per air layer
7. Leaf Area per layer
8. Survival percentage of transplanted air layers.

3.4.7.1 Number of days taken for rooting

The date of appearance of first roots in air layers, which were visible through the transparent polythene sheet, was recorded by periodic observation. The number of days taken for rooting of air layers in each treatment was calculated.

3.4.7.2 Number of air layers rooted

After two and half months, air-layers were detached from the mother plants, rooted and unrooted layers were separated and counted for further calculation of percentage of rooted layers in each treatment lot.

3.4.7.3 Mean number of primary and secondary roots per air layer

Five rooted layers were randomly sampled from each treatment. The polythene sheet and the rooting media were removed carefully using forceps and care was taken to avoid damage to roots. Number of primary and secondary roots were counted in each rooted layer.

3.4.7.4 Mean length of primary roots per air layer

Length of primary roots was measured from the collar region to the tip of primary root. Length of the secondary roots developed from the primary root was also measured in centimetres (cm).

3.4.7.5 Mean length of longest primary root per air layer

Length of longest primary root was measured from collar region to the tip of longest primary root was also measured in centimetres (cm).

3.4.7.6 Number of side shoots per air layer

Total number of side shoots produced per air layer after transplanting was recorded from the tagged air layers.

3.4.7.7 Leaf Area

Leaf area was recorded with the help of leaf area meter (LICOR 10 Model) by selecting leaves randomly and equal from top, middle and bottom of the air layers. The readings were taken from the tagged air layers and leaf area index was expressed in square centimetres (cm^2) per layer.

3.4.7.8 Survival percentage of transplanted air-layers and establishment study

After detaching the layers from mother plant, the layers were defoliated retaining the petioles intact and carefully transplanted into polythene bags (25 x 20 cm) filled with potting mixture comprising Sand : Soil : Farm Yard Manure in 1: 1: 1 ratio by volume. These bagged layers were allowed to grow inside greenhouse and watered daily. After 15 days, they transferred to shade house. The observations on the number of shoots, number of leaves and length of sprouts in bagged layers were recorded at 30 days and 60 days after bagging. The survival percentage was taken two months after bagging.

3.4.8 Biochemical analysis

Biochemical analysis was done for total sugar, starch content, carbohydrates, total nitrogen, C: N ratio, total phenols. The basal 2.5 – 3.0 cm portion root zone of the rooted layers was used for all biochemical estimations.

Sampling for this study was made at two stages as under:

1. Just before giving growth regulator and microbial inoculants treatment that is before air layering stage.
2. At the time of separation of air layers from mother plant.

The samples were oven- dried and ground to a fine powder and stored until used for the biochemical analysis. The methods used for the estimation of different chemical constituents are described below.

3.4.8.1 Starch content

The residue obtained after alcohol extraction of the sample was dried of which 200 mg of was digested with perchloric acid (52%) and distilled water (3.5: 1.5) and the mixture was subjected to centrifugation for 10 minutes at 3000 rpm. The extraction was repeated by changing the centrifugation time to 20 and 30 minutes respectively and volume was made upto 25 ml with distilled water. It was neutralized and one ml of aliquot was used to estimate the reducing sugars by Dinitro-Salicyclic acid (DNS) method (Miller, 1972). The amount of starch was computed by multiplying the value of this fraction by 0.9 (Steel, 1949). The results were expressed as percentage on dry weight basis.

3.4.8.2 Total sugars

For the estimation of total sugars, 2 g of oven-dried finely ground sample was extracted successively thrice using 80 per cent ethanol. One ml of this extract was taken in a test tube and the alcohol was evaporated. Distilled water was added and the volume made upto 10 ml. The total sugar content in the alcohol- free extract was determined after hydrolysing this extract with 1 N HCl on a hot water bath at 50^0 C for 20 minutes. The hydrolysed extract was neutralized with 1 N NaOH and the total sugar was estimated by Dinitro-Salicylic acid (DNS) method (Miller, 1972). The result was expressed on percentage dry weight basis.

3.4.8.3 Total carbohydrates

The total carbohydrates were computed by summing up the values of total sugars and starch and were expressed on percentage dry weight basis.

3.4.8.4 Total Nitrogen

Dried and powdered shoot samples were used for estimation of total nitrogen was determined by Micro-Kjeldahl method (Anon., 1970) and results were expressed on percentage dry weight basis.

3.4.8.5 Carbohydrates/Nitrogen (C/N) ratio

This was computed by dividing the value of total carbohydrates by that of total nitrogen.

3.4.8.6 Total phenols

The amount of total phenols was estimated by Folin-ciocalteau reagent (FCR) method (Mahadevan, 1975).

3.5 Statistical Analysis

The Statistical data analysis to different parameters were tabulated and statistically analysed as per the methods outlined by Panse and Sukhatme (1961) by adopting “Fisher’s Analysis of variance technique”. The data relating to percentage of rooting and survivability of rooted air –layers were transformation values before analysis.

IV. EXPERIMENTAL RESULTS

The results of the propagation studies in guava through mature shoot air-layers as influenced by growth regulators and microbial inoculants are presented under the following headings.

Effect of IBA, NAA and microbial inoculants applied singly and in combination on induction of rooting in air layers of guava cv. Lucknow-49

4.1 Root parameters

4.1.1 Number of days taken for root emergence

4.1.2 Percentage of layers rooted

4.1.3 Mean number of primary roots per air-layer

4.1.4 Mean length of primary roots per air-layer

4.1.5 Mean length of longest primary roots per air-layer

4.1.6 Mean girth of primary roots

4.1.7 Mean number of secondary roots per air-layer

4.1.8 Mean length of secondary roots

4.2 Biochemical studies

4.2.1 Starch

4.2.2 Total sugars

4.2.3 Carbohydrates

4.2.4 Total phenols

4.2.5 Nitrogen

4.2.6 Carbohydrates / Nitrogen ratio

4.3 Post-seperation studies

4.3.1 Mean number of side shoots in air-layer

4.3.2 Mean number of healthy side shoots in air-layer

4.3.3 Mean number of leaves in air-layer

4.3.4 Leaf area per layer

4.3.5 Mean length of side shoot in air-layer

4.3.6 Mean length of longest side shoot in air-layer

4.3.7 Survival percentage of transplanted air-layers

4.1 Root parameters

4.1.1 Number of days taken for root emergence

The data pertaining to the number of days taken for root emergence in air-layers as influenced by different growth regulators and microbial inoculants are furnished in Table 2 and depicted in Fig. 1.

The data revealed that the number of days taken for root emergence in relation to different formulations of growth regulator and microbial inoculants treatment was found to be significant. Among the different treatments, combination of *Azospirillum* 37.5 g with equal proportion (3000 ppm each) of IBA and NAA recorded the shortest duration of 30.75 days as against the longest duration of 51.75 days in untreated layers. The combination treatment of IBA and NAA 3000 ppm each also recorded the shortest duration of 31.25 days for root emergence as compared to treatment where the growth regulator was applied singly.

Table 2. Influence of growth regulators and *Azospirillum* on number of days taken for root emergence in guava air-layers

Treatments	Average number of days taken for root emergence
T_1 - IBA, 2000 ppm	44.75
T_2 - IBA, 4000 ppm	39.40
T_3 - IBA, 6000 ppm	44.50
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	39.38
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	37.40
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	31.25
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	42.75
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	38.40
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	46.76
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	37.25
T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	35.75
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	30.75
T_{13} - *Azospirillum* 37.5 g	48.25
T_{14} - Control	51.75
Mean	40.60
S.Em$\pm$	1.53
CD at 5%	4.45

* Measured which are more than 0.5 cm

4.1.2 Percentage of layers rooted

The data on the influence of growth regulators and microbial inoculants on rooting of guava layers are presented in Table 3 and depicted in Fig. 2 (Plate 2).

The data showed that all the formulations of growth regulators and microbial inoculants treatment formulations promoted rooting in air layers as compared to untreated layers. The better success was noted in treatment with *Azospirillum* + IBA + NAA as compared to treatments where the growth regulators were applied singly. The highest percentage of rooting (91.68) was noted in the layers which had received equal proportion (3000 ppm) each of IBA, NAA and *Azospirillum* 37.5 g. This is followed by IBA, 3000 ppm + NAA, 3000 ppm combination treatment.

With regard to effect of application of growth regulator singly, the highest (81.76) percentage was noted in IBA.

4.1.3 Mean number of primary roots per air-layer

The data on the mean number of primary roots per air layer as influenced by growth regulators and microbial inoculants are furnished in Table 4 and depicted in Fig. 3.

The data revealed that the mean number of primary roots per air layer in relation to different formulations of growth regulators and microbial inoculant treatments were found to be significant. Among the different treatments, combination treatment of *Azospirillum* 37.5 g with equal proportion (3000 ppm) each of IBA and NAA recorded significantly the highest mean number of primary roots per air layer as against the lowest mean number of primary roots (3.82) in sole treatment of control.

Among the other treatment combinations, the treatment T_6 recorded numerically the highest number of primary roots (11.34) which was on par with T_2, T_8, T_9, T_{11} (10.72, 11.04, 10.25 and 10.42).

LEGEND -1

T_1 - IBA, 2000 ppm

T_2 - IBA, 4000 ppm

T_3 - IBA, 6000 ppm

T_4 - IBA, 1000 ppm + NAA, 1000 ppm

T_5 - IBA, 2000 ppm + NAA, 2000 ppm

T_6 - IBA, 3000 ppm + NAA, 3000 ppm

T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm

T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm

T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm

T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm

T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm

T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm

T_{13} - *Azospirillum* 37.5 g

T_{14} - Control

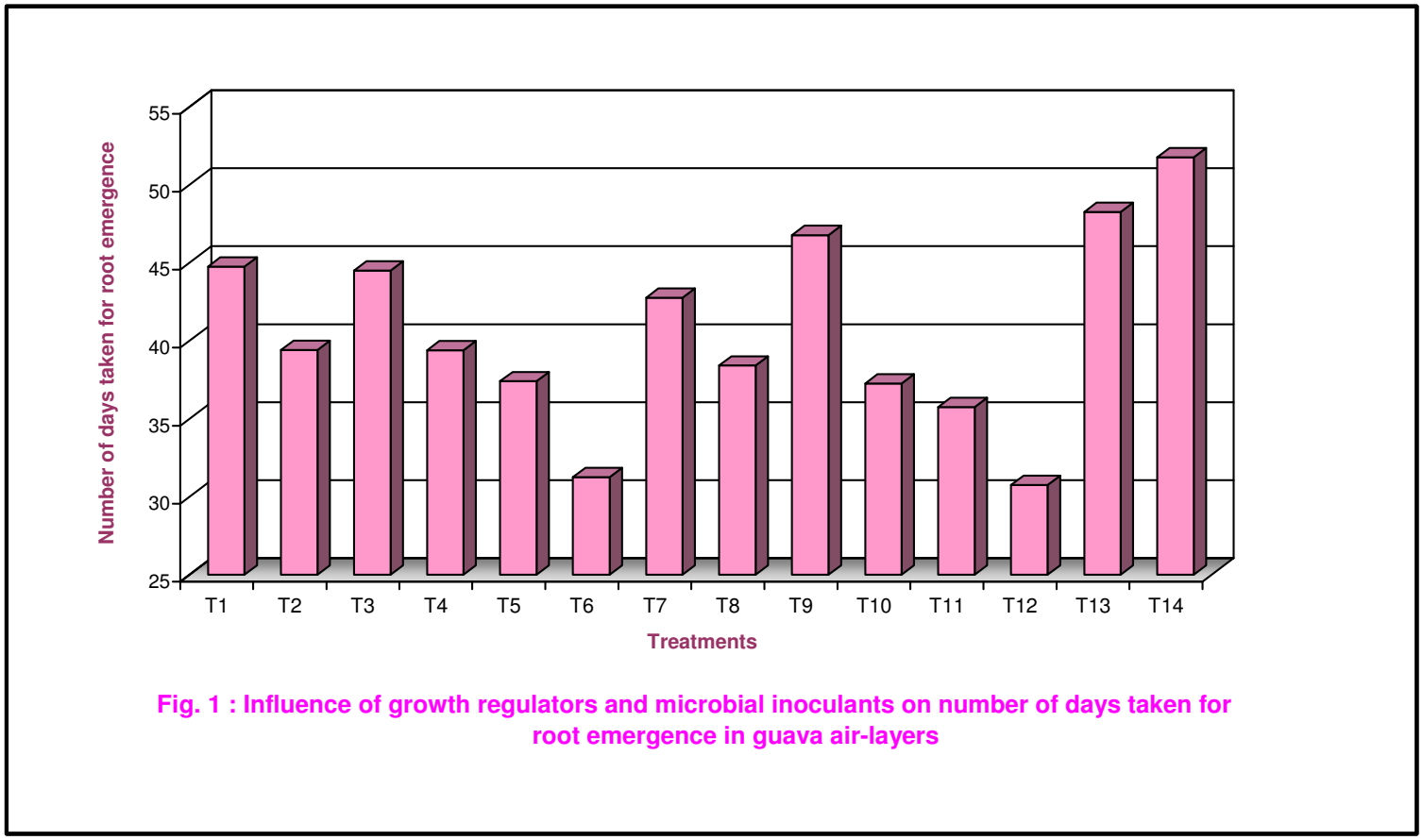

Fig. 1 : Influence of growth regulators and microbial inoculants on number of days taken for root emergence in guava air-layers

Fig. 1 : Influence of growth regulators and microbial inoculants on number of days taken for root emergence in guava air-layers

Plate 2. Rooting in mature shoot air layers in guava cv. L – 49 in relation to different treatments

Table 3. Influence of growth regulators and *Azospirillum* on rooting in air-layers of guava

Treatments	Rooting percentage
T_1 - IBA, 2000 ppm	66.38
T_2 - IBA, 4000 ppm	81.76
T_3 - IBA, 6000 ppm	79.84
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	73.15
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	79.07
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	88.49
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	67.68
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	82.45
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	80.84
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	74.38
T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	80.46
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	91.68
T_{13} - *Azospirillum* 37.5 g	43.42
T_{14} - Control	38.51
Mean	73.44
S.Em$\pm$	1.06
CD at 5%	3.15

4.1.4 Mean length of primary roots per air-layer

The data on the mean length of primary roots in air layers of guava as influenced by growth regulators and microbial inoculants are furnished in Table 4.

The overall mean length of roots developed on the treated layers (with the exception of IBA applied singly at 2000 or 6000 ppm) was in the range of 4.98 to 8.59 cm which was significantly higher as compared to (2.92 cm) developed on untreated layers. The maximum mean length of primary roots per air-layer in *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm treated layers was 8.59 cm.

4.1.5 Mean length of longest primary root per air-layer

The results regarding the mean length of the longest primary root per air-layer as influenced by growth regulators and microbial inoculants are presented in Table 4 and depicted in Fig. 4.

The data showed that the treatments promoted longer mean length of longest primary root as compared to control. The mean length of longest primary root was in the range of 6.80 to 9.16 cm in different treatments as against (3.29 cm) control. The mean length of longest primary root that developed on layers which received 37.5 g *Azospirillum* + IBA, 3000 ppm + NAA, 3000 ppm was 9.16 cm which was significantly higher over control.

LEGEND -2

T_1 - IBA, 2000 ppm

T_2 - IBA, 4000 ppm

T_3 - IBA, 6000 ppm

T_4 - IBA, 1000 ppm + NAA, 1000 ppm

T_5 - IBA, 2000 ppm + NAA, 2000 ppm

T_6 - IBA, 3000 ppm + NAA, 3000 ppm

T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm

T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm

T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm

T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm

T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm

T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm

T_{13} - *Azospirillum* 37.5 g

T_{14} - Control

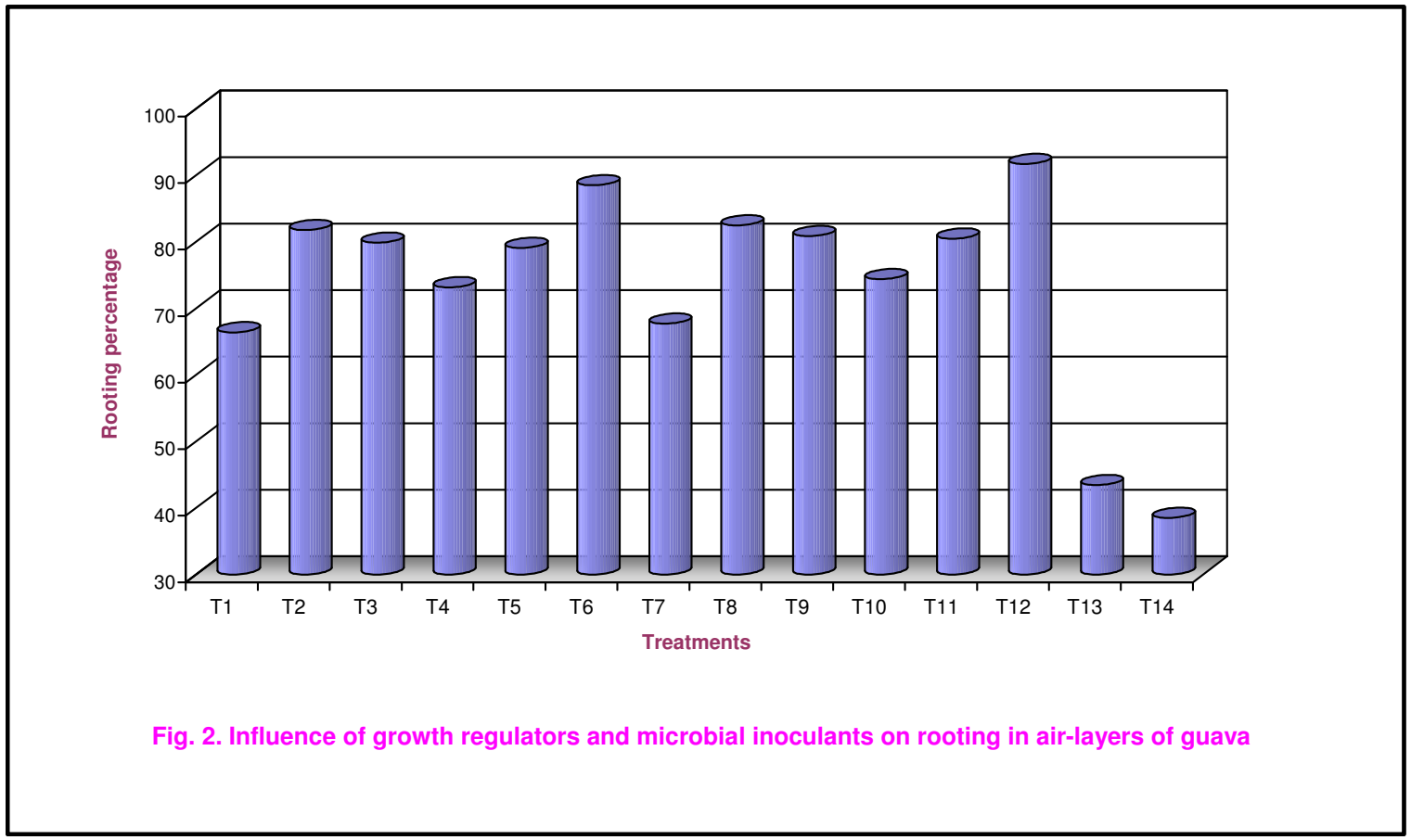

Fig. 2. Influence of growth regulators and microbial inoculants on rooting in air-layers of guava

Fig. 2. Influence of growth regulators and microbial inoculants on rooting in air-layers of guava

Table 4. Influence of growth regulators and *Azospirillum* on parameters of primary roots in air-layers of guava

.Treatments	Mean number of primary roots	Mean length of primary roots* (cm)	Mean length of longest primary root (cm)	Mean girth of primary roots (mm)
T_1	7.96	4.98	6.80	5.23
T_2	10.72	6.24	7.95	6.03
T_3	9.85	6.11	7.63	5.37
T_4	8.76	5.83	6.98	5.42
T_5	9.42	5.97	7.27	5.54
T_6	11.34	7.26	8.24	6.28
T_7	8.19	5.17	7.11	5.41
T_8	11.04	6.45	8.06	6.13
T_9	10.25	6.32	7.83	5.62
T_{10}	9.72	6.24	7.26	5.68
T_{11}	10.42	7.01	7.79	5.76
T_{12}	12.85	8.59	9.16	7.59
T_{13}	6.96	3.41	4.57	4.88
T_{14}	3.82	2.92	3.29	4.15
Mean	9.38	5.89	7.14	5.65
S.Em$\pm$	0.40	0.29	0.32	0.37
CD at 5%	1.18	0.83	0.92	1.07

*Measured which are more than 0.5 cm

T_1 IBA, 2000 ppm
T_2 IBA, 4000 ppm
T_3 IBA, 6000 ppm
T_4 IBA, 1000 ppm + NAA, 1000 ppm
T_5 IBA, 2000 ppm + NAA, 2000 ppm
T_6 IBA, 3000 ppm + NAA, 3000 ppm
T_7 *Azospirillum* 37.5 g + IBA 2000 ppm
T_8 *Azospirillum* 37.5 g + IBA, 4000 ppm
T_9 *Azospirillum* 37.5 g + IBA, 6000 ppm
T_{10} *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm
T_{11} *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm
T_{12} *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm
T_{13} *Azospirillum* 37.5 g
T_{14} Control

4.1.6 Mean girth of primary roots

The data on mean girth of primary roots as influenced by growth regulators and microbial inoculant treatments are presented in Table 4.

The data on mean girth of primary roots was found to be significantly higher. The mean girth of primary root was in the range of 5.23 to 7.59 mm in different growth regulator and microbial inoculant treatments as against (4.15 mm) control layers. The maximum mean girth of primary root that developed on layers which received *Azospirillum* 37.5 g and equal proportion (3000 ppm) each of IBA and NAA was 7.59 mm which was significantly higher than control.

4.1.7 Mean number of secondary roots per air-layer

The data pertaining to the mean number of secondary roots per air-layer as influenced by different growth regulators and microbial inoculants are furnished in Table 5 and depicted in Fig. 5.The data revealed that the mean number of secondary roots induced by different growth regulators and microbial inoculant treatments was significantly higher. The highest number (25.22) of secondary roots was recorded in layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm followed by IBA, 3000 + NAA, 3000 ppm treatment (23.18). The lowest number (9.47 and 8.98) of secondary roots was recorded in *Azospirillum* 37.5 g and untreated layers.

4.1.8 Mean length of secondary roots

The data on the mean length of secondary roots as influenced by growth regulators and microbial inoculants are presented in Table 5 and depicted in Fig. 5.

The overall mean length of secondary roots developed on treated layers (with the exception of IBA applied singly at 2000/6000 ppm) was in the range of 10.92 – 18.33 cm which was significantly higher than the ones (4.90 cm) developed on untreated layers. The mean length of secondary roots in *Azospirillum* 37.5 + IBA, 3000 ppm + NAA, 3000 ppm treated layers was highest (18.33 cm) as compared with other treatments.

LEGEND -3

T_1 - IBA, 2000 ppm

T_2 - IBA, 4000 ppm

T_3 - IBA, 6000 ppm

T_4 - IBA, 1000 ppm + NAA, 1000 ppm

T_5 - IBA, 2000 ppm + NAA, 2000 ppm

T_6 - IBA, 3000 ppm + NAA, 3000 ppm

T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm

T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm

T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm

T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm

T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm

T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm

T_{13} - *Azospirillum* 37.5 g

T_{14} - Control

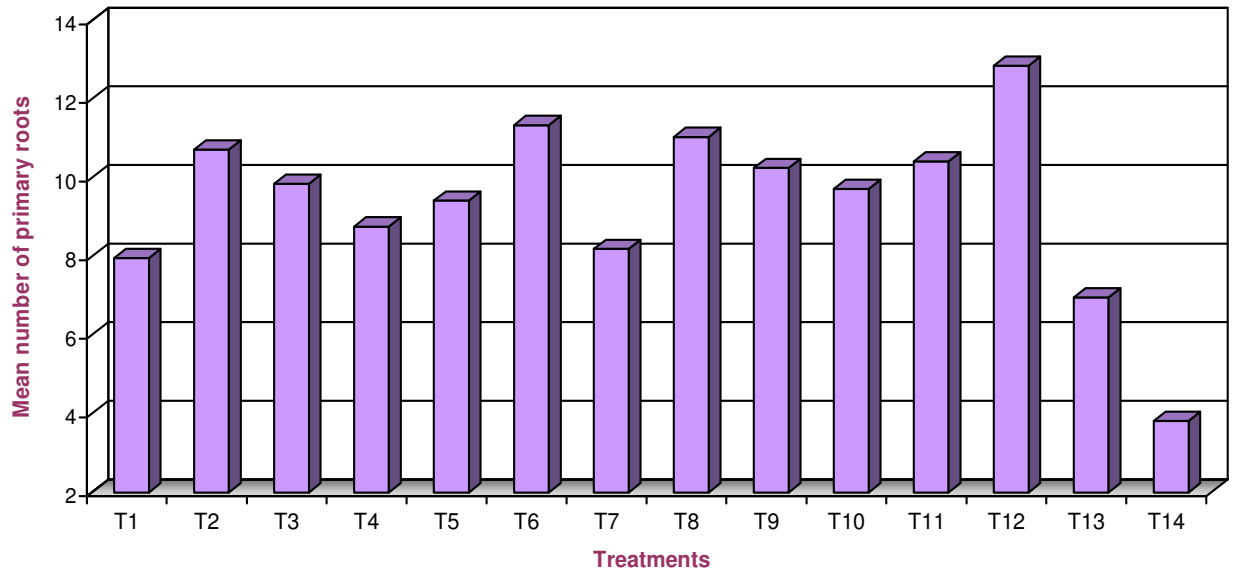

Fig. 3. Influence of growth regulators and microbial inoculants on number of primary roots in air-layers of guava

Fig. 3. Influence of growth regulators and microbial inoculants on number of primary roots in air-layers of guava

4.2 Biochemical studies

The data on biochemical changes during rooting of guava air layers as influenced by growth regulators and microbial inoculants are given in Table 6, 7 and 8.

4.2.1 Starch

The changes in starch content during rooting are presented in Table 6 and 7. The starch content of the guava shoots at the time of layering was 7.45 per cent, and showed a marginal reduction during the process of rooting. The decline was more (from 7.45 to 7.15%) in case of layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm as compared to control (from 7.45 to 7.36%). In all the combination treatments of *Azospirillum*, IBA and NAA, showed a greater decline in starch content as compared to their individual application.

4.2.2 Total sugars

The data on total sugars are presented in Table 6 and 7. At the time of layering, the selected shoots were containing 8.96 per cent total sugars. During the process of rooting there was a marginal reduction in the total sugar content in both treated and untreated layers. The layers treated with growth regulators and microbial inoculants showed slightly higher sugars content at the end of rooting stage as compared to untreated shoots. The layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm also followed a similar trend.

4.2.3 Carbohydrates

The total carbohydrate content was found to be 16.41 per cent initially but showed marginal reduction during rooting process (Table 6 and 8). The carbohydrate content decreased from 16.41 to 15.95 per cent as against the control which reduced to 16.01 per cent.

LEGEND -4

T_1 - IBA, 2000 ppm

T_2 - IBA, 4000 ppm

T_3 - IBA, 6000 ppm

T_4 - IBA, 1000 ppm + NAA, 1000 ppm

T_5 - IBA, 2000 ppm + NAA, 2000 ppm

T_6 - IBA, 3000 ppm + NAA, 3000 ppm

T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm

T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm

T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm

T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm

T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm

T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm

T_{13} - *Azospirillum* 37.5 g

T_{14} - Control

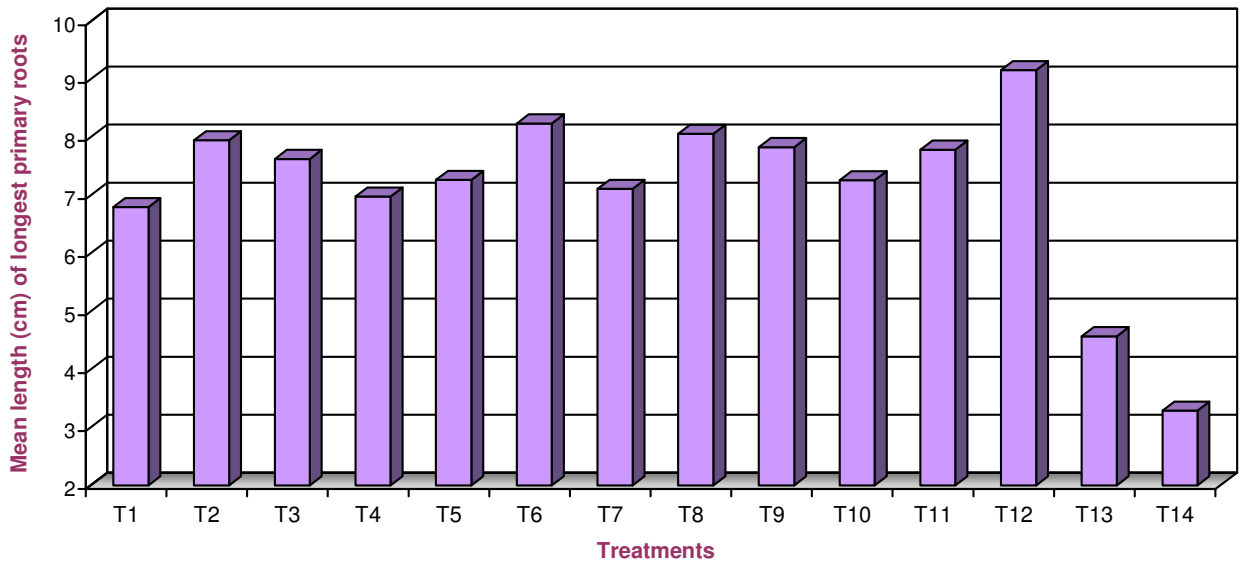

Fig. 4. Influence of growth regulators and microbial inoculants on mean length of longest primary roots in air-layers of guava

Fig. 4. Influence of growth regulators and microbial inoculants on mean length of longest primary roots in air-layers of guava

Table 5. Influence of growth regulators and *Azospirillum* on secondary root characters in air-layers of guava

Treatments	Number of secondary roots	Mean length of secondary roots* (cm)
T_1 - IBA, 2000 ppm	16.29	10.92
T_2 - IBA, 4000 ppm	22.83	14.67
T_3 - IBA, 6000 ppm	20.35	13.79
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	17.39	13.37
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	19.37	14.45
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	23.18	16.36
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	16.76	11.12
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	23.11	15.54
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	20.89	14.15
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	17.96	13.66
T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	20.14	15.16
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	25.22	18.33
T_{13} - *Azospirillum*	9.47	5.38
T_{14} - Control	8.98	4.90
Mean	18.71	12.99
S.Em$\pm$	0.68	0.76
CD at 5%	2.02	2.23

*Measured which are more than 2cm

Table 6. Biochemical constituents of guava shoots prior to inoculation

Biochemicals	cv. L- 49 (Percentage dry weight)
Starch	7.45
Total sugars	8.96
Total phenols (mg/g dry wt.)	1.46
Carbohydrates	16.41
Nitrogen	1.35
C/N ratio	12.5

Table 7. Influence of growth regulators and Azospirillum at seperation stage on biochemical constituents in air-layers of guava

Treatments	Starch (% dry wt.)	Total sugars (% dry wt.)	Total phenols (mg/g dry wt.)
T_1	7.28	8.68	1.29
T_2	7.24	8.78	1.36
T_3	7.27	8.71	1.33
T_4	7.24	8.73	1.32
T_5	7.22	8.78	1.34
T_6	7.19	8.80	1.36
T_7	7.23	8.72	1.32
T_8	7.19	8.83	1.37
T_9	7.21	8.75	1.35
T_{10}	7.18	8.78	1.34
T_{11}	7.17	8.82	1.36
T_{12}	7.15	8.85	1.37
T_{13}	7.31	8.67	1.24
T_{14}	7.36	8.65	1.21
Mean	7.23	8.75	1.32
S.Em+	0.58	0.63	0.14
CD at 5%	NS	NS	NS

NS - Non significant

T_1 IBA, 2000 ppm
T_2 IBA, 4000 ppm
T_3 IBA, 6000 ppm
T_4 IBA, 1000 ppm + NAA, 1000 ppm
T_5 IBA, 2000 ppm + NAA, 2000 ppm
T_6 IBA, 3000 ppm + NAA, 3000 ppm
T_7 *Azospirillum* 37.5 g + IBA 2000 ppm
T_8 *Azospirillum* 37.5 g + IBA, 4000 ppm
T_9 *Azospirillum* 37.5 g + IBA, 6000 ppm
T_{10} *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm
T_{11} *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm
T_{12} *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm
T_{13} *Azospirillum* 37.5 g
T_{14} Control

4.2.4 Total phenols

The data on the changes in total phenols content during rooting is presented in Table 6 and 7.

The shoots initially contained 1.46 mg/g total phenols on dry weight basis. The content was reduced during the process of rooting. However, the convesion was lesser in the treated layers. The layers treated with different combination treatments of *Azospirillum* 37.5 g, IBA and NAA and IBA singly at 4000 ppm maintained the total phenol at a higher level (1.32 to 1.37 mg/g dry wt.).

4.2.5 Nitrogen

The data relating to change in nitrogen level during rooting are presented in Table 6 and 8.

It was seen that the percentage of nitrogen was higher (1.35) at the time of layering. The level was reduced during the process of rooting from 1.35 to 1.14 per cent. There was no appreciable differences due to treatments.

4.2.6 Carbohydrates / Nitrogen ratio (C/N ratio)

The data regarding the changes in C/N ratio during rooting are presented in Table 6 and 8.

The shoots were containing a C/N ratio of 12.15 at layering stage. The final C/N ratio at the end of rooting was higher in the treated layers than in untreated layers. The layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm maintained a C/N ratio of 14.03 as compared to 12.60 recorded in untreated layers.

LEGEND -5

T_1 - IBA, 2000 ppm

T_2 - IBA, 4000 ppm

T_3 - IBA, 6000 ppm

T_4 - IBA, 1000 ppm + NAA, 1000 ppm

T_5 - IBA, 2000 ppm + NAA, 2000 ppm

T_6 - IBA, 3000 ppm + NAA, 3000 ppm

T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm

T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm

T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm

T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm

T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm

T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm

T_{13} - *Azospirillum* 37.5 g

T_{14} - Control

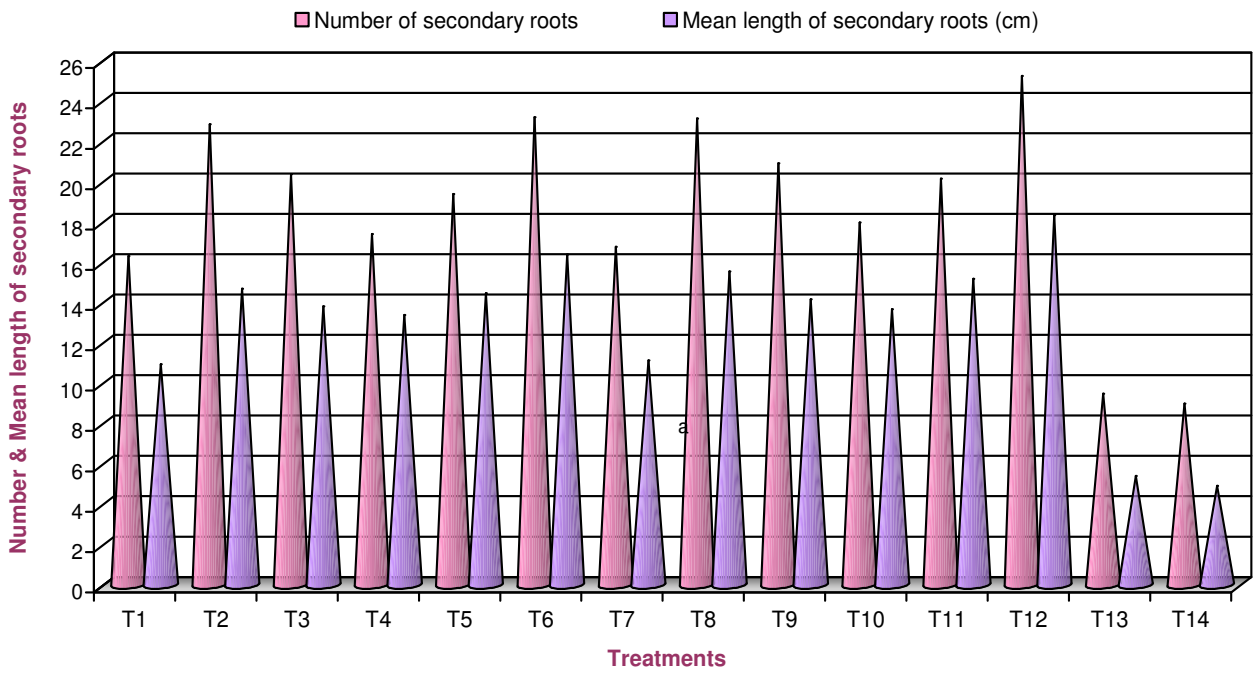

Fig. 5. Influence of growth regulators and microbial inoculants on secondary root characters in air-layers of guava

Fig. 5. Influence of growth regulators and microbial inoculants on secondary root characters in air-layers of guava

Table 8. Influence of growth regulators and *Azospirillum* at seperation stage on biochemical constituents in air layers of guava

Treatments	Carbohydrates (% dry wt.)	Nitrogen (% dry wt.)	Carbohydrate/ Nitrogen ratio
T_1	15.96	1.22	13.08
T_2	16.02	1.20	13.35
T_3	15.98	1.21	13.20
T_4	15.98	1.22	13.09
T_5	16.00	1.20	13.33
T_6	15.99	1.19	13.43
T_7	15.95	1.20	13.29
T_8	16.02	1.18	13.57
T_9	15.96	1.18	13.52
T_{10}	15.96	1.19	13.41
T_{11}	15.99	1.17	13.66
T_{12}	16.00	1.14	14.03
T_{13}	15.98	1.25	12.78
T_{14}	16.01	1.27	12.60
Mean	15.99	1.20	13.31
S.Em$\pm$	0.98	0.11	0.79
CD at 5%	NS	NS	NS

NS - Non significant

T_1 IBA, 2000 ppm
T_2 IBA, 4000 ppm
T_3 IBA, 6000 ppm
T_4 IBA, 1000 ppm + NAA, 1000 ppm
T_5 IBA, 2000 ppm + NAA, 2000 ppm
T_6 IBA, 3000 ppm + NAA, 3000 ppm
T_7 *Azospirillum* 37.5 g + IBA 2000 ppm
T_8 *Azospirillum* 37.5 g + IBA, 4000 ppm
T_9 *Azospirillum* 37.5 g + IBA, 6000 ppm
T_{10} *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm
T_{11} *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm
T_{12} *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm
T_{13} *Azospirillum* 37.5 g
T_{14} Control

Table 9. Effect of growth regulators and *Azospirillum* on number of side shoots in seperated air-layers of guava

Treatments	Number of side shoots at 30 days after seperation	Number of healthy side shoots at 60 days after separation
T_1 - IBA, 2000 ppm	4.94	3.96
T_2 - IBA, 4000 ppm	6.89	5.84
T_3 - IBA, 6000 ppm	6.72	5.72
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	5.83	4.95
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	6.31	5.31
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	8.17	7.19
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	5.22	4.21
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	7.12	5.98
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	6.91	5.88
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	6.32	5.08
T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	7.13	5.79
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	9.67	8.43
T_{13} - *Azospirillum*	3.11	2.89
T_{14} - Control	2.38	2.14
Mean	6.19	5.24
S.Em$\pm$	0.48	0.39
CD at 5%	1.42	1.14

Plate 3a. At 60 days after separation (T_1-T_7)

Plate 3b. At 60 days after separation (T_8-T_{14})

Table 10. Effect of growth regulators and *Azospirillum* on number of leaves produced in seperated air-layers of guava

Treatments	Number of leaves produced at 30 days after seperation	Number of leaves produced at 60 days after seperation
T_1 - IBA, 2000 ppm	9.98	12.69
T_2 - IBA, 4000 ppm	15.36	19.92
T_3 - IBA, 6000 ppm	13.76	18.83
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	11.83	14.51
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	12.78	18.67
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	16.45	23.76
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	10.31	13.35
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	15.92	20.11
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	13.82	19.47
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	12.21	14.73
T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	13.32	19.19
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	18.41	25.84
T_{13} - *Azospirillum* 37.5 g	6.75	8.22
T_{14} - Control	5.36	7.37
Mean	12.59	16.90
S.Em$\pm$	0.42	0.56
CD at 5%	1.22	1.64

4.3 Post-seperation studies in air-layers

4.3.1 Mean number of side shoots in air layer

The data pertaining to mean number of side shoots at 30 days after seperation (DAS) in air-layers as influenced by growth regulators and microbial inoculants are presented in Table 9.

The data on the number of side shoots per air layer was significant. The highest number of side shoots (9.67) was recorded in layers treated with *Azospirillum* 37.5 g and equal proportion (3000 ppm) each of IBA and NAA as against lowest number (2.38) recorded in untreated layers.

4.3.2 Mean number of healthy side shoots in air-layer

The data related to number of healthy side shoots in air-layer at 60 days after seperation (DAS) are presented in Table 9.

It was found that number of healthy side shoots per layer at 60 (DAS) was significant. The maximum number of healthy side shoots (8.43) was noted in layer which had received *Azospirillum* 37.5 g and equal proportion (3000 ppm) each of IBA and NAA as against lowest number (2.14). Here also application of IBA + NAA, 3000 ppm each affected the number of healthy side shoots.

4.3.3 Mean number of leaves in air-layer

The data on the mean number of leaves produced per layer at the end of 30 and 60 days after seperation (DAS) are presented in Table 10 and depicted in Fig. 6.It was found that the mean number of leaves produced by the treated layers at both the stages of 30 and 60 days after seperation (DAS) was significant. The layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm produced maximum mean number (18.41) of leaves at 30 days after seperation (DAS) as against the lowest (5.36) in untreated layers. The use of IBA alone at different concentrations affected the production of leaves specially IBA, 2000 ppm. A similar trend was noted with respect to mean number of leaves produced at 60 days after seperation (DAS).

LEGEND - 6

T_1 - IBA, 2000 ppm

T_2 - IBA, 4000 ppm

T_3 - IBA, 6000 ppm

T_4 - IBA, 1000 ppm + NAA, 1000 ppm

T_5 - IBA, 2000 ppm + NAA, 2000 ppm

T_6 - IBA, 3000 ppm + NAA, 3000 ppm

T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm

T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm

T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm

T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm

T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm

T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm

T_{13} - *Azospirillum* 37.5 g

T_{14} - Control

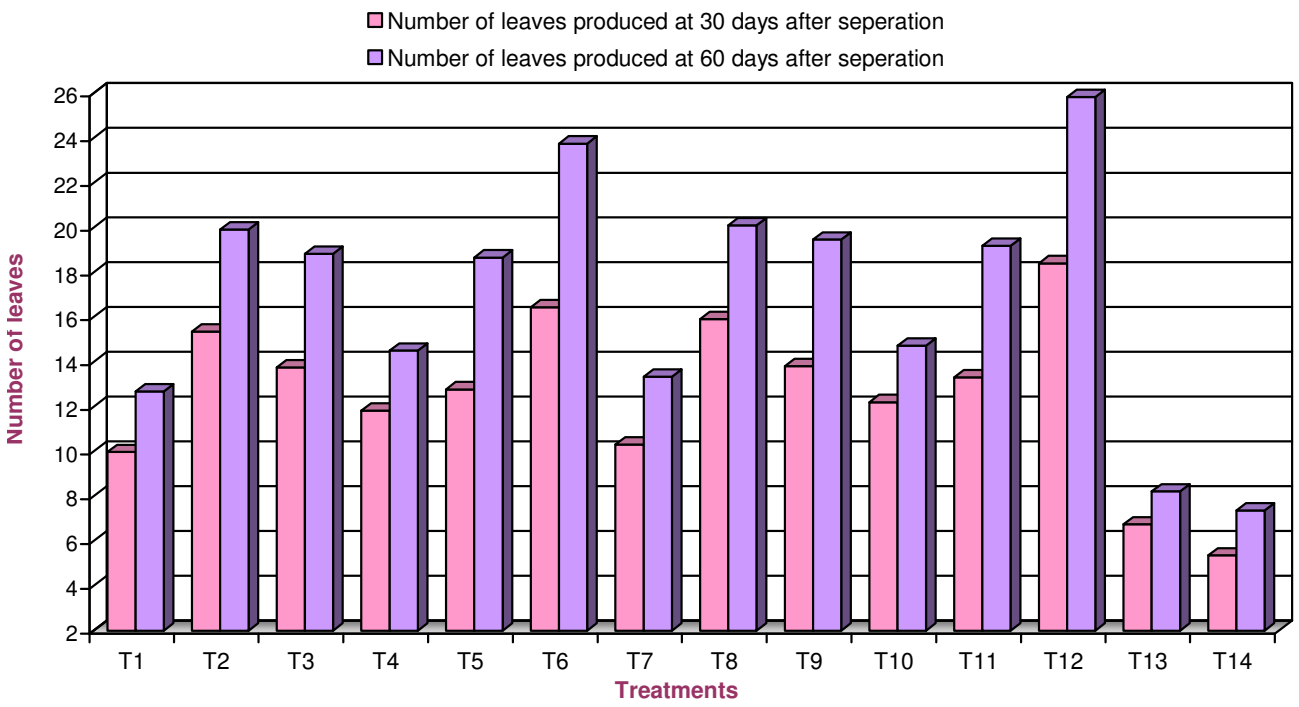

Fig. 6. Effect of growth regulators and microbial inoculants on number of leaves produced in seperated air-layers of guava

Fig. 6. Effect of growth regulators and microbial inoculants on number of leaves produced in seperated air-layers of guava

Table 11. Effect of growth regulators and *Azospirillum* on leaf area per layer in seperated air-layers of guava

.Treatments	Leaf area per layer (cm^2)
T_1 - IBA, 2000 ppm	39.92
T_2 - IBA, 4000 ppm	41.51
T_3 - IBA, 6000 ppm	40.80
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	41.31
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	42.56
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	43.82
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	43.87
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	45.89
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	46.47
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	46.64
T_{11}- *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	51.59
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	55.32
T_{13} - *Azospirillum* 37.5 g	38.04
T_{14} - Control	33.41
Mean	43.65
S.Em$\pm$	1.27
CD at 5%	3.69

Table 12. Length of side shoots as influenced by growth regulators *Azospirillum* air layers of guava

.Treatments	Mean length of side shoots at 60 days after seperation (cm)	Length of longest side shoots at 60 days after seperation (cm)
T_1 - IBA, 2000 ppm	4.11	4.98
T_2 - IBA, 4000 ppm	7.32	8.79
T_3 - IBA, 6000 ppm	7.12	8.62
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	5.19	7.01
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	6.51	8.93
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	7.99	10.31
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	4.82	5.27
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	7.97	8.98
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	7.93	8.87
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	5.57	7.44
T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	6.89	9.21
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	9.10	11.81
T_{13} - *Azospirillum*	3.11	4.17
T_{14} - Control	2.21	3.66
Mean	6.14	7.72
S.Em$\pm$	0.38	0.42
CD at 5%	1.10	1.22

4.3.4 Leaf area

The data with respect to leaf area per air-layers as influenced by growth regulators and microbial inoculants are presented in Table 11.

The data on the leaf area per layer was significant. The highest leaf area (55.32 cm^2) was noted in layers treated with *Azospirillum* 37.5 g and equal proportion (3000 ppm) each of IBA and NAA as against lowest leaf area (33.41 cm^2) recorded in control layers.

Application of growth regulators and *Azospirillum* recorded the highest leaf area as compared to other treatments.

4.3.5 Mean length of side shoot in air layer

The data relating to mean length of side shoots per layer as influenced by growth regulators and microbial inoculants are furnished in Table 12 and depicted in Fig. 7 (Plate 3a and 3b).

The differences in the mean length of side shoots produced under influence of various growth regulators and microbial inoculant treatments were statistically significant. The layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm recorded maximum (9.10 cm) mean length as compared to control layers which produced lowest (2.21 cm) mean length of side shoots.

4.3.6 Mean length of the longest side shoot in air layer

The results obtained regarding the mean length of the longest side shoots as influenced by growth regulators and microbial inoculants are presented in Table 12 and depicted in Fig. 7.

The data showed that all the treatments promoted higher mean length of longest side shoots as compared to control. The overall mean length of longest side shoots was in the range of 4.98 to 11.81 cm in different treatments with the exception of IBA applied singly at 2000/ 6000 ppm as against (3.66 cm) recorded in control. The mean length of longest side shoots that developed on layers which received *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm was 11.81 cm which was significantly higher as compared to rest of the treatments.

4.3.7 Survival percentage of transplanted air layers

The data pertaining to survival percentage of seperated rooted layers as influenced by growth regulators and microbial inoculants are presented in Table 13 and depicted in Fig. 8 (Plate 4).

The data revealed that all the growth regulators and microbial inoculants treatments significantly recorded higher proportion of survival of rooted layers. The highest percentage of survival (98.14%) was noted in the layers which had received *Azospirillum* 37.5 g and equal proportion (3000 ppm) each of IBA and NAA which had also recorded highest percentage of rooting. This is followed by IBA, 3000 ppm + NAA, 3000 ppm (88.43%).

LEGEND - 7

T_1 - IBA, 2000 ppm

T_2 - IBA, 4000 ppm

T_3 - IBA, 6000 ppm

T_4 - IBA, 1000 ppm + NAA, 1000 ppm

T_5 - IBA, 2000 ppm + NAA, 2000 ppm

T_6 - IBA, 3000 ppm + NAA, 3000 ppm

T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm

T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm

T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm

T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm

T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm

T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm

T_{13} - *Azospirillum* 37.5 g

T_{14} - Control

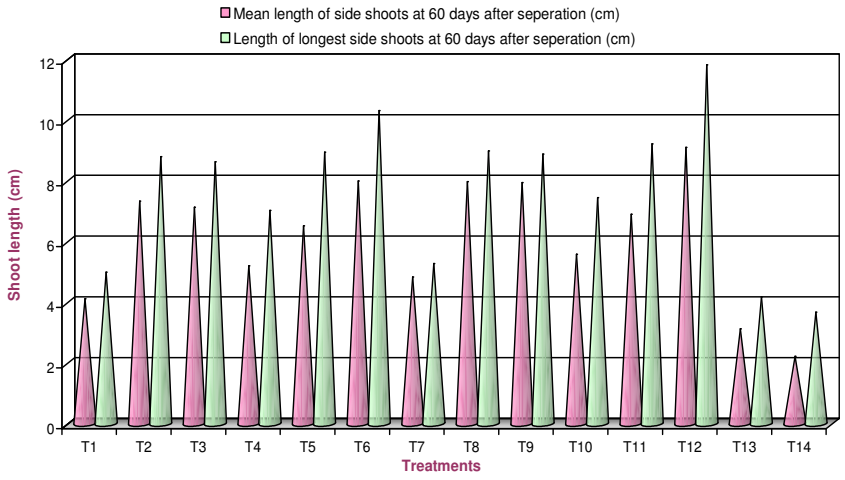

Fig. 7. Effect of growth regulators and microbial inoculants on number of side shoots in seperated air-layers of guava

Fig. 7. Effect of growth regulators and microbial inoculants on number of side shoots in seperated air-layers of guava

Table 13. Survival percentage of seperated air-layers in guava as influenced by growth regulators and *Azospirillum*

.**Treatments**	**Survival percentage of seperated air layers**
T_1 - IBA, 2000 ppm	69.76
T_2 - IBA, 4000 ppm	82.75
T_3 - IBA, 6000 ppm	76.93
T_4 - IBA, 1000 ppm + NAA, 1000 ppm	75.81
T_5 - IBA, 2000 ppm + NAA, 2000 ppm	80.09
T_6 - IBA, 3000 ppm + NAA, 3000 ppm	88.43
T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm	72.00
T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm	84.75
T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm	79.81
T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm	78.00
T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm	86.61
T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm	98.14
T_{13} - *Azospirillum* 37.5 g	56.48
T_{14} - Control	52.12
Mean	**77.26**
S.Em$\pm$	2.47
CD at 5%	7.17

LEGEND - 8

T_1 - IBA, 2000 ppm

T_2 - IBA, 4000 ppm

T_3 - IBA, 6000 ppm

T_4 - IBA, 1000 ppm + NAA, 1000 ppm

T_5 - IBA, 2000 ppm + NAA, 2000 ppm

T_6 - IBA, 3000 ppm + NAA, 3000 ppm

T_7 - *Azospirillum* 37.5 g + IBA, 2000 ppm

T_8 - *Azospirillum* 37.5 g + IBA, 4000 ppm

T_9 - *Azospirillum* 37.5 g + IBA, 6000 ppm

T_{10} - *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm

T_{11} - *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm

T_{12} - *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm

T_{13} - *Azospirillum* 37.5 g

T_{14} - Control

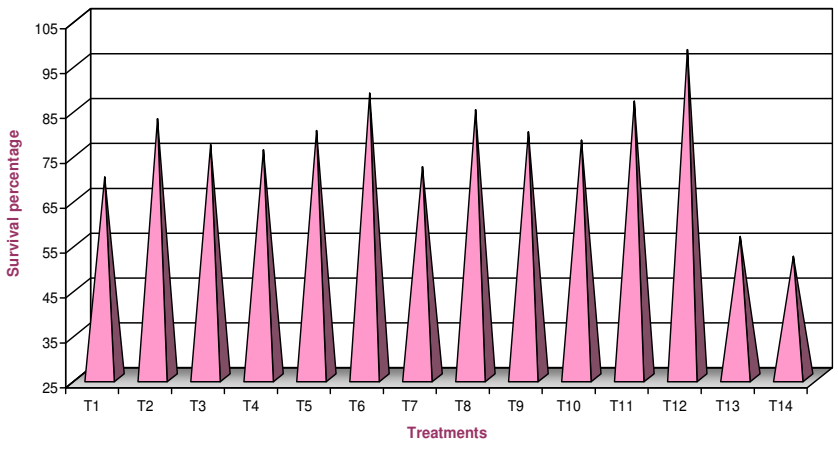

Fig. 8. Survival percentage of seperated air-layers in guava as influenced by growth regulators and microbial inoculants

Fig. 8. Survival percentage of seperated air-layers in guava as influenced by growth regulators and microbial inoculants

Plate 4; Bagging air layers of guava after separation from mother plant

V. DISCUSSION

The results of the experiment carried out to find out the optimum level of growth regulator, microbial inoculant and their combinations for better rooting and the biochemical changes related to rooting of mature shoot air-layers in guava cv. Lucknow-49 are discussed as under.

5.1 Root parameters

Both the growth regulators *viz*., IBA and NAA and *Azospirillum* favoured rooting. However, the favourable effect was more marked when both the growth regulators used in combination and in equal proportion with *Azospirillum.* The percentage of rooting with IBA 2000 ppm alone was 66.38 which was raised significantly to 79.07 per cent when it was mixed with equal proportion of NAA. Similarly, the treatment combination IBA, 3000 ppm + NAA, 3000 ppm recorded the higher rooting percentage of 88.49. For the same treatment when the *Azospirillum* 37.5 g per treatment was mixed resulted in significantly highest 91.68 rooting percentage. The growth regulators are known to exert synergistic influence when used in combination. Probably, in the present study also the higher percentage of rooting may be due to synergistic effect of two growth regulators used in combination in addition with *Azospirillum.* Bhandary and Kololgi (1960), Karunakara (1997), Kunal Kumar and Syamal (2005), also had made similar observation in guava air-layering and attributed the higher success in air-layers to the synergistic effect of two growth regulators IBA and NAA. Sateesh Kumar (1999) reported that dipping of pomegranate cuttings in *Azospirillum brasilense* gave maximum rooting percentage.

It is interesting to note that use of medium concentration of (IBA, 4000 ppm) when applied singly was also effective as compared to their lower (2000 ppm) or higher (6000 ppm) concentration.

Besides giving higher percentage of rooting, the layers which received combination treatments of equal proportion (3000 ppm each) of IBA and NAA along with *Azospirillum* 37.5 g excelled in all root characters. The treatments involving combination of IBA, 3000 ppm +NAA, 3000 ppm, IBA, singly at 4000 ppm, *Azospirillum* 37.5 g + IBA, 4000 ppm, *Azospirillum* 37.5 g + IBA, 6000 ppm and *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm were the next in the order with respect to root characters.

The *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm showed root emergence in shorter period (30.75 days). This was followed by IBA, 3000 ppm + NAA, 3000 ppm (31.25 days). Probably the synergistic influence could have been there for induction of early rooting, since NAA is known for promoting emergence of roots when used at optimum concentration in combination with IBA (Sulladmath and Kologi, 1969 in sapota., Karunakar, 1997., in guava and Athani *et al*., 2001 in guava air-layering).

Regarding the primary and secondary roots, the layers treated with combination of *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm produced significantly higher number of roots. This is followed by the treatments IBA, 3000 ppm + NAA, 3000 ppm, IBA 4000 ppm, *Azospirillum* 37.5 g + IBA, 4000 ppm, *Azospirillum* 37.5 g + IBA, 6000 ppm, *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm. These results got the support from finding in other crops *viz*., Bid and Mukharjee (1969) in mango and Uthaiah *et al*. (1976) in sapota. Similar trend was observed by Karunakara (1997) in guava, Patil *et al*. (2004) in pomegranate air-layers and Gowda *et al*. (2006b) in sapota.

With respect to mean length of primary and secondary roots and longest primary root, the treatments *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm and IBA, 3000 ppm + NAA, 3000 ppm was found to be better than all other treatments. This may be due to the fact that early root initiation might have provided enough time for higher rate of cell division and cell elongation which ultimately might have promoted higher length of roots. Similar trend was observed by Sulladmath and Kologi (1969) in sapota and Rajan and Sant Ram (`1985) in mango and Karunakara (1997) in guava air layers and Patil *et al*. (2004) in pomegranate air layers

The foregoing indicate that combination treatment of *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm has been found to induce better root system in guava mature shoot air- layers.

Azospirillum is nitrogen fixing bacterium that lives in symbiotic (associative) relationship in the rhizosphere of several tropical crops. It stimulates plant growth through nitrogen fixation and production of growth substances like auxins, gibberellins and cytokinin. It is estimated that almost 10 to 15 % of the required nitrogen can be met by *Azospirillum* (Tanuja and Purohit, 2008).

5.2 Biochemical studies

It is a well known fact that the different biochemical constituents have an important role during rooting. This may be mainly related to sugars, total carbohydrates, total nitrogen, C/N ratio and total phenols. Rooting co-factors which act synergistically with auxins have also been reported to promote rooting. These biochemical factors as related to the different treatments in the present work are discussed below

In all the estimation of biochemical factors done in the present study, there was only a marginal change during the process of rooting both in the treated and untreated layers (Tables 6, 7 and 8).

The steady decline in the level of sugars and starch during the initiation and growth of the roots indicates breakdown of carbohydrates during root development. Arslonov (1979) observed breakdown of carbohydrates during the initial stages of root growth in lemon cuttings, and also noted a rise in catalase and peroxidase activities which accompanied the breakdown of carbohydrates.

There was a reduction in starch content both in treated and untreated layers. But the reduction was more in case of treated layers. The difference in the magnitude of decline of starch content between the treated and untreated layers showed that the exogenous application of auxins might have enhanced the hydrolysis of starch. According to Arslonov (1979) the exogenous application of auxins produced changes in the redox regime and this resulted in the utilization of the stored food substances for quicker root formation in lemon cuttings.

Further, among different growth regulators used at different concentrations, there has been difference in the rate of decline in the starch content. This indicates that there exists differential capacity of growth regulators in enhancing the hydrolysis of starch. In the present study *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm showed higher magnitude of hydrolysis of starch whereas, the layers which had received *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm recorded lowest percentage of nitrogen as compared to the untreated layers. This indicates higher utilization of nitrogen in treated layers. A similar trend was noted by Rao *et al*. (1990) in cashew and Karunakara (1997) in guava air-layers respectively and opined that low nitrogen brings about an increase in the activity of rooting co-factors, thus causing better rooting.

The carbohydrate/nitrogen ratio was found to be slightly higher in treated layers as compared to untreated layers in the present study. It has been opined that higher the C/N ratio, the greater would be the activity of rooting cofactors and thereby better rooting (Uthaiah, 1975 and Telang, 1981).

With regard to total phenol content, it was marginally higher in the treated layers as compared to untreated layers at the end of the rooting period, after a steady decline from the initial level. The layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm maintained a higher level of total phenols in which rooting was maximum. Similarly, several workers (Bose *et al*., 1972., Roy *et al*., 1973 and Karunakara, 1997) were of the opinion that the phenolic compounds act as synergists to the auxin action in root promotion. Mokashi (1977) also attributed the higher percentage of rooting observed in Gulabi cultivar of grape to the higher level of phenolic compounds present as compared to its content in Thompson seedless cultivar of grape.

5.3 Post- separation studies

The growth regulator and microbial inoculants treatments which produced better rooting seem to influence the survival percentage also. The treatment *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm, which was found to be good for root promotion, also recorded highest significantly (98.14%) magnitude of survival. The previous workers also had made similar observations in mango (Bid and Mukherjee, 1969), in guava (Singh *et al*., 1996), (Karunakara, 1997), (Kunal Kumar and Syamal, 2005) and (Prabhar Singh *et al*., 2007).

The higher percentage of survival after transplanting the rooted air-layers can be attributed to the possession of better root characters like higher number and length of roots.

Azospirillum sp. are considered to be important plant growth promoting rhizobacteria that can improve the growth and economically important. *Azospirillum* plant association leads to the enhanced development and yield of different host plants under appropriate growth condition. The increase in yield is attributed mainly to an improvement in root development, an increase in the rate of water and mineral uptake by roots.

Phytohormones synthesized by *Azospirillum* influence the host root respiration rate, metabolism and root proliferation and hence better the mineral and water uptake in inoculated plants.

An organism to be used as an inoculant for crop improvement should be capable of quick and firm establishment in the root region. To establish the affinity of *Azospirillum* to crop plant roots

The number of new side shoots as well as leaves, leaf area and length of new side shoots produced were also highest in the layers treated with *Azospirillum* 37.5 g + IBA 3000 ppm + 3000 ppm. Since these seperated layers are having better root system, they could absorb sufficient water and other nutrients, which could excell in all the characters when compared to other treatments.

Govind and Pandey (1985) have found that pepper cuttings inoculated with *Azospirillum* spp. had higher germinated cuttings, length of sprout and more number of fully opened leaves. According to them the bacteria apart from producing root hormone (IAA), it also synthesises gibberellic acid which had enhanced vegetative growth.

Apart from the reasons mentioned earlier enhanced growth parameter like plant height, leaf area and number of branches due to *Azospirillum* may also be attributed to the influence of nitrogen, the chief constituent of protein-essential for formation of protoplasm, which enhance cell division and cell enlargement, moreover, nitrogen is an important component of amino acids and co-enzymes, which are of considerable biological importance (Sattar and Gaur, 1987). Also by providing protection against the non-parasitic pathogens and transforming unavailable mineral and organic compounds into available forms in plants. Any of these effects would have lead to increase in plant growth and better survival percentage.

Future line of work

1. Different concentrations of IBA, NAA and *Azospirillum* in different combinations may be tried for inducing rooting in guava air layers.
2. The microbial inoculants other than *Azospirillum may* be tried in combination with IBA, NAA and other rooting growth regulators for inducing rooting in guava air layers.

VI. SUMMARY AND CONCLUSION

The investigation on propagation of guava (*Psidium guajava* L.) by air-layering as influenced by growth regulators and microbial inoculants were carried out at the Silver Jubilee Orchard (S.J.O.), Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad during 2010-2011.

The study aimed to find out suitable root inducing treatment and its optimum concentration for maximum survival percentage in mature shoot air layers of guava cv. L-49.

In mature shoot air-layering experiment, the growth regulator and microbial inoculant treatments comprised of application of Indole Butyric Acid (IBA) and Naphthelene Acetic Acid (NAA) singly or in combination with different concentrations added with *Azospirillum.* The treatment combination of growth regulators and microbial inoculants were IBA, 1000 ppm + NAA, 1000 ppm, IBA, 2000 ppm + NAA, 2000 ppm, IBA, 3000 ppm + NAA, 3000 ppm, *Azospirillum* 37.5 g + IBA, 2000 ppm, *Azospirillum* 37.5 g + IBA, 4000 ppm, *Azospirillum* 37.5 g + IBA, 6000 ppm, *Azospirillum* 37.5 g + IBA, 1000 ppm + NAA, 1000 ppm, *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm, *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm and single application concentrations ranged from as low a concentration as 2000 ppm to as high as 6000 ppm. In all, there were 14 treatment combinations with three replications laid out in RBD statistical design.

Observations were recorded in respect of rooting percentage, root parameters, biochemical constituents during the rooting process of air-layers and success and surviaval percentage of air-layers seperated from mother plant.

The salient finding of the study are summarized below.

1. In general, both growth regulators *viz*. IBA, NAA alone and in combination with *Azospirillum* favoured rooting in air-layers.
2. A synergistic effect was noted in case of higher percentage of rooting where the two growth regulators were used in combination with *Azospirillum.* Among the different combinations, the layers which had received *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm recorded the significantly higher percentage (91.68%) of rooting with desirable root characters such as higher number of primary and secondary roots, longer length of primary roots higher girth of primary roots.
3. Next to this treatment, the other favourable treatments were IBA, 3000 ppm + NAA, 3000 ppm, IBA, 2000 ppm, *Azospirillum* 37.5 g + IBA, 4000 ppm, *Azospirillum* 37.5 g + IBA, 6000 ppm, *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm.
4. The use of medium (3000 ppm) concentration of both the growth regulators (IBA and NAA) with *Azospirillum* 37.5 g applied singly was more effective as compared to either lower (2000 ppm) or higher (6000 ppm) concentrations.
5. Exogenous application of auxins favoured the breakdown of carbohydrates which might have helped in better rooting, as evidenced by lower content of nitrogen and higher carbohydrates / nitrogen ratio maintained in the treatments involving growth regulator, microbial inoculants. Especially in case of phenols, the content was higher in layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm, IBA, 3000 ppm + NAA, 3000 ppm which indicates its role in favouring induction and maturity of roots by way of synergistic effect.
6. With regard to survivability of rooted layers, maximum survival (98.14%, 60 days after seperation) was noted in the layers treated with *Azospirillum* 37.5 g + IBA, 3000 ppm + NAA, 3000 ppm. Besides this, treatment also produced more number of new shoots, leaves, leaf area, and higher length of new shoots in seperated air layers.

Finally it may be concluded that the mature shoots or forced shoots in the germplasm block (Gene Bank) may be used for multiplication of plant material by air-layering. The higher percentage of rooting could be obtained by application of equal proportion of 3000 ppm each of IBA and NAA with *Azospirillum* 37.5 g per treatments.

REFERENCES

Abnave, R. B., Dhumal, S. S., Chavan, S. K., More, A. J. and Thorat, D. N., 2007, Effect of IBA and NAA in propagation of guava by tongue layering. J. Maharashtra Agric. Univ., 32 (3): 414-416.

Ahamed, R., 1964, Propagation of guava by aerial layering. W. Pakistan J. Agric. Res., 2 (3): 82-84.

Albany, V. N. R., Vilchez, P. J. A., Viloria, Z. J., Castro, C. and Gadea, L. J., 2004, Effect of vegetative propagation of guava using air layering technique. Agronomia Trop. Maracay, 54 (1): 63-73.

Alila, P., Chiesotsu, S., Kar, P. L. and Sanyal, D., 2000, Influence of growth regulators and etiolation on air layering in Jackfruit. Hort. J., 13 (2): 9-13.

Animesh Sankar and Bikash ghosh, 2006, Air layering in guava cv. L-49 as affected by plant growth regulators, wrappers and time of operation. Environ and Eco., 24 S (Special 3A): 820-823.

Anonymous, 1961, Rooting in guava. Indian Hort., 3 (4): 11.

Anonymous, 1970, *Official Methods of Analysis* of the "Association of Official Analytical Chemists", 11th Edition, Washington.

Arslonov, M. N., 1979, Physiological changes occuring during root formation in lemon cuttings. Uzabekskii Biologicheskii Zhurnal, 5: 24-26.

Athani, S. I., Swamy, G. S. K. and Patil, P. B., 2001, Effect of different pre-treatment's on rooting of air layers in Guava cv. Sardar. Karnataka J. Agric. Sci., 14 (1): 199-200.

Bar, T. and Okon, Y., 1993, Tryptophan conversion to Indole-3-acetic acid via Indole-3-acetamide pathway in *Azospirillum brasilense*. Canadian J. Microbiol, 39: 81-86.

Barholia, A. K., 1995, Effect of growth regulators on the rooting and survival of air layers of jackfruit (*Artocarpus heterophyllus* Lam.). Gujarat Agric. Univ. Res. J., 20 (2): 108-111

Basu, R. N., Lahari, B. and Sen, P. K., 1967, Biochemical changes during regeneration of roots in air layers of mango (*Mangifera indica* L.). Curr. Sci., 36: 413-415.

Bhagat, B. K., Jain, B. P., Singh, C. and Choudhary, B. M., 1999, Studies on the propagation of guava (*Psidium guajava L.)* cv. Sardar by ground layering in polybags. Orissa J. Hort., 27 (1): 19-21.

Bhandary, K. R. and Kololgi, S. D., 1960, Studies on seasonal effect of plant regulators in combination on the rooting of air-layers of guava (*Psidium guajava* L.) var. 'Lucknow-49'. The Lal-Baugh, 5 (2): 8-10.

Bhujbal, B. G., 1972, Effective concentration of IBA in the air layering of guava. Res. J. Mahatma Phule Agric. Univ., 3 (1): 53-56.

Bhullar, S. S., 1962, Litchi likes layering. Indian Hort., 6 (2): 5- 6.

Bid, N. N. and Mukherjee, S. K., 1969, Varietal response to etiolation and growth regulator treatment in air-layering of mango. Indian J. Agric. Sci., 39: 1013-1019.

Bose, T. K., Roy, B. N. and Basu, R. N., 1972, Synergism between auxins and phenolic compounds in the rooting of cuttings. Indian Agric., 16 (2): 171-176.

Chandrappa and Nache Gouda, V., 1998, Influence of Auxins and 1, 2, 3, Acid on rooting of guava air layers. Mysore J. Agric. Sci., 32: 59-66.

Chinnappa, K. S. and Kololgi, S. D., 1961, Effect of plant regulators on cell wall constituents of bark of chiku (*Achras zapota* L.) above the gootee girdle. The Lal-Baugh, 6(4): 29-32.

Chonkar, V. S. and Singh, R. K., 1972, Propagation of *Mangifera indica* L. by air-layering. Acta Hort., 24: 89-92.

Duarte, O. and Sachini, G., 2003, Effect of season, type of material and on cuttings and air layers of litchi (*Litchi sinensis* Sonn.) 'Brewster'. *Proc. of the Interamerican* Society for Trop. Hort., 46: 29-31.

Dutta, P., 2000, Effect of growth regulators on air-layering of sweet (*Citrus sinensis* L.). Osbeck. Environ. and Ecol., 18 (4): 899-901.

Fallik, E. and Okon, Y., 1984, Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize roots. Soil Biol. and Bioche., 21: 147-153.

Ghosh, S. N. and Ranjan Tarai., 2005, Air layering in guava in red laterite zone of West Bengal. Hort. J., 18 (2) 91-93.

Goodwin, T. W., 1976, Chemistry and Biochemistry of Plant Pigments. Vol. I, Academic Press, p. 736.

Govind, M. and Pandey, K. C., 1985, Utilization of the diazotroph. *Azospirillum* for inducing rooting in Pepper cuttings (*Piper nigrum* L.). Curr. Sci., Nov., 20, 54: 1186-1188.

Gowda, V. N., Shyamalamma, S. and Prakash, G. N., 2006a, Influence of auxin and 1, 2, 4 acid on rooting of Rose apple (*Syzygium Jambos* L.). Acta Hort., (727): 89-94.

Gowda, V. N., Vasanth, K. R. and Shyamalamma, S., 2006b, Studies on vegetative propagation of khirni (*Manilkara hexandra* (Roxb) Dub.) by air layering. Acta Hort., (727): 85-88.

Harari, A., Kigel, J. and Okon, Y., 1988, Involvement of IAA in the interaction between *Azospirillum brasilense* and *Panicum mileaceum* roots. Pl. and Soil, 110: 275-282.

Hedge, N. K. and Sulikeri, G. S., 1989, Effect of IBA on the rooting of air layers of pomegranate. Curr. Res. Univ. Agric. Sci., Bangalore (India), 18 (11): 161-162.

Hore, J. K. and Sen, S. K., 1991, Role of growth regulating chemicals on air-layering of rose apple (*Syzygium jambos* (Linn.) Alston). Orissa J. Hort., 19 (1-2): 99-106.

Hore, J. K. and Sen, S. K., 1993, Effect of growth regulators and synergists on air layering of water apple. Orissa J. Agric. Res., 5 (1-2): 20-29.

Hore, J. K. and Sen, S. K., 1997, Effect of etiolation and plant growth regulators on the success of air-layering of pomegranate (*Punica granatum* L.). Haryana J. Hort. Sci., 26 (1-2): 63-66.

Jan, N. E., Wazir, F. K., Mahmmad, Ishtiaq., Haji Mohammad and Asghar, Ali., 2003, Effect of different concentration of IBA on rooting of Litchi in air layering. Sarhad J. Agri., 19 (1): 47-54.

Kadman, A., 1985, Improvements in the air layering propagation methods for lychee and macadamia trees. Acta Hort., 158: 143-149.

Kakon, A. J., Rahim, M. A. and Alam, M. S., 2005, Effect of steps of cutting on the survivability of growth of two varieties of guava air-layers. J. Subtrop. Agric. Res. and dev., 3 (1): 34-38.

Kamleshkar Singh and Jain, B.P., 1996, Propagation of Guava (*Psidium Guajava* L.) by air layering. Scientific Hort., 5: 49-50.

Karunakara, J., 1997, Studies on induction of rooting in shoots of juvenile plants in guava (*Psidium guajava* L.). *M. Sc. (Agri.) Thesis,* Univ. Agric. Sci., Dharwad (India).

Kunal Kumar and Syamal, M. M., 2005, Effect of etiolation and plant growth substances on rooting and survival of air layering of guava. Indian J. Hort., 62 (3): 290-292.

Lal, S., Tiwari, J. P., Awasthi, P. and Singh, G., 2007, Effect of IBA and NAA on rooting potential of stooled shoots of guava (*Psidium guajava* L.) cv. Sardar. Acta Hort., (735): 193-196.

Mahabir Singh, 2001, Efficacy of plant growth regulators, their concentrations and wrappers on rooting success and survival of air-layered guava twig. Crop Res., 21 (2): 153-156.

Mahabir Singh., Patel, Y. R. and Yadava, H. S., 1995, Success and survival of air layering as influenced by growth regulators and wrappers in guava (*Psidium guajava* L.). Adv. Plant Sci., 8 (1): 183-188.

Mahadevan, S., 1975, Methods in Physiological Plant Pathology, Shivakasi Publication, Madras, pp. 63-64.

Mascarua-Esparca, M. A., Yilla-Gonzaler, R. and Cabalaellado, J., 1988, Acetylen reduction and indole acetic acid production by *Azospirillum* isolates from cactaceous plants. Pl. and Soil, 106: 91-95.

Miller, G. L., 1972, Use of Dinitro-salicylic acid reagent for determination of reducing sugar. Ann. Chem., 31: 426-428.

Mishra, R. S. and Singh, S. P., 1995, High value propagation. Horticulturist, 4 (4): 32-33.

Mokashi, A. N., 1977, Studies on propagation of Thompson Seedless grape (*Vitis vinifera* L.) by cuttings. *M.Sc (Agri.) Thesis*, Univ. Agric. Sci., Bangalore (India).

Mukherjee, S. K., Rao, D. P., Chaladar, B. P. and Chatterjee, B. K., 1986, Effect of growth regulators, invigoration and etiolation on rooting of air layers of Bael (*Aegle marmelos* corre). Indian J. Horti., 43: 9-12.

Namita Boro., Lal, R. L. and Singh, A. K., 2006, Effect of IBA and planting containers on shoot and root characters and survival of litchi air layers. Indian J. Hort., 63 (2): 155-158.

Nanda, K. K., 1975, Physiology of adventitious root formation. Indian J. Pl. Physiol., 18: 8-9.

Omay, S. H., Schmidt, W. A. and Martin, P., 1992, Indole acetic acid production by the rhizosphere bacterium *Azospirillum brasilense* under in-vitro conditions. Canadian J. Microbiol., 39: 187-192.

Pandey, G., 1996, Response of IBA and NAA on rooting of Pineapple guava. Adv. Hort. and For., 5: 75-79.

Panse, V. G. and Sukhatme, P. V., 1961, *Statistical Methods for Agricultural Workers*, Indian Council of Agri. Res., New Delhi, p. 381.

Patel, N. B. and Pasaliya, Y. M., 1995, Effect of plant growth regulators and advanced ringing on rooting in air-layering of guava (*Psidium guajava* L.) cv. L-49. Recent Hort., 2 (2): 33-36.

Patel, R. K., Bose, U. S. and Tripathi, S. K., 1996, Effect of growth regulators and wrappers on success and survival of air layering in guava cv. Allahabad safeda. Crop Res., 12 (1): 56-60.

Patel, R. M., Patel, R. B. and Patel, M. P., 1989, Effect of growth regulators and polythene wrappers on rooting of air layers of guava. Bharatiya Krishi Anusandhan Patrika, 4 (3): 141-148.

Patil, A. B., Nirmalnath, P. J. and Patil, S. R., 2004, Studies on promotion of rooting in air layers of Pomegranate as influenced by microbial inoculants. Karnataka J. Agric. Sci., 17 (4): 861-863.

Pawan Kumar, Thakur, S., Singh, U. K. and Singh, R. R., 2004, Effect of growth regulators on the rooting and survival of air layers of jackfruit. J. Applied Biol., 14 (1): 39-41.

Prabhar Singh, Chandrakar, J., Singh, A. K., Vijay Jain and Agrawal, S., 2007, Effect on rooting in guava cv. L-49 through PGR and organic media under Chhattisgarh condition. Acta Hort., (735): 197-200.

Rahman Noor., Hussain Ijaz., Mohammod Imran Taslin Jan. and Azmat Ali Awan., 2000, Effect of different Concentrations of IBA on Rooting of Litchi (*Litchi chinensis*) in Air Layering. Pakistan J. Biol. Sci., 3 (2): 330-331.

Rajan, S. and Sant Ram, 1985, Interaction of Indole-3-butyric acid with other chemicals in the rooting of air-layers. Abtr. Papers, *Second Int. Symp. on Mango*, Bangalore (India) pp. 20-21.

Ram Chandra and Sheo Govind, 1993, Efficacy of cane sugar and auxins in relation to rooting and survival of guava layers in arid Alfisol. Prog. Hort., 23 (1-4): 22-25.

Rao, M. B. N., Satyanarayana, G., Raj, A. E. and Rameshwar, A., 1990, Influence of post-ringing period on auxin activities, carbohydrate and nitrogen contents in ringed shoot cuttings cashew. Trop. Agri., 67 (3): 283-285.

Roy, B. N., Bose, T. K. and Basu, R. N., 1973, Studies on auxin synergists in rooting of cuttings, Pl. Sci., 5: 117-122.

Sadhu, M. K., Naskar, B. B. and Basu, R. N., 1972, Auxin synergists in the rooting of air-layers of tropical fruit trees. Indian Agri., 16 (3): 251-257.

Salaria, A. and Salaria, B. S., 2010, Facts and figure of Indian horticulture. In: *Fruit and plantation crops. pp. I.*

Saroj, P. L. and Pathak, R. K., 1994, Propagation of wild guava through stooling. Adv. Agric. Res., India, 2: 74-80.

Sateesh Kumar, K., 1999, Role of microorganisms in rooting of pomegranate (*Punica granatum* L.) cv. Allahabad surkha. Prog. Hort., 28 (1-2): 51-53.

Sattar, M. A. and Gaur, A. C., 1987, Production of auxins and gibberellins by phosphate dissolving mcrooganisms. Zentralbl Microbiol., 142: 393-395.

Sengupta, S. and Thakur, S., 2001, Studies on the effect of growth regulators on rooting of air-layers of jackfruit (*Artocarpus heterophyllus* Lam.). Orissa J. Hort., 29 (1): 63-65.

Sharfuddin, A. F. M. and Husain, A., 1973, Effect of growth regulators, rooting and wrapping materials on the success of air layering in Litchi. Bangladesh Hort., 1 (2): 45-51.

Sharfuddin, A. F. M., 1983, Effect of pruning on the survival of detached rooted air layerinh Litchi. Bangladesh Hort., 11 (1): 39-40.

Sharma, K. K., Jawanda, J. S. and Gill, S. S., 1978, Propagation of guava by mound layering. Punjab Hort. J., 18 (1/2): 65-67.

Sharma, R. S., Sharma, T. R. and Sharma, R. C., 1991, Influence of growth regulators and time of operation on rooting of air layering in guava (*Psidium guajava* L.) cv. 'Allahabad Safeda'. Orissa J. Hort., 19 (1-2): 41-45.

Sharma, S. B., Ray, P. K. and Singh, B. K., 1990, Note on rooting and survival of Litchi layers. Indian J. Hort. 47 (2): 174-176.

Singh, A. K. and Singh, G. N., 2004, Effect of IBA on rooting of air layers of Jackfruit (*Artocarpus heterophyllus* Lam.). Scientific Hort., 9: 41-46.

Singh, B. P. and Singh, I. J., 1970, Studies on the effect of source and plant growth regulators on performance of air layers of guava (*Psidium guajava* L.). Balwant Vidyapeeth J. Agric. Scientific. Res., 12 (1): 12-15.

Singh, D. B. and Singh, R. K., 1996, "Effect of IBA rooting of stem-cutting and Air- layering of Guava (*Psidium Guajava* L.)"cv. Allahabad surkha. Prog. Hort., 28 (1-2): 51-53.

Singh, D. K., Maurya, V. N. and Singh, A. R., 1996, A note on Regeneration of Guava (*Psidium Guajava* L.) cultivars of stooling with aid of IBA. Haryana J. Hort. Sci., 25 (1): 34-36.

Singh, S. P., 2006, Effect of time of ringing of shoots and application of root promoters on success of air layering in Jamun (*Syzygium cumini* Skeel). Scientific Hort., 10: 165-170.

Sinha, R. and Ray, S. K. D., 2002, Effect of different growth regulators in air layering of Litchi cv. Bombai. Hort. J., 15 (1): 63-67.

Smarsi, S. C., Chagas, E. A., Reis, L. L. dos., Oliveira, G. F. De., Mendonca, V., Tropaldi, L., Pio, R. and Scarpare - Filho, J. A., 2008, Concentrations of IBA and kinds of substances in the vegetative propagation of litchi. Revista Brasileirade Fluri., 30 (1): 7-11.

Storey, W. B., 1973, The Lychee. Year b. Calif. Avocado Soc., 56: 75-86.

Sulladmath, U. V. and Kololgi, S. D., 1969, Synergistic influence of IBA and NAA on rooting of air-layers of chiku (*Achras zapota* L.) var. Kalipatti. South Indian Hort., 17: 14-17.

Suryanarayana, V. and Venkateswara Rao, K., 1984, Effect of growth regulators on air layering of fig and pomegranate. Andra Agric. J., 31 (1): 54-58.

Syamal, M. M. and Singh, S. K., 1993, Effect of etiolation and plant growth substances on rooting of air-layers in litchi. Indian J. Hort., 50 (3): 241-244.

Tanuja Singh and Purohit, S. S., 2008, *Biofertilizer Technology*. Published by Agrobios (India), vol. 631.847.

Telang, V. V., 1981, Studies on propagation in seedless lime (*Citrus latifolia* Tanaka) by air layering. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad, Karnataka (India).

Tien, T. M., Gaskins, M. H. and Hubbell, D. H., 1979, plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearlmillet. Applied and Environ. Microbiol., 37: 1016-1024.

Tomar, K. S., Gurjar, B. S. and Tomar, R. S. S., 1999, Response of different concentrations of IBA and NAA on rootage and growth of air layers of guava (*Psidium guajava* L.) cv. Gwarlior-27. Adv. Plant Sci., 12 (2): 535-538.

Uthaiah, B. C., 1975, Studies on propagation of sapota (*Achras zapota* L.) by air layering. *M.Sc. (Agri.) Thesis,* Univ. Agric. Sci., Bangalore (India).

Uthaiah, B. C., Bhandary, K. R. and Rao, M. M., 1976, Effect of certain pre-conditioning treatments on the rooting of air-layers of sapota (*Achras zapota* L). South Indian Hort., 24: 135-139.

Venkatesh, H. C., 1983, Studies on propagation of guava (*Psidium guajava* L.) by air layering and cuttings. *M. Sc. (Agri) Thesis*, Univ. Agric. Sci., Bangalore, Karnataka (India).

Verma, A. N., Srivastava, D. K., Ram, K. and Sharma, R. K., 1970, Effect of plant regulators on air-layering in mango guava and kagzi lime. Allahabad Farmer, 44: 139-142.

*Yee, W., 1972, The Lychee in Hawaii. Circ. Univ. Hawii. Agric. Coop. Exp. Serv., 366: 1-24.

Zimmer, W. and Bothe, H., 1988, The phytohormonal interaction between *Azospirillum* and wheat plant. Pl. and Soil, 110: 239-247.

---

* Original not seen

Appendix-I

Monthly meteorological data for the experimental year 2010-2011 and the average of 60 years (1950-2009) at Meteorological Observatory, Main Agricultural Research Station, College of Agriculture, University of Agricultural Sciences, Dharwad.

Months	Rainfall (mm)		Temperature (^{0}C)				Relative humidity (%)	
			Mean maximum		Mean minimum			
	2010	1950-2009	2010	1950-2009	2010	1950-2009	2010	1950-2009
January	0.8	0.062	29.67	28.2	15.4	14.07	63	64.81
February	0.4	0.547	32.20	32.4	17.3	16.56	50	54.41
March	Trace	15.65	33.73	35.6	20.3	19.71	49	64.24
April	43.8	39.27	36.00	37.6	22.0	20.11	55	78.05
May	63.1	68.39	34.41	35.7	22.4	20.95	63	75.78
June	63.4	108.51	28.77	31.2	21.8	21.68	75	86.29
July	155.0	138.70	28.64	27.7	20.8	20.85	84	89.18
August	190.7	154.45	26.92	27.9	20.7	20.16	84	88.60
September	164.9	135.23	28.15	27.9	20.2	19.96	83	86.68
October	177.0	94.43	30.13	29	19.5	18.65	77	79.40
November	92.8	52.49	29.67	28.4	19.0	15.93	79	73.62
December	0.6	2.83	28.94	27.4	14.1	13.18	65	69.24
Total	**952.5**	**810.55**						

AIR LAYERING IN GUAVA AS INFLUENCED BY GROWTH REGULATORS AND AZOSPIRILLUM

SANJEEV KUMAR **2011** **Dr. S. G. ANGADI**

MAJOR ADVISOR

ABSTRACT

An investigation on air layering in guava as influenced by growth regulators and *Azospirillum* was carried out in the mother plants block of guava at the Silver Jubilee Orchard (SJO), Department of Horticulture, College of Agriculture, University of Agricultural Sciences, Dharwad during 2010-11. The study aimed to find out suitable root inducing treatment and its optimum concentration for maximum survival percentage in mature shoot air-layers of guava Cv. L-49. There are 14 treatment combinations with three replications laid out in Randomised Block Design. In general, both growth regulators *viz*. IBA, NAA alone and in combination with *Azospirillum* favoured rooting in air-layers. A synergestic effect was noticed in terms of higher percentage of rooting where the two growth regulators were used in combination with *Azospirillum*. Among the different combinations, the layers which had received *Azospirillum* 37.5g + IBA (Indole butyric acid) 3000 ppm + NAA (Naphthalene acetic acid) 3000 ppm (T_{12}) recorded significantly higher percentage (91.68%) of rooting with desirable root characters such as higher number of primary and secondary roots, longer length of primary roots and higher girth of primary roots. Next to this treatment, the other favourable treatments were IBA, 3000 ppm + NAA, 3000 ppm (T_6), IBA, 2000 ppm (T_1), *Azospirillum* 37.5g + IBA, 4000 ppm (T_8), *Azospirillum* 37.5 g + IBA, 6000 ppm (T_9), *Azospirillum* 37.5 g + IBA, 2000 ppm + NAA, 2000 ppm (T_{11}). The use of medium concentration (3000 ppm) of both the growth regulators (IBA and NAA) with *Azospirillum* 37.5g was more effective as compared to either lower (2000 ppm) or higher (6000 pm) concentrations of IBA and NAA.

With regard to survivability of rooted layers, maximum survival percentage (98.14%, 60 days after separation) was noted in the layers treated with *Azospirillum* 37.5g + IBA, 3000 ppm + NAA, 3000 ppm (T_{12}).