Effect of different concentration of IBA and rooting media on callusing and survival of air layering in acid lime (*Citrus aurantifolia* Swingle)

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(FRUIT SCIENCE)

By

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CERTIFICATE – I

This is to certify that the thesis entitled, **"Effect of different concentration of IBA and rooting media on callusing and survival of air layering in acid lime (*Citrus aurantifolia* Swingle)"** *submitted in partial fulfillment of the requirement for the Degree of* **MASTER OF SCIENCE** *in* **HORTICULTURE (Fruit Science)** *of Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior.* **Mr. AJAY PRAKASH BHARDWAJ** *under my guidance and supervision. The subject of the thesis has been approved by the Student's Advisory Committee and the Director of Instruction.*

No part of the thesis has been submitted for any degree or diploma or has been published. All the assistance and help received during the course of the investigation has been acknowledged by the Scholar.

Place: Gwalior **(K. K. Yadav)**
Chairman of the Advisory Committee

Date:

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Member	(Dr. P. S. Tomar)	-------------------------------------
Member	(Dr. Arvinder Kaur)	-------------------------------------
Member	(Dr. V.B. Singh)	-------------------------------------

CERTIFICATE – II

This is to certify that the thesis entitled, **"Effect of different concentration of IBA and rooting media on callusing and survival of air layering in acid lime (*Citrus aurantifolia* Swingle)"** *submitted by* **Mr. AJAY PRAKASH BHARDWAJ** *to the Rajmata Vijayaraje ScindIa Krishi Vishwa Vidyalaya, Gwalior in partial fulfillment of the requirements for the degree of* **MASTER OF SCIENCE** in **HORTICULTURE (Fruit Science*)*** *in the* **Department of Horticulture, College of Agriculture, Gwalior** *has been accepted after evaluation, approved by the External Examiner and approved by the Student's Advisory Committee after an oral examination on the same.*

Place: Gwalior

Date:

(K. K. Yadav)
Chairman of the Advisory Committee

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Date:

Place: Gwalior **(AJAY PRAKASH BHARDWAJ)**

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LIST OF ABBREVIATIONS

%	:	Per Cent
/	:	Per
@	:	At the rate of
C.D.	:	Critical difference
cm	:	Centimeter
cv.	:	Cultivar
d.f.	:	Degree of freedom
et al.	:	And other
Fig.	:	Figure
g	:	gram(s)
ha	:	Hectare
i.e.	:	That is
viz.	:	Namely
IBA	:	Indole-3 butyric acid
kg	:	Kilogram(S)
M.P.	:	Madhya Pradesh
Max.	:	Maximum
Min	:	Minimum
mg	:	milligram
ml	:	milliliter
mm	:	millimeter
MSS	:	Mean sum of squares
NHB	:	National Horticulture Board
ppm	:	Parts per million

CHAPTER – I

INTRODUTION

The acid lime *(Citrus aurantifolia* Swingle*)* is an important citrus crop which is grown on commercial scale in India. It belongs to the family *Rutaceae,* subfamily Aurantioidae*.* It is adaptable to a wide range of soil and climatic conditions and hard enough to withstand considerable neglect as compared to other fruit crops. Besides, it is also a cheap and very rich source of vitamin C carbohydrate, fat and contains a fair amount of calcium and phosphorus as well (Souci *et al.*, 2000).

In India, acid lime is cultivated in all parts of the country mainly in Andhra Pradesh, Telangana, Karnataka, Odisha and Madhya Pradesh. It occupied an area of 259.3 thousand hectares and production 2789 thousand tones in India (Anonymous, 2017). Madhya Pradesh has 5th position in lime cultivation with an area of 17.7 thousand hectares and production 230.84 thousand tones (Anonymous, 2017). Among the citrus crops, lime is one of the important fruit. Lime cv. Kagzi lime is the principal lime cultivar of the north and central region of India (Babu, 2001). Fruits are small to medium; pulp is juicy greenish with strong adherence to the skin; skin yellowish green, thin or papery and shiny*.* It is used for processing purpose mainly for preparations of limeade mixed drinks, pies and iced tea and is squeezed on to seafood to bring out the flavor (Cheema *et al.*, 1954). It is also use in bottled lime juice and carbonated beverages etc. Its juice is used for the preparation of "Sharbat". These qualities make Kagzi lime an important and one of the most popular fruit of India.

Although citrus being essentially a crop of the subtropical region is also grown under favorable parts of the temperate and tropical region. Due to high consumption of lime, its rapid multiplication is very necessary. Acid lime plants are commercially propagated by seeds, budding and air layering. These propagation methods have their own merits and demerits. Air layering seems to be easier rather than certain methods of propagation of this crop. Now acid lime is usually propagated vegetatively by air layering which ensures true to type plants.

The auxin *indole- butyric acid* has been identified as a rooting hormone. Exogenous application of IBA induces rooting of stem cuttings. It promotes rooting as well as number of roots. In many fruit species, formation of adventitious roots can be induced by given treatment with auxin. The cell elongation in roots is also significantly improved with the application of exogenous auxin. Now-a-days, auxin has been employed as commercial application of rooting of cuttings. Auxin affects a variety of physiological process in plants *i.e.*, nucleic acid, directed protein synthesis, enzyme activity and membrane permeability, either directly or through some other metabolic activities (Cooper, 1940). The success of air layering in lime can be achieved by application of exogenous application of growth regulators have been found to hasten the initiation of roots, increase percentage of rooting and number of roots per layer (Olimen *et al.*, 1971). Many workers have studied upon these growth substances for vegetative propagation among which auxins like IBA has been found to be the most effective in rooting with varied success. However, the response of citrus to different treatments with growth regulators varied with species, changing in physiological and environmental conditions of plant.

Similarly, rooting media is also play an important role for better root formation and development. An ideal rooting media should be loose, porous with high water holding capacity. Rooting media holds moisture content for new emerged roots, congenial conditions for respiration and maintains optimum temperature for the root initiation. The nature of roots arising from the air layerings is also influenced by the type of rooting medium (Chattopadhyay, 1994). Soil is the most common rooting media. However, other rooting materials like sand and vermi-compost were taken in appropriate ratio for air layering. Among the rooting medium, the vermi-compost is important key element of rooting media because they have various major and micro-nutrients, provide simultaneously sufficient level of oxygen and water to the roots, adequate storage of water and nutrients for the balancing of physical, chemical, and biological requirements for good and uniform plant growth (Kumar *et al.*, 2015). The information on these aspects is lacking hence, the comparative efficiency of rooting media for better root development over soil alone need to be evaluated.

With the view to find out optimum concentration of IBA along with different combination of rooting media for better callusing, rooting and survival of air layers in the lime, the present investigation on **"Effect of different concentration of IBA and rooting media on callusing and survival of air layering in acid lime (*Citrus aurantifolia* Swingle)"** was conducted at University orchard, Department of Horticulture, College of Agriculture, RVSKVV, Gwalior (M.P.) with the following objectives:

1. To find out the most suitable concentration of IBA for rooting and survival of air layers of acid lime.
2. To determine the most suitable rooting media for callusing, rooting and survival of air layers of acid lime.
3. To find out the best combination of IBA and rooting media for better performance of air layers of acid lime.
4. To assess the economics of the treatments.

CHAPTER – II

REVIEW OF LITERATURE

In the present chapter, efforts have been made to review the work done by other workers in India and abroad on propagation of lime and other fruit plants by air layering with the use of different concentrations of IBA along with different rooting media. The observations and results recorded by the mere great importance in the context of present investigation and have been presented under separate appropriate headings.

2.1 Effect of IBA

Cooper (1940) presumed that the application of growth substances resulted in accumulation of certain growth substance at the base of cutting which stimulates the meristem to divide quickly and from root.

Gautheret (1969), Olimen *et al.* (1971) and Haising (1971) stated that *auxin* naturally or artificially applied is a requirement for initiation of adventitious roots on stem and indeed it has been showed that the division of first root initial cell is dependent upon either applied or endogenous *auxin*.

Haising (1971) observed that root initials in stem, is apparently dependent upon the native *auxin* in plant plus *auxin* synergist together these lead to synthesis of ribo-nucleic acid (RNA) which is involved initiation of the root primordial.

Yadav (1989) found that highest concentration of IBA (5000ppm) proved significantly better for rooting and survival of air layers of kagzi lime.

Mishra (1990) reported that the concentration of growth regulators G_2 (IBA 5000ppm) showed great improvements in most of the characters like callusing, rooting and survival of air layers of kagzi lime and found statistically better than other treatments.

Kumar (1992) reported better rooting, growth and survival of kagzi lime air layer with 2400ppm IBA.

Lavania *et al.* (1995) reported that the best rooting was observed in the 7500ppm IBA treatment. The highest survival of plants (52.5%) was also observed in this treatment.

Singh (1996) reported that the concentration of the growth regulators (IBA 5000ppm) showed great improvement in most of the character like callusing, rooting and survival of air layers of kagzi lime and found statistically better than other treatments.

Ghosh (1998) found that the air layering done during July with the application of rooting hormone (IBA) recorded the highest percentage of rooting and survival in sweet orange.

Mukhtar *et al.* (1998) reported that maximum rooting (93%) was obtained with IBA at 4000ppm in soft wood cuttings of guava.

Tomar *et al.* (1999) reported that air layering in Kagzi lime was more successful when exposed areas treated with 2400ppm concentration of IBA and wrapped in a mixture of sand, farmyard manure and soil held in polyethylene film.

Brahmam *et al.* (2000) observed that air-layering of *Madhuca indica* as influenced by the plant growth regulators IAA, IBA and NAA, in various combinations and concentrations and using various time schedules. Maximum rooting percentage (88%), a high survival rate (82%), and better root characteristics and vigour attributes were obtained with the IBA+NAA combination. Concentrations of 1000ppm of and mid monsoon season were ideal for achieving the optimum results.

Eganathan *et al.* (2000) attempted large-scale propagation of three mangrove species (*Excoecaria agallocha*, *Heritiera fomes* and *Intsia bijuga*) using cutting and air layering. Maximum rooting was recorded when the cuttings and air layers were treated with IBA alone up to 2500ppm in all the three species.

Sengupta *et al.* (2000) studied that effect of different concentrations (5000, 7500 and 10000ppm) of plant growth regulators (IBA and NAA, either alone or in combination) on the survival and regeneration of roots in layering of jackfruit under an etiolated conditions. IBA + NAA at 5000ppm produced the highest number of rooted layers (90%), primary and secondary roots per layer, length of primary and secondary roots and fresh and dry weight of roots. The lowest primary and secondary root diameters were also observed in IBA + NAA at 5000ppm treatments.

Sengupta *et al.* (2001) studied that the effect of growth regulators, either alone or in combination, on the survival and regeneration of roots in the layering of jackfruit under etiolated conditions. IBA and NAA, alone or in combinations, were applied at 5000, 7500 and 10000ppm. Etiolated shoots treated with growth regulators produced roots considerably better than the control. Etiolation + 5000ppm IBA + 5000ppm NAA (1:1) produced the highest success of rooted layers (90.00%), primary roots per layer (19.00), number of secondary roots per layer (46.30), length of primary roots (11.39 cm), length of secondary roots (8.29 cm), and fresh (6.59 g) and dry (0.878) root weight. Etiolation + 10000ppm NAA produced the highest primary (0.23 cm) and secondary (0.14 cm) root diameters.

Singh (2001) studied that the efficacy of plant growth regulators (5000, 10000, 15000 and 20000ppm of IAA, IBA and NAA). Their concentrations and wrappers (white and black polyethylene) on rooting success and survival of air layered guava (cv. Allahabad Safeda) twigs. The results revealed that use of IBA was beneficial in enhancing the callus formation, number, length and diameter of both primary and secondary roots and survival of air -layered twigs. 20000ppm of plant growth regulators was found optimum for better rooting success and survival and was significantly superior over 5000, 10000 and 15000ppm concentrations of different plant growth regulators. The use of black polythene as wrapper resulted in better rooting success and survival of air-layered guava twigs as compared to white polythene.

Gangawar *et al.* (2002) observed that 300 or 600ppm IBA and/or 2500ppm IAA on the clonal propagation of wild pear (*Pyrus pashia*, commonly used as a rootstock) by stool layering. IBA at 600ppm enhanced the layering success over other treatments and recorded 83.33% rooting. The same treatment recorded the minimum number of days to callusing (10.75) and rooting (40.00). Treatment with IAA recorded the minimum rooting percentage (72.30%) and the maximum number of days to rooting (48.50), while the control treatment recorded the maximum number of days to callusing (17.75). IBA also significantly increased the number of primary roots, i.e. 11.75 (600ppm) and 9.50 (300ppm) as compared to 3.50 and 3.75 under the IAA and control treatments, respectively. The primary root lengths were recorded 15.72, 13.67, 8.98 and 8.15 cm under 300ppm IBA, 600ppm IBA, control and IAA treatments, respectively. Highly fibrous, medium fibrous and low fibrous rooting were recorded upon treatment with 600ppm IBA, control, and 300ppm IBA+IAA or 600ppm IBA+IAA, respectively. However, treatment with either 300ppm IBA or IAA resulted in non-fibrous roots.

Singh (2002) reported that effect of different concentrations (5000, 10000, 15000 and 20000ppm) of IAA, NAA and IBA and use of black or white polyethylene film on the vegetative growth and physiology of air-layered guava. Application of IBA resulted in better vegetative growth, survival and physiology of air-layered guava compared to the application of IAA and NAA. Increasing rates of plant growth regulators resulted in increasing vegetative growth and enhanced physiology of the air layers. Compared to the white polyethylene film, the use of black polyethylene film resulted in better performance of guava air layers.

Bhojvaid and Negi (2003) reported that IBA enhanced the root initiation and induced rooting in a shorter duration in *Elaeocarpus ganitrus*. Air layering trials in pre monsoon season under 500 mg $liter^{-1}$ treatment exhibited maximum rooting percentage followed by monsoon and post monsoon season under 500 mg $litre^{-1}$ IBA treatment. Profuse rooting was observed in air layers treated with IBA in pre monsoon season.

Jan *et al.* (2003) observed that different concentrations of IBA (0, 1000, 1500, 2000, 2500, 3000 and 3500ppm) on the rooting of litchi (*Litchi chinensis*) propagated by air layering. The ringed shoots of litchi were treated with IBA for 5 seconds. IBA significantly affected the various parameters evaluated for the air layers. The greatest number of roots per plant (32.97), root length (9.59 cm), root weight (4.00 g), root volume (4.86 ml), root diameter (1.77 mm), shoot length (29.27 cm), shoot diameter (4.48 mm), number of leaves (15.63) and percent plant survival (75.23%) were recorded for layers treated with 2500ppm of IBA. The lowest number of roots per plant (10.53), root length (4.53 cm), root weight (1.56 g), root volume (1.90 ml), root diameter (0.32 mm), shoot length (17.28 cm), shoot diameter (2.98 mm), number of leaves (5.13) and percent plant survival (36.20) were recorded in the control.

Pandey *et al.* (2003) reported that seedless lemon resulted species produced the highest number of roots (12.44), length (13.44 cm) and diameter (3.54 mm) of roots per cutting. IBA at 2000ppm was superior in terms of success percentage of cutting (84.00%), root growth and survival percentage (96.66%).

Ray *et al.* (2003) founded that survival of 8 cultivars of *Litchi chinensis* (Muzaffarpur, Nefarpal, Elaichi, Bombai, Purbi, Bedana, Rose-Scented and China) in air layering propagation was investigated, with the application of NAA at 5000ppm + poly hydroxyl benzoic acid (PHB) at 200ppm, IBA at 5000 + PHB, and the control. Shoots treated with plant growth regulators showed better survival (90.56 and 80.26% under NAA + PHB and IBA + PHB, respectively) compared to the control (37.45%).

Purohit *et al.* (2004) examined the rooting ability of air layered shoots of *Myrica esculenta*, a commercially important and difficult to root species, using different chemicals. The lower concentration of IBA (100 and 500ppm) only resulted in rooting (20.0 and 53.33% respectively, compared to no rooting in control shoots); the highest number of roots per air-layered shoot was formed when 500ppm IBA was applied.

Singh and Singh (2004) conducted studies on the effect of IBA and NAA, at 2500, 5000, 7500 and 10000ppm, and their combination (1:1) on the air layering of jackfruit was conducted in Kanpur, Uttar Pradesh, India during 1998-99. The combination of IBA + NAA at 5000ppm each showed the best effect on the rooting of the air layers of jackfruit. IBA alone at 5000ppm improved root initiation of the air layers. Rooting under the growth regulator treatments was superior compared to the control.

Castillo *et al.* (2005) studied the effects of IBA concentration (0, 3000, 4000, or 5000 µ g/g) on the rooting of *M. Paniculata* propagated by air layering. After 60 days, 100% percent rooting was obtained in all treatments, but IBA at 4000 µ g/g resulted in the greatest weight, number and total length of roots.

Kumar and Syamal (2005) reported that the upper cut ends of air layers were treated with plant growth regulators, i.e. IBA and NAA at 1000, 2000, 3000 and 4000ppm, applied singly or in combination. Pre-soaked moss grass in water was used as rooting medium. IBA at 3000ppm recorded the highest values for mean number (14.80) and length (11.30 cm) of primary roots per air layer, average number of secondary roots (10.72), percentage of success of air layers (93.34%) and survival of air layers (75.90%), while IBA at 4000ppm recorded the highest value for diameter of roots (2.30 mm).

Wagner *et al.* (2005) observed that IBA (0, 1, 2, 3 and 4 mg/litre) on the rooting of air layered peach cv. Biuti were determined in a field experiment conducted in Minas Gerais, Brazil from July to November 2003. Rooting, and root number and length were highest with the application of 4 mg IBA/litre.

Chovatia and Singh (2006) reported that percentage of rooted layers in Jamun and their survival under field conditions were highest under the influence of instant ringing and application of IBA at 10000ppm. 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 10000ppm. Control produced fewer and shorter root.

Gowda *et al.* (2006) conducted a study on the propagation of Khirni (*Manilkra hexandra*) by air layering in Banglore, Karnatka, India. The maximum rooting percentage (76%) of Khirni air layers was recorded with 5000 mg litre-1 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 10000 mg litre-1 IBA recorded the maximum number of primary (9.2), secondary (49.4) and tertiary (34.3) roots.

Bitencourt *et al.* (2007) conducted studies to analyze of the efficiency of the air layering technique in *G. biloba* for vegetative propagation. The air- layered branches were accomplished by a complete ringing of the woody branches and employing IBA with lanolin paste at 0, 1.5 and 3.0 mg/kg concentration. The experiment or analysis was made after 70 and 90 days from its installation. The Treatment with 1.5 and 3.0 mg IBA/kg have shown high percentages of rooted air-layered branches compared to the control. The presence of air-layered branches with callus was 100% for all the treatment. The treatment with 3.0 mg IBA/kg resulted in higher values for all the others variables and can thus be recommended for the vegetative propagation of *Ginkgo biloba*.

Kumar *et al.* (2007) observed that effect of IBA (2000, 4000, 6000, 8000, 10000 and 12000ppm) in lanolin paste on the success of air layering in jackfruit cv. Pant Garima. The application of IBA increased rooting percentage to 87.5%, the number of roots/air layered (AL) shoot (38.3), length of primary root/AL shoot

(6.8 cm), fresh weight of root per AL shoot (47.7 g), dry weight of root/AL shoot (20.8 g), root:shoot ratio (0.46) and survival percentage (76.7%).

Kakon *et al.* (2008) studied guava mound layering as affected by different variety and growth regulators. It was a two factor experiment consisting of three varieties viz., BARI guava-1, BARI guava-2 and Kashi and four three growth regulators (IBA, NAA and IAA) with four concentrations viz., 300, 500, 800 and 1200ppm of each and one control. Among different varieties BARI guava-1 showed the best performance. Different concentrations of growth regulators had significant effect on almost all parameters. IBA at 1200ppm showed the best performance among the treatments.

Chaukiyal *et al.* (2009) observed that suitable concentration of IBA in response to the number and length of *Pongamia pinnata* roots so that in future, air layered rooted as well as cuttings can be used for the multiplication of trees with the desirable characters. As the concentration of IBA increased from 100ppm to 500ppm the number of days for callus formation also increased. Maximum root length (23.2 cm) was observed in the IBA 100ppm and as IBA concentration increased the root length decreased. The increase in the number of roots and leaves after 60 days followed near about the same trend as the root length. It was observed that IBA (100ppm) was the best for the rooting of *Pongamia pinnata* cuttings.

Singh *et al.* (2009) carried out an experiment to determine the response of different cultivars of litchi to IBA alone and in combination with PHB on different rooting parameters and survival of layers. All the rooting parameters i.e. length of roots, number and weight of roots both fresh and dry and survival of rooted layers were improved significantly with 5000ppm IBA+ 1000ppm PHB treatment. These traits were however recorded significantly maximum in Rose Scented when compared with other cultivars.

Rymbai and Reddy (2010) found that effect of IBA concentrations, time of layering and rooting media on rooting, root characters and survival percentage of rooted air layers under different growing nursery conditions i.e open and polyhouse conditions on guava, cv. L-49 were carried out during 2008-09. Three different concentration of IBA viz., 2000, 3000 and 4000ppm were used in three time of layering viz., 15th June, 15th July and 15th August with two rooting media viz., sphagnum moss and coco peat. It was revealed that high percentage of rooting and root characters of air layers of guava have been successfully achieved by exogenous application of IBA at 4000ppm. Regarding the time of layering, 15^{th} August gave the maximum rooting success and root attributes. Among the rooting media, moist sphagnum moss produced highest rooting percentage and root characters. The survival of rooted air layers was found maximum with the treatment combination of IBA at 4000ppm, layering on 15^{th} August and sphagnum moss in polyhouse conditions produced higher survival than open conditions after 45 days of detachment from the mother plants.

Sathyanarayana (2010) conducted an experiment in India, to evaluate the response of different rooting media on guava air-layers. Three different concentration of IBA viz., 2000, 3000 and 4000ppm were used in three time of layering viz., 15^{th} June, 15th July and 15th August with two rooting media viz., sphagnum moss and coco peat. Among the rooting media, moist sphagnum moss produced highest rooting percentage and root characters. The survival of rooted air layers was found maximum with the treatment combination of IBA at 4000ppm, 15^{th} August and sphagnum moss in polyhouse conditions produced higher survival than open conditions after 45 days of detachment from the mother plants.

Tomar and Singh (2011) reported that effects of seasons and rooting hormone on air layering were investigated in *Ficus krishnae* and *Ficus auriculata*. Trials were conducted in three different seasons, viz. pre monsoon, monsoon and post monsoon using IBA rooting hormone @ 100, 300 and 500ppm. Results revealed that IBA enhanced the root initiation and induced rooting in a shorter duration. Air layering trials in pre monsoon season with 500ppm IBA treatment exhibited maximum rooting percentage followed by monsoon and post monsoon season.

Tomar (2011) found that different concentrations of IBA and NAA on rooting and survival percentage of pomegranate (*Punica granatum* L.) air layers revealed that IBA in better than NAA. Application of IBA @ 2000ppm is best for successful propagation of pomegranate air layers.

Costa *et al.* (2012) found that propagation of the litchi is undertaken preferably through air layering, thus providing higher yield and the maintenance of its genetic characteristics. Therefore, the objective of this work was to study three types of branches and the use of a growth regulator on the vegetative propagation of litchi by air layering. Branches were girdled, completely removing the bark, forming approximately 2 cm thick rings, which were treated with different concentrations of stimulate sp. and covered with substrate comprising plantmax Reg. mixture, vermiculite and common soil. The experimental design used randomized blocks, in a 5x3 factorial outline, with three replications and one air layering per portion. Treatments consisted of five concentrations of Stimulate Reg. (0, 25; 50; 75 and 100%) and three types of branches (thin, medium and thick). It was possible to infer that the use of thick branches and a 50% concentration of Stimulate Reg. in the air layering of litchi, Bengal cultivar, favor good results in the development of air layers.

2.2 Effect of rooting media

Puccini (1954) compared vermiculite with sand, soil leaf mould and sphagnum moss for herbaceous, semi woody and woody ornamental and observed that vermiculite had a better water absorbing and water holding capacity than any of the other materials except sphagnum moss, but it generally was not a good medium.

Bhojvaid and Negi (2003) transplanted the callused and rooted air layers after detachment from the mother plants separately in polyethylene bags in three different growing media: M_1 - sand, nursery soil and farmyard manure (1:2:1); M_2 - sand and nursery soil (1:1); and M_3 - nursery soil under nursery conditions. A highly significant variation ($P<0.05$) was observed within the effect of season and callus formation as well as root initiation. Maximum survival of callused and rooted air layers (air layering conducted in pre monsoon season) was observed in the growing medium containing sand, nursery soil and farmyard manure (1:2:1).

Alam *et al.* (2004) evaluated the effects of rooting media (100% cow dung (CD), 75% CD + 25% soil, 50% CD + 50% soil, 25% CD + 75% soil, and 100% soil) and wrapping materials (polythene sheet, gunny bag and straw) on air-layering of litchi cv. China-3. Rooting media and wrapping materials significantly affected the time of root emergence, number of roots, and length and thickness of roots. Application of 100% CD as rooting medium and gunny bag as wrapping material resulted in the best rooting performance of litchi.

Tyagi and Kumar (2006) reported that various growth and flowering parameters increased significantly with the application of vermicompost. Significant maximum plant height, stem diameter, number of branches, flower diameter, number of flowers and yield of flower per hectare was reported by the application of 8 t /ha vermicompost. The favourable effect of vermicompost might be attributed as vermicompost enhances soil fertility and moisture retention capacity of the soil.

Kumar and Sharma (2007) reported that the efficiency of FYM and vermicompost on *Jatrofa curcus* raised through cutting and comparison were made between two type of soil mix i.e. (soil + FYM) and soil mix II (soil + vermicompost) between the different treatments soil + vermicompost was found to be better on vegetative growth i.e. survival percentage, plant height, diameter of stem, number of branches per plant of *Jatrofa curcus.*

Bisen *et al.* (2010) revealed that vermicompost as rooting media was beneficial in increasing the number (16.39 and 24.26 cm), length (13.44 and 5.45 cm) and diameter (0.502 and 0.097 cm) of primary and secondary roots respectively. The rooting percentage (91.25%), survival percentage (80.08%), number of leaves (14.19), length (15.36%), diameter (0.835 cm) of emerging shoots. Leaf area index (11.38 cm_2), vigour index (2308) and length of leaves (14.48 cm) also recorded better over remaining treatments.

Kumar *et al.* (2015) reported that a combination of garden soil + sand + vermicompost (1:1:1) was gave significant results on rooting of stem cuttings and survival percentage of lemon (*Citrus limon* Burm) cv. Pant Lemon-1 as compared to control (Garden soil) ones under western UP conditions.

2.3 Effect of IBA and rooting media

Kumar *et al.* (2001) found that three different rooting media, namely gootee medium, moss grass and sawdust, alone and in combination with 500ppm IBA, on the propagation of custard apple cv. Sitaphal by air layering were evaluated. Layers with combination of sawdust and IBA resulted in 53.76% success, whereas the minimum (22.45%) was recorded in gootee medium without IBA. There was a significant effect of IBA on rooting with all the 3 rooting media (44.72% success). Sawdust with combination of IBA produced the maximum number of roots (10.80) and the least number (7.00) was recorded in gootee mixture with IBA. The maximum survival percentage (31.00%) was recorded in sawdust combined with 500ppm IBA.

Bhojvaid and Negi (2003) conducted trials in three different seasons, viz., pre monsoon, monsoon and post-monsoon using IBA as the rooting hormone (control, 100, 250 and 500 mg $litre^{-1}$). Results reveal that IBA enhanced the root initiation and induced rooting in a shorter duration. Air layering trials in pre monsoon season under 500 mg $litre^{-1}$ treatment exhibited maximum rooting percentage followed by monsoon and post-monsoon season under 500 mg $litre^{-1}$ IBA treatment. Profuse rooting was observed in air layers treated with IBA in pre monsoon season. The callused and rooted air layers after detachment from the mother plants were transplanted separately in polyethylene bags in three different growing media: M_1 - sand, nursery soil and farmyard manure (1:2:1); M_2 - sand and nursery soil (1:1); and M_3 - nursery soil under nursery conditions. A highly significant variation ($P<0.05$) was observed within the effect of season and callus formation as well as root initiation. Maximum survival of callused and rooted air layers (air layering conducted in pre monsoon season) was observed in the growing medium containing sand, nursery soil and farmyard manure (1:2:1).

Alam *et al.* (2004) evaluated the effects of rooting media (100% cow dung (CD), 75% CD + 25% soil, 50% CD + 50% soil, 25% CD 75% soil, and 100% soil) and wrapping materials (polythene sheet, gunny bag and straw) on air layering of litchi cv. China-3. Rooting media and wrapping materials significantly affected the time of root emergence, number of roots and length & thickness of roots. Application of 100% CD as rooting medium and gunny bag as wrapping material resulted in the best rooting performance of litchi.

Chandrakar *et al.* (2007) studied that air layering of guava with IBA concentration of 6000ppm with soil : sand : poultry manure rooting media produced maximum percentage (76.75%) of survival of 60 days old plants grown in poly bags. This combination of IBA with rooting media helped in producing maximum number of primary roots (18.57), secondary roots (23.91), leaves on 60 days (14.36) and length of shoots on 60 days (5.31 cm). IBA at 5000ppm and poultry manure combination was found to be second best for survival of air layering (73.25%).

Kanpure *et al.* (2015) conducted field experiment during 2012–13 at RVSKVV, Department of Fruit Science, College of Horticulture, Mandsaur (M.P.). The results revealed that the highest success in rooting percentage (94.75), callus formation (0.42cm), number of primary and secondary roots (28.40 and 100.59) respectively, length of primary and secondary roots (11.16cm and 6.07cm) respectively fresh and dry weight of roots (0.76g. and 0.22g.) respectively were recorded in T_{17} (IBA 10000 PPM+sphagnum moss+ black polythene) while the minimum value of these characters were recorded under T_1 (soil + white polythene). The maximum survivability (88.77%) of air layering of Kagzi lime was noted under T_{17} followed by 85.92% in T_{20} (IBA 10000 PPM + vermicompost + FYM + soil + black polythene) whereas the minimum survivability of air layers (42.36%) were recorded under T_1. Similarly the highest numbers of new sprouts (10.93) were noted under T_{17} followed by 10.43, 9.95 and 9.37 under treatments of T_{13}, T_{20} and T_{16} respectively. The effect of black polythene was pronounced as compared to white polythene in all characters studied.

Kumar *et al.* (2015) conducted an experiment consisted of ten treatment viz., T_1 - 400ppm IBA +garden soil + sand (1:1),T_2 - 400ppm IBA + garden soil + sand + FYM (1:1:1), T_3 - 400ppm IBA + garden soil + sand + vermicompost (1:1:1), T_4 - 800ppm IBA + garden soil + sand (1:1), T_5 - 800ppm IBA + garden soil + sand + FYM (1:1:1) ,T_6 - 800ppm IBA + garden soil + sand + vermicompost (1:1:1), T_7 - 1200ppm IBA + garden soil + sand (1:1), T_8 -1200ppm IBA + garden soil + sand + FYM (1:1:1), T_9 - 1200ppm IBA + garden soil + sand + vermicompost (1:1:1), T_{10} - Control (Garden soil). Out of these, the treatment 800ppm IBA + garden soil + sand + vermicompost (1:1:1) was gave significant results on rooting of stem cuttings and survival percentage of lemon (*Citrus limon* Burm) cv. Pant Lemon-1 as compared to control ones under western UP conditions.

Singh *et al.* (2015) conducted a trial to evaluate the effect of growing media, hormonal treatments and growing seasons on the shoot and root characters and survival percentage of cuttings of lemon cv. Pant Lemon-1. Pooled data of two seasons (rainy and spring) has shown that soil+sand+FYM growing medium resulted maximum length of sprouts and average number of leaves per cutting. Whereas, average diameter of shoot and average length of longest roots were maximum in soil+cocopeat based medium. Average diameter of root was higher in soil+cocopeat and soil+FYM medium, but did not differ significantly.

Maximum survival percentage of cuttings was noted with soil+sand+FYM. IBA (500ppm) was found superior regarding for all parameters except on average diameter or roots per cutting with IBA+NAA (500ppm each). Taken as a whole, rainy season was found superior over spring season in respect to all shoot and root characteristics.

Parmar *et al.* (2018) studied the effect of different concentrations of Indole 3-butyric acid (0, 2000, 4000, 8000 and 10000ppm), different types of rooting media (i.e. sphagnum moss, coco peat, vermicompost, sphagnum moss + coco peat and sphagnum moss + coco peat + vermin-compost) and the interaction of these in different combinations on rooting and growth characteristic of guava, cv. L-49. The results revealed that the exogenous application of Indole 3-butyric acid (IBA) 8000ppm with media combination of sphagnum moss + coco peat + vermicompost (I_3M_5) significantly increased the root characters i.e. success percent of air layers (83.3%), callus formation (23.7), number of primary roots (24.21) and secondary roots (32.57); and growth characters i.e. number of leaves (48.17), branches (7.92) and sprouts (8.48) and survival percentages (83.18) over the other treatments and control. Among the rooting media, the combination of sphagnum moss + coco peat + vermicompost (M_5) produced the highest rooting percentage (71.27), root characters and growth characters during months of July and August.

2.4 Economics of treatments

Gautam *et al.* (2010) study revealed potential of clonal propagation technique in guava for nursery stock production at commercial scale. The selection of appropriate site adapted phenotypes and elite genotypes with excellent fruit bearing and flesh quality and manipulation of cultural environment are important to improve the quality of planting stock for optimum gains. Further the propagation technique of nursery stock production is simpler and cheaper than *in vitro* culture and can be used even by unskilled nursery growers.

Maurya *et al.* (2012) evaluated different organic media and water holding materials with IBA on rooting and survival of air layering in guava (*Psidium guajava* L.) cv. Allahabad Safeda. The treatments comprised the combination of media *i.e*. soil, organic media (Vermicompost/ Poultry manure) and water holding materials (Sphagnum moss/Cocopeat) in the ratio of 40:20:5 g along with various

IBA levels (4000, 5000 and 6000 mgl^{-1}) and compared with soil alone (control). Maximum survival percentage (90.67%), length of shoots (7.93cm) and number of leaves (18.33) was calculated at 60 days of air layers in the poly bag after transferring with highest economics (Net CBR 1:3.59).

Reddy *et al.* (2014) reported that the treatments comprised the combinations of rooting media i.e. soil, organic media (Vermicompost/Poultry manure) and water holding materials (Sphagnum moss/Coco peat) in the ratio of 60:30:10 g along with various IBA levels (1000, 2000 and 3000 mgl^{-1}) and compared with soil alone (control). The air layers made with soil + poultry manure + sphagnum moss + 3000 mgl^{-1} IBA showed early root initiation (8.73 days), minimum days required for bulk appearance of roots (20.80), highest number of primary roots (39.20 and 58.87), maximum secondary roots (155.93 and 250.73), maximum primary root length (16.53 and 17.48 cm), maximum secondary root length (2.36 and 3.37 cm) at 30 and 45 days, respectively. Maximum fresh weight of shoot biomass (34.10, 35.96 and 43.53 g), maximum fresh weight of root biomass (5.63, 6.63 and 7.73 g), maximum dry weight of shoot biomass (16.49, 24.91 and 30.88 g), maximum dry weight of root biomass (1.65, 2.13 and 2.81 g), maximum survival percentage of air layers (90.93, 88.53 and 83.46) and number of new leaves (4.60, 6.53 and 8.86) at 45, 60 and 75 days of air layers in the poly bag after planting, respectively with highest economics (Net CBR 1:3.34).

Azad and Matin (2015) reported that the *Swietenia macrophylla* is very important from ecological, economical, aesthetic point of view for Bangladesh and clonal propagation through branch cutting might be effective method for its conservation. They recommended that branch cutting with 0.4% IBA treatment may be applicable for this species.

Mali *et al.* (2015) reported that the wide range was observed for net income. Although, the high density increases the cost of cultivation per hectare, the much higher yield from close spacing indicates that the added cost is economically feasible. Increased density from 1111 to 2500 plants/ha reduced the per quintal cost of cultivation at cost C from Rs.1696/–to Rs.541/– respectively. All these findings are in close agreement with those of Yadav (1978) and according to him close planting reduce the cost of production, produce the larger profit and increase the returns on investment. The maximum (1.84) Benefit: Cost ratio at cost C was observed in treatment M_2S_1 while minimum (0.54) Benefit: Cost ratio was observed in treatment M_4S_4. It confirmed the returns per rupee invest is the greatest from the closest planting.

Soni *et al.* (2015) revealed that production of guava plants in net house condition proved to be the best for rapid and cheapest method for multiplication of guava true-to-type plants. Significant results were obtained when guava nursery produced by semi hard and hard wood cuttings after application of 3000ppm IBA. The plants produced by this technique can be planted in high density planting. The technique was developed in this study is simpler, rapid, less labour intensive and economical, as root promoting hormones are required for root initiation. It is useful as compared to conventional method of propagation (grafting/budding) of guava because of higher success rate, independence of season and climate, small size of cuttings, use of juvenile shoot cuttings, disease free nature and production of large number of uniform true to mother type plants in a short period of time.

CHAPTER – III

MATERIALS AND METHODS

The present investigation entitled **"Effect of different concentration of IBA and rooting media on callusing and survival of air layering in acid lime (*Citrus aurantifolia* Swingle)"** was conducted at University orchard, Department of Horticulture, College of Agriculture, Gwalior during the year 2018-19. A detailed account of the materials employed and methods followed during the course of investigation is embodied in this chapter.

3.1 Location, climate and weather data

Gwalior is located at 26˚ 13' N latitude and 78˚ 14' E longitude and 208 meters above mean sea level. The climate of Gwalior is subtropical with hot and dry summers where maximum temperature exceeds 45˚C in May and June. The winters are cool and minimum temperature reaches as low as 2˚C in December and January; occurrence of frost is expected from the last week of December to the first week of February. Usually the monsoon arrives in the second fortnight of June and lasts till September.

Occasionally light rains are expected during winter. The annual rainfall ranges between 650 to 751 mm, most of which received from end of June to end of September. Drought is the common feature due to the scanty and uneven distribution of rainfall. The total rainfall received during the experimentation period was 357 mm. The mean weather parameters viz. Temperature, relative humidity and rainfall during the experimental period were recorded as per meteorological observatory, College of Agriculture, Gwalior. The relevant meteorological data during crop season are presented in Table 3.1 and depicted in Fig. 3.1.

Table 3.1: Weekly meteorological data during experiment (August to December 2018)

SMW	Duration SMW	Temperature (^{o}C)		Relative Humidity (%)		Rainfall (mm)
		Max.	Min.	Morning	Evening	
31	30 July- 05 Aug	31.3	24.6	86.6	70.3	30.8
32	06 Aug- 12 Aug	33.5	26.5	87.1	62.3	13.0
33	13 Aug- 19 Aug	33.8	26.3	91.0	65.4	64.8
34	20 Aug- 26 Aug	31.6	25.7	90.7	78.6	82.0
35	27 Aug- 02 Sep	30.5	25.0	92.9	73.7	84.4
36	03 Sep- 09 Sep	28.9	23.8	91.4	81.4	50.0
37	10 Sep- 16 Sep	31.8	24.6	79.9	67.4	0.0
38	17 Sep- 23 Sep	33.3	23.8	83.0	66.8	32.0
39	24 Sep- 30 Sep	33.8	27.7	81.1	56.8	0.0
40	01 Oct- 07 Oct	36.7	18.5	63.6	44.1	0.0
41	08 Oct- 14 Oct	35.4	18.7	81.5	38.5	0.0
42	15 Oct- 21 Oct	36.5	17.4	76.8	27.5	0.0
43	22 Oct- 28 Oct	34.5	14.9	80.7	28.7	0.0
44	29 Oct- 04 Nov	33.3	14.4	82.1	28.2	0.0
45	05 Nov- 11 Nov	29.6	10.6	83.6	33.6	0.0
46	12 Nov- 18 Nov	31.6	13.5	80.3	28.3	0.0
47	19 Nov- 25 Nov	35.3	11.4	88.3	30.7	0.0
48	26 Nov- 02 Dec	26.7	9.8	99.3	39.1	0.0
49	03 Dec- 09 Dec	26.8	7.2	93.6	33.3	0.0
50	10 Dec- 16 Dec	22.4	6.8	90.4	57.7	0.0
51	17 Dec- 23 Dec	23.5	4.5	90.7	38.4	0.0
52	24 Dec- 31 Dec	22.8	3.5	94.5	36.8	0.0

Source: Meteorological Observatory, College of Agriculture, Gwalior

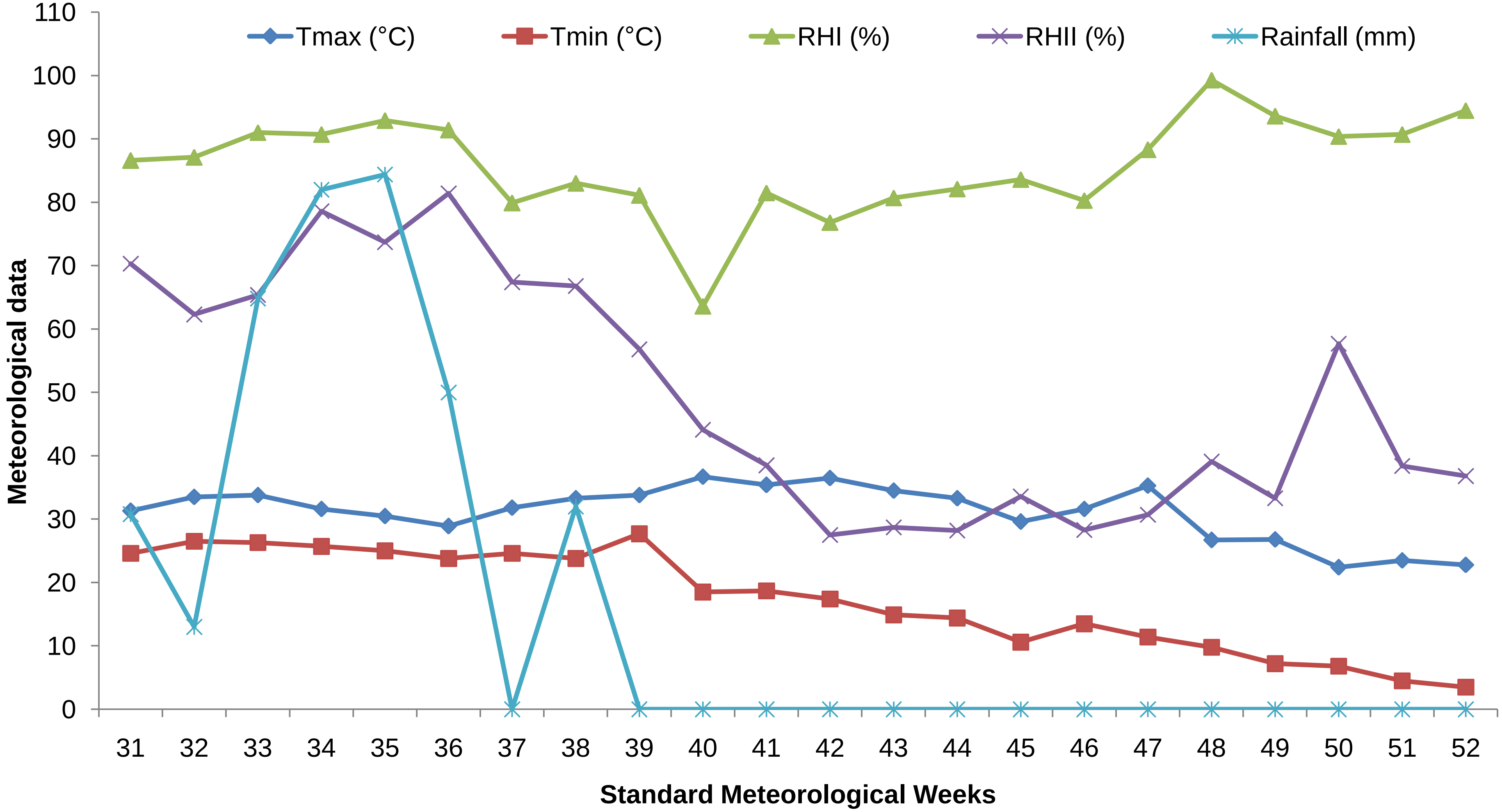

Fig 1: Climatic variables during the period of investigation (August to December 2018)

3.2 Details of experiment:

The experiment was laid out in Randomized Block Design with thirteen treatments and three replications. The treatments consisted of five levels of IBA and three levels of rooting media. All the treatments were randomized separately in each replication (Fig. 2). The details of the experiments are given below:

3.2.1 Details of layout

Name of the crop	:	Acid Lime *(Citrus aurantifolia* Swingle*)*
Variety	:	Kagzi Lime
Design	:	RBD
No. of Treatments	:	13
Number of replications	:	03
No. of layers per treatment	:	30
Total number of plants	:	39
Total number of layers	:	1170
Date of operation of air layering	:	7th August, 2018
Time of detachment of air layers	:	After 45 days of operation

3.2.1 Detail of treatments

Treatment factors

Concentrations of IBA

1. 0ppm
2. 500ppm
3. 1000ppm
4. 1500ppm
5. 2000ppm

Different rooting media

1. Soil
2. Soil + Sand
3. Soil + Sand + Vermicompost

Treatments	Symbol
IBA 0ppm + soil (Control)	T_0
IBA 500ppm + soil	T_1
IBA 500ppm + Soil + Vermicompost	T_2
IBA 500ppm + Soil + Sand + Vermicompost	T_3
IBA 1000ppm + Soil	T_4
IBA 1000ppm + Soil + Vermicompost	T_5
IBA 1000ppm + Soil + Sand + Vermicompost	T_6
IBA 1500ppm + Soil	T_7
IBA 1500ppm + Soil + Vermicompost	T_8
IBA 1500ppm + Soil + Sand + Vermicompost	T_9
IBA 200ppm + Soil	T_{10}
IBA 2000ppm + Soil + Vermicompost	T_{11}
IBA 2000ppm + Soil + Sand + Vermicompost	T_{12}

3.3 Selection of plants and their branches for making air-layers

For the experiment, 7 years old 39 plants of lime cv. Kagzi Lime having uniform vigour and size were selected. Thirteen plants were taken under each replication and about 1-2 years old well matured healthy branches having pencil's like thickness were selected on each plant for air layering. The length of branches was 45-60 cm and diameter 1 cm approximately, 30 air-layers under each treatment, 390 air layers under each replication and 1170 air layers under the whole experiment were operated.

3.4 Preparation of IBA solution

In the present experiment, solutions of the IBA were prepared in talcum powder base.

A. IBA 500ppm

First of all stock powder IBA solution of 500ppm strength was prepared. For this purpose, 0.05 g of growth regulator was weighed on electronic balance and then dissolved in about 10.00 cc absolute alcohol. This solution was then thoroughly mixed with 99.95 g of talcum powder.

B. IBA 1000ppm

Second stock powder IBA with 1000ppm strength was prepared. For this purpose, 0.10 g of growth regulator was weighed on electronic balance and then dissolved in about 10.00 cc absolute alcohol. This solution was then thoroughly mixed with 99.90 g of talcum powder.

C. IBA 1500ppm

Third stock powder IBA with 1500ppm strength was prepared. For this purpose, 0.15 g of growth regulator was weighed on electronic balance and then dissolved in about 10.00 cc absolute alcohol. This solution was then thoroughly mixed with 98.50 g of talcum powder.

D. IBA 2000ppm

Fourth stock powder of IBA with 2000ppm strength was prepared. For this purpose, 0.2 g of growth regulator was weighed on electronic balance and then dissolved in about 10.00 cc absolute alcohol. This solution was then thoroughly mixed with 98.00 g of talcum powder.

After preparation, the powder was kept in broad mouthed bottles covered with paper to protect it from light.

3.5 Preparation of rooting media

Soil was taken from University Orchard, Department of Horticulture, Gwalior. Colour of soil was medium black with clay loam texture. Fine river sand and well prepared vermicompost was purchased from market for experiment purpose.

1. Soil soaked into water for half an hour i.e. soaked till wet was ready to use.
2. Soil and well prepared vermicompost were mixed in the ratio of 1:1 by volume, and then water is added and mixed thoroughly to develop a friable medium.
3. Soil, sand and well prepared vermicompost were mixed in the ratio of 1:1:1 by volume, then water is added and mixed thoroughly to develop a friable medium.

3.6 Method of preparing and treating of air-layers

After selection of branches, a ring of bark about 2 cm wide was removed carefully from the selected branches of lime just below the bud without injuring the under wood. Previously prepared powder containing the growth regulator was applied evenly on all sides of uppercut of the ring with camel hairbrush. For different treatments, different brushes were used then the cut portion was covered with rooting media and immediately wrapped with transparent polythene film (400 gauges) and then tied with the help of jute rope (Sutli). Only talcum powder was used for treating air layers under control and covered with soil then wrapped with same material. All the plants were given treatment on August 7, 2018. Each treatment was replicated thrice.

3.7 Detachment of air-layers

After 45 days from the date of operation, air-layers were detached by making a cut just below the lower end of the ringed surface with sharp secateurs. The air-layers were brought under shade after detachment and their polythene covers were removed gently. Care was taken to ensure that the roots were not injured at the time of removing polythene wrapper. After this, rooted air-layers were just dipped in 0.1% solution of carbendazim and then planted in polythene bags containing mixture of soil + sand + vermicompost prepared in 2:1:1 ratio.

3.8 Observations recorded

Five air layers per treatment were selected randomly and following observations were recorded at different intervals. The root related observations were made at the time of detachment of air layers except callus formation. The growth observations were recorded at 30 days intervals in nursery after planting of air layers.

3.8.1 Rooting Studies

(i) Callus formation

Observations on callusing of air-layers were recorded after 20 days of operation; callus cells were measured with the help of venier callipers.

(ii) Number of primary roots

Number of primary roots was counted at 45 days of layering by taking 5 random samples from each treatment under each replication after detachment of air layers from the mother plant.

(iii) Length of primary roots (cm)

The length of primary roots of selected air layers was measured after detachment from mother plant at 45 days of layering with the help of scale from base up to the tip of the root and average length of primary roots per air-layer was calculated from each treatment under each replication for this purpose.

(iv) Diameter of primary roots (mm)

The diameter of primary roots was measured at center of the root length with the help of vernier callipers at 45 days after layering and average diameter of primary roots per air-layer was calculated.

(v) Number of secondary roots

Secondary roots were also counted at 45 days after layering as per procedure adopted for primary roots from same selected air layers.

(vi) Length of secondary roots (cm)

Length of secondary roots was measured as same procedure adapted to primary roots.

(vii) Diameter of secondary roots (mm)

Diameter of secondary roots was measured after detachment of air layers from mother plant with similar procedure adapted to primary roots.

(viii) Fresh weight of roots (g)

The fresh weight of roots was measured by weighing with the help of electronic balance at 45 days after layering. Average weight was calculated and expressed in grams.

(ix) Dry weight of roots (g)

The same roots used for fresh weight measurement were put into butter paper packet and kept in an oven maintained at 80°C±1°C for twenty four hours. After drying the roots were kept in desiccators for cooling. The weight of dried roots was recorded and average weight was calculated and expressed in grams. It was observed at 45 days after layering.

(x) Success in rooting (%)

The number of rooted air-layers was counted after detachment of air-layered twigs from the mother plants after forty five days of operation. The data were compiled and success in rooting percentage was calculated by the following formula:

$$\text{Success in rooting (\%)} = \frac{\text{No. of rooted air layers}}{\text{Total number of air layers}} \times 100$$

3.8.2 Growth studies

(i) Number of Branches per layer

Five established plants were selected randomly from each treatment under each replication and number of branches was counted at 30, 60 and 90 days after planting of air layers in polythene bags.

(ii) Number of leaves per layer

The number of leaves were counted at 30, 60 and 90 days after planting to selected plants from each treatment under each replication and then average number of leaves per air layer was calculated.

(iii) Survival of air layer (%)

The air-layered branches were planted in the polythene bags on 21st September 2018. It was also observed whether these layers have been established in the polythene bags or not at 90 days after planting. The survival percentage of air-layers was calculated by the following formula.

$$\text{Survival (\%)} = \frac{\text{Total number of established layered plants}}{\text{Total number of layered plants planted}} \times 100$$

3.8.3 Economic study of treatments

Economics of different treatments was calculated for 60 air layers.

3.9 Irrigation

Irrigation was given to the plants at 7 days interval in the nursery, starting from the date of transplanting of air-layers regularly without fail.

3.10 Weeding/ intercultural operations

Weeding or intercultural operations were done at 15 days interval after transplanting of air layers keeping care not to cause any injury to root system.

3.11 Statistical analysis and presentation of data

The data of different characters were recorded and analyzed using the method of analysis of variance as directed by R.A. Fisher (1954) in his book "Design of Experiment". Skeleton of analysis of variance table of the experiment is given below.

Table3.2 Analysis of variance table

Source of variance	Degree of freedom	Sum of squares	Mean sum of squares	'F' value calculated	'F' value (table) at 5% level of significance
Replication	2	RSS	RMS	RMS/EMS	
Treatment	12	TSS	TMS	TMS/EMS	
Error	24	ESS	EMS		
Total	38				

The significance of the treatment was judged by employing 'F' test. The treatment mean were distinguished with the help of critical difference (CD) which was computed as follows

(i) S.E.m± and C.D. for treatment

S.E.m ± = Error/treatment

C.D. = √2 × S.E.m × t (at 5%) for error d.f.

(ii) S.E.m± and C.D. for replication

S.E.m ± = √Error/replication

C.D. = √2 × S.E.m × t (at 5%) for error d.f.

Where,

Error variance = MSS due to error

The mean sum of square (MSS) was calculated by dividing the sum of square (SS) by corresponding degree of freedom (d.f.). The ratio 'F' was calculated by the following formula:

F = MSS/EMSS

The data have been presented in the form of summary tables along with C.D. at 5% level of significance. Suitable diagrammatical illustrations of the data have also been given at appropriate places in the text. The analysis of variance tables have been given as appendices.

CHAPTER – IV

RESULTS

The present investigation entitled "Effect of different concentration of IBA and rooting media on callusing and survival of air layering in acid lime (*Citrus aurantifolia* Swingle)" was carried out during the rainy season of 2018-19. The results obtained during the course of investigation have been described in this chapter under appropriate headings. In this chapter, an endeavor has been made to elicit the influence of various treatments of different concentrations of IBA and rooting media on root and growth of lime air layers. The observations recorded during study are summarized in the form of tables and illustrated through figures where ever found necessary. The ANOVA of statistically analyzed data are given in the appendix section.

4.1 ROOT STUDIES

4.1.1 Callus formation (cm)

Callus formation in the air-layers is the first apparent symptom of root formation process in auxin adenine balance. Growth regulators especially auxin help in the fast healing of wound caused by development of callus cells and ultimately roots.

The data on the callus formation summarized in Table 4.1 and illustrated graphically in Fig. 3 revealed that the callus formation in lime air layers was significantly affected by different treatments over control.

Different concentrations of IBA along with different rooting media significantly influenced the callus formation. Callus formation increased with increasing concentration of IBA with same rooting media. Maximum callus formation (0.67 cm) was recorded with T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{12} (IBA 2000ppm + soil + vermicompost) (0.55 cm) which was significantly at par with T_{10} (IBA 1500ppm + soil + sand + vermicompost) (0.51 cm). The minimum callus formation (0.16 cm) was recorded under T_1 (IBA 0ppm + soil). Results also indicated that the T_{13} recorded four times more callus formation over control. It was also observed that the rooting media containing soil + sand + vermicompost found superior over rooting media contained soil + vermicompost or soil alone.

Table 4.1: Effect of IBA and rooting media on callus formation

Treatments	Symbol	Callus formation (cm)
IBA 0ppm + soil (Control)	T_1	0.16
IBA 500ppm + soil	T_2	0.24
IBA 500ppm + Soil + Vermicompost	T_3	0.31
IBA 500ppm + Soil + Sand + Vermicompost	T_4	0.37
IBA 1000ppm + Soil	T_5	0.28
IBA 1000ppm + Soil + Vermicompost	T_6	0.35
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	0.42
IBA 1500ppm + Soil	T_8	0.33
IBA 1500ppm + Soil + Vermicompost	T_9	0.42
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	0.51
IBA 2000ppm + Soil	T_{11}	0.39
IBA 2000ppm + Soil + Vermicompost	T_{12}	0.55
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	0.67
S.E.(m) ±		**0.02**
C.D. (at 5%)		**0.05**

4.1.2 Number of primary roots

The perusal of the data presented in Table 4.2 and depicted through Fig. 3 revealed that all the treatments significantly increased the number of primary roots per air-layer over control. Number of primary roots per air-layer increased significantly with the increase in the concentration of IBA except lower concentration with the same rooting media. Highest concentration of IBA along with rooting media contained soil + sand + vermicompost was recorded maximum number of primary roots per air-layer over their respective treatments. Among the treatments, T_{13} (IBA 2000ppm + soil + sand + vermicompost) resulted in significantly highest number of primary roots (24.58) per air-layer followed by T_{10} (IBA 1500ppm + soil + sand + vermicompost) (22.53) which was significantly at par with T_{12} (IBA 2000ppm + soil + vermicompost) (21.25) and T_{12} was also at par with T_9 (IBA 1500ppm + soil + vermicompost) (19.31). The minimum number of primary roots (8.52) was noted under T_1 (control).

Table 4.2: Effect of IBA and rooting media on number of primary roots

Treatments	Symbol	Number of primary roots
IBA 0ppm + soil (Control)	T_1	8.52
IBA 500ppm + soil	T_2	11.98
IBA 500ppm + Soil + Vermicompost	T_3	14.61
IBA 500ppm + Soil + Sand + Vermicompost	T_4	15.11
IBA 1000ppm + Soil	T_5	12.87
IBA 1000ppm + Soil + Vermicompost	T_6	15.11
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	18.11
IBA 1500ppm + Soil	T_8	15.66
IBA 1500ppm + Soil + Vermicompost	T_9	19.31
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	22.53
IBA 2000ppm + Soil	T_{11}	17.22
IBA 2000ppm + Soil + Vermicompost	T_{12}	21.25
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	24.58
S.E.(m) ±		**0.07**
C.D. (at 5%)		**2.07**

4.1.3 Length of primary roots (cm)

The data on length of primary roots are presented in Table 4.3 and depicted by Fig. 4. Analysis of variance (Appendix-I) indicates that length of primary roots significantly varied due to various treatments.

The maximum length of primary roots (5.98 cm) was measured in T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{12} (IBA 2000ppm + soil + vermicompost) (5.13 cm). There was no significant difference found between T_{12} and T_{10} (IBA 1500ppm + soil + sand + vermicompost) (4.68 cm). All the similar concentrations of IBA along with rooting media contained soil + sand + vermicompost were found significantly at par with rooting media contained soil + vermicompost, except higher concentration. Minimum length of primary roots was noted in control (2.11 cm).

Table 4.3: Effect of IBA and rooting media on length of primary roots

Treatments	Symbol	Length of primary roots (cm)
IBA 0ppm + soil (Control)	**T_1**	2.11
IBA 500ppm + soil	**T_2**	2.78
IBA 500ppm + Soil + Vermicompost	**T_3**	3.41
IBA 500ppm + Soil + Sand + Vermicompost	**T_4**	3.62
IBA 1000ppm + Soil	**T_5**	3.11
IBA 1000ppm + Soil + Vermicompost	**T_6**	3.65
IBA 1000ppm + Soil + Sand + Vermicompost	**T_7**	4.08
IBA 1500ppm + Soil	**T_8**	3.57
IBA 1500ppm + Soil + Vermicompost	**T_9**	4.31
IBA 1500ppm + Soil + Sand + Vermicompost	**T_{10}**	4.68
IBA 2000ppm + Soil	**T_{11}**	4.31
IBA 2000ppm + Soil + Vermicompost	**T_{12}**	5.13
IBA 2000ppm + Soil + Sand + Vermicompost	**T_{13}**	5.98
S.E.(m) ±		**0.17**
C.D. (at 5%)		**0.49**

4.1.4 Diameter of primary roots (mm)

It is clear from the data presented in Table 4.4 and depicted through Fig. 5 that increase in concentrations of IBA along with same rooting media was significantly increased the diameter of primary roots over control. Maximum diameter of primary roots (2.21 mm) was noted with T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{10} (IBA 1500ppm + soil + sand + vermicompost) (1.82 mm), T_{12} (IBA 2000ppm + soil + vermicompost) (1.73 mm) and T_9 (IBA 1500ppm + soil + vermicompost (1.58 mm). However, each follower treatment was significantly at par with their next follower. The minimum diameter of primary roots (0.68 mm) was observed under T_1 (control).

Table 4.4: Effect of IBA and rooting media on diameter of primary roots

Treatments	Symbol	Diameter of primary roots (mm)
IBA 0ppm + soil (Control)	T_1	0.68
IBA 500ppm + soil	T_2	0.95
IBA 500ppm + Soil + Vermicompost	T_3	1.16
IBA 500ppm + Soil + Sand + Vermicompost	T_4	1.48
IBA 1000ppm + Soil	T_5	1.11
IBA 1000ppm + Soil + Vermicompost	T_6	1.41
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	1.65
IBA 1500ppm + Soil	T_8	1.30
IBA 1500ppm + Soil + Vermicompost	T_9	1.58
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	1.82
IBA 2000ppm + Soil	T_{11}	1.45
IBA 2000ppm + Soil + Vermicompost	T_{12}	1.73
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	2.21
S.E.(m) ±		**0.06**
C.D. (at 5%)		**0.17**

4.1.5 Number of secondary roots

The data pertaining to the number of secondary roots per air-layer are summarized in Table 4.5 and illustrated in Fig. 6 indicated that the number of secondary roots per air-layer varied significantly due to different treatments. An increase in concentration of IBA with same rooting media was significantly increased the number of secondary roots, except lower concentration. The maximum number of secondary roots (52.31) was produced under T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{10} (IBA 1500ppm + soil + sand + vermicompost) (45.67), which was significantly at par with T_{12} (IBA 2000ppm + soil + vermicompost) (44.34). Minimum number of secondary roots (22.24) was noted under T_1 (control).

Results also indicate that each concentration of IBA along with rooting media contained soil + sand + vermicompost was at par with their next higher concentration along with rooting media contained soil + vermicompost.

Table 4.5: Effect of IBA and rooting media on number of secondary roots

Treatments	Symbol	Number of secondary roots
IBA 0ppm + soil (Control)	T_1	22.24
IBA 500ppm + soil	T_2	26.12
IBA 500ppm + Soil + Vermicompost	T_3	31.55
IBA 500ppm + Soil + Sand + Vermicompost	T_4	35.46
IBA 1000ppm + Soil	T_5	29.21
IBA 1000ppm + Soil + Vermicompost	T_6	34.91
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	39.42
IBA 1500ppm + Soil	T_8	32.33
IBA 1500ppm + Soil + Vermicompost	T_9	38.22
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	45.67
IBA 2000ppm + Soil	T_{11}	36.78
IBA 2000ppm + Soil + Vermicompost	T_{12}	44.34
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	52.31
S.E.(m) ±		**1.18**
C.D. (at 5%)		**3.47**

4.1.6 Length of secondary roots (cm)

It is clear from data presented in Table 4.6 and illustrated with Fig. 7 that the length of the secondary roots was significantly affected by different treatments over control. The maximum length of secondary roots (1.21 cm) was noted under T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{12} (IBA 2000ppm + soil + vermicompost) (0.98 cm) and T_{10} (IBA 1500ppm + soil + sand + vermicompost) (0.91 cm) whereas, minimum length of secondary roots (0.46 cm) was noted with the T_1 (IBA 0ppm + soil). Each increasing dose of IBA up to 1000ppm with same rooting media was at par with each other. It was also observed that T_{13} (IBA 2000ppm + soil + sand + vermicompost) was recorded four times more length of secondary roots over control.

Table 4.6: Effect of IBA and rooting media on length of secondary roots

Treatments	Symbol	Length of secondary roots (cm)
IBA 0ppm + soil (Control)	**T_1**	0.46
IBA 500ppm + soil	**T_2**	0.55
IBA 500ppm + Soil + Vermicompost	**T_3**	0.67
IBA 500ppm + Soil + Sand + Vermicompost	**T_4**	0.76
IBA 1000ppm + Soil	**T_5**	0.61
IBA 1000ppm + Soil + Vermicompost	**T_6**	0.73
IBA 1000ppm + Soil + Sand + Vermicompost	**T_7**	0.81
IBA 1500ppm + Soil	**T_8**	0.67
IBA 1500ppm + Soil + Vermicompost	**T_9**	0.80
IBA 1500ppm + Soil + Sand + Vermicompost	**T_{10}**	0.91
IBA 2000ppm + Soil	**T_{11}**	0.75
IBA 2000ppm + Soil + Vermicompost	**T_{12}**	0.98
IBA 2000ppm + Soil + Sand + Vermicompost	**T_{13}**	1.21
S.E.(m) ±		**0.02**
C.D. (at 5%)		**0.07**

4.1.7 Diameter of secondary roots (mm)

The data presented in Table 4.7 and depicted through Fig. 8 clearly indicated that all the treatments significantly increased the diameter of secondary roots over control. The maximum diameter of secondary roots (0.73 mm) was noted under T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{12} (IBA 2000ppm + soil + sand + vermicompost) (0.65 mm), which was significantly at par with T_{10} (IBA 1500ppm + soil + sand + vermicompost) (0.62 mm). Among the various concentrations of IBA, diameter of secondary roots was increased with increase in the concentration. However, there was no significant difference found under lower concentrations up to 1000ppm with same rooting media. The minimum diameter (0.27 mm) was recorded with T_1 (IBA 0ppm + soil).

Table 4.7: Effect of IBA and rooting media on diameter of secondary roots

Treatments	Symbol	Diameter of secondary roots (mm)
IBA 0ppm + soil (Control)	T_1	0.27
IBA 500ppm + soil	T_2	0.35
IBA 500ppm + Soil + Vermicompost	T_3	0.42
IBA 500ppm + Soil + Sand + Vermicompost	T_4	0.48
IBA 1000ppm + Soil	T_5	0.39
IBA 1000ppm + Soil + Vermicompost	T_6	0.46
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	0.54
IBA 1500ppm + Soil	T_8	0.45
IBA 1500ppm + Soil + Vermicompost	T_9	0.56
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	0.62
IBA 2000ppm + Soil	T_{11}	0.55
IBA 2000ppm + Soil + Vermicompost	T_{12}	0.65
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	0.73
S.E.(m) ±		**0.02**
C.D. (at 5%)		**0.06**

4.1.8 Fresh weight of roots (g)

The data on fresh weight of roots was computed and presented in Table 4.8 and illustrated graphically in Fig. 9 revealed that the fresh weight of roots increased significantly with each increase in the concentration of IBA as well as well as by different rooting media also at similar IBA concentration. Maximum fresh weight of roots (4.21 g) was recorded with T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{10} (IBA 1500ppm + soil + sand + vermicompost) (3.67g) and T_{12} (IBA 2000ppm + soil + sand + vermicompost) (3.64 g), which was significantly at par with each other. Minimum fresh weight (1.37 g) of roots per air layer was noted with the control (IBA 0ppm + soil). Results also indicated that different rooting media was significantly increased fresh weight of roots at same IBA concentrations.

Table 4.8: Effect of IBA and rooting media on fresh weight of roots (g)

Treatments	**Symbol**	**Fresh weight of roots (g)**
IBA 0ppm + soil (Control)	T_1	1.37
IBA 500ppm + soil	T_2	1.72
IBA 500ppm + Soil + Vermicompost	T_3	2.34
IBA 500ppm + Soil + Sand + Vermicompost	T_4	2.68
IBA 1000ppm + Soil	T_5	2.28
IBA 1000ppm + Soil + Vermicompost	T_6	2.52
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	3.11
IBA 1500ppm + Soil	T_8	2.59
IBA 1500ppm + Soil + Vermicompost	T_9	2.93
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	3.67
IBA 2000ppm + Soil	T_{11}	2.78
IBA 2000ppm + Soil + Vermicompost	T_{12}	3.64
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	4.21
S.E.(m) ±		**0.09**
C.D. (at 5%)		**0.26**

4.1.9 Dry weight of roots (g)

The data pertaining to the dry weight of roots per air-layer summarized in Table 4.9 and illustrated in Fig. 10 showed that the dry weight of roots deviated significantly due to different treatments. Each increase in the concentration of IBA was significantly increased dry weight of roots. Different rooting media was also performed significantly better at similar IBA concentrations. The maximum dry weight of roots (1.87 g) was observed with T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{10} (IBA 1500ppm + soil + sand + vermicompost) (1.58g) and T_{12} (IBA 2000ppm + soil + vermicompost), whereas, minimum dry weight of roots (0.51 g) per air layer was recorded with T_1 (control).

Table 4.9: Effect of IBA and rooting media on dry weight of roots (g)

Treatments	Symbol	Dry weight of roots (g)
IBA 0ppm + soil (Control)	T_1	0.51
IBA 500ppm + soil	T_2	0.73
IBA 500ppm + Soil + Vermicompost	T_3	0.99
IBA 500ppm + Soil + Sand + Vermicompost	T_4	1.25
IBA 1000ppm + Soil	T_5	0.90
IBA 1000ppm + Soil + Vermicompost	T_6	1.21
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	1.41
IBA 1500ppm + Soil	T_8	1.09
IBA 1500ppm + Soil + Vermicompost	T_9	1.25
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	1.58
IBA 2000ppm + Soil	T_{11}	1.17
IBA 2000ppm + Soil + Vermicompost	T_{12}	1.42
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	1.87
S.E.(m) ±		**0.05**
C.D. (at 5%)		**0.15**

4.1.10 Success in rooting percentage of air-layers

Data presented in Table 4.10 and illustrated with Fig 11 revealed that the significant effect of different treatments was found on rooting percentage of lime air layers over control. The maximum rooting (92.17%) was noted with T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{12} (IBA 2000ppm + soil + sand + vermicompost) (87.31) and T_{10} (IBA 1500ppm + soil + vermicompost) (82.11%). However, there was no significant difference was found between T_{13} and T_{12}; T_{12} and T_{10}. While, minimum rooting (58.11%) was recorded under T_1 (control), which was significantly at par with T_2 (IBA 500ppm +soil). It was also observed that the rooting media contained soil + sand + vermicompost was given statistically similar results with rooting media contained soil + vermicompost at same IBA concentration except 1000ppm.

Table 4.10: Effect of IBA and rooting media on rooting percentage

Treatments	Symbol	Percent air layers rooted
IBA 0ppm + soil (Control)	T_1	58.11
IBA 500ppm + soil	T_2	62.75
IBA 500ppm + Soil + Vermicompost	T_3	66.12
IBA 500ppm + Soil + Sand + Vermicompost	T_4	70.1
IBA 1000ppm + Soil	T_5	64.23
IBA 1000ppm + Soil + Vermicompost	T_6	68.88
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	75.33
IBA 1500ppm + Soil	T_8	69.37
IBA 1500ppm + Soil + Vermicompost	T_9	78.12
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	82.11
IBA 2000ppm + Soil	T_{11}	79.03
IBA 2000ppm + Soil + Vermicompost	T_{12}	87.31
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	92.17
S.E.(m) ±		**2.02**
C.D. (at 5%)		**6.45**

4.2 GROWTH STUDIES

4.2.1 Number of branches per air layer

Results given in Table 4.11 (Fig. 12) indicate that number of branches was significantly increased with different treatments over control. The maximum number of branches (3.21, 7.28 and 13.42) was counted under T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{12} (IBA 2000ppm + soil + vermicompost) (2.98, 6.47 and 11.67) and T_{10} (IBA 1500ppm + soil + sand + vermicompost) (2.92, 6.33 and 9.28) at 30, 60 and 90 DAT, respectively. However, there was no significant difference found between T_{12} and T_{10} at 30 and 60 DAT. The minimum number of branches (1.42, 2.21 and 5.11 at 30, 60 and 90 DAT, respectively) was counted under T_1 (IBA 0ppm + soil).

Table 4.11: Effect of IBA and rooting media on number of branches

Treatments	Sym.	Number of branches per air layer		
		30 Days	**60 days**	**90 days**
IBA 0ppm + soil (Control)	T_1	1.42	2.21	5.11
IBA 500ppm + soil	T_2	1.78	3.11	5.47
IBA 500ppm + Soil + Vermicompost	T_3	2.27	4.16	6.23
IBA 500ppm + Soil + Sand + Vermicompost	T_4	2.41	4.48	7.13
IBA 1000ppm + Soil	T_5	2.14	4.17	6.07
IBA 1000ppm + Soil + Vermicompost	T_6	2.43	4.62	7.49
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	2.62	5.36	8.14
IBA 1500ppm + Soil	T_8	2.36	4.87	7.52
IBA 1500ppm + Soil + Vermicompost	T_9	2.68	5.81	8.11
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	2.92	6.33	9.28
IBA 2000ppm + Soil	T_{11}	2.51	5.44	8.72
IBA 2000ppm + Soil + Vermicompost	T_{12}	2.98	6.47	11.67
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	3.21	7.28	13.42
S.E.(m) ±		**0.07**	**0.20**	**0.32**
C.D. (at 5%)		**0.21**	**0.58**	**0.95**

4.2.2 Number of leaves per air layer

Results presented in Table 4.12 and depicted through Fig. 13 clearly indicated that the number of leaves per air layer was significantly increased by different treatments over control. The maximum number of leaves per air layer (15.12, 25.54 and 30.87 at 30, 60 and 90 DAT, respectively) was obtained by T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{10} (13.47) and T_{12} (12.98) for 30 DAT; and followed by T_{12} (22.84 and 34.81) and T_{10} (22.00 and 33.10) for 60 and 90 DAT, respectively. However, there was no significant difference observed between T_{12} and T_{10} at 30, 60 and 90 DAT. While, minimum number of leaves (4.81, 8.28 and 13.11) was noted with control (T_1) at 30, 60 and 90 DAT respectively.

Table 4.12: Effect of IBA and rooting media on number of leaves

Treatments	Sym.	Number of leaves per air layer		
		30 Days	60 days	90 days
IBA 0ppm + soil (Control)	T_1	4.81	8.28	13.11
IBA 500ppm + soil	T_2	6.15	12.11	19.91
IBA 500ppm + Soil + Vermicompost	T_3	8.71	14.78	22.32
IBA 500ppm + Soil + Sand + Vermicompost	T_4	9.28	17.21	25.10
IBA 1000ppm + Soil	T_5	8.80	15.11	23.11
IBA 1000ppm + Soil + Vermicompost	T_6	10.22	17.52	25.32
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	12.11	19.68	27.61
IBA 1500ppm + Soil	T_8	10.88	18.32	26.82
IBA 1500ppm + Soil + Vermicompost	T_9	12.22	20.11	29.16
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	13.47	22.00	33.10
IBA 2000ppm + Soil	T_{11}	11.76	19.67	28.11
IBA 2000ppm + Soil + Vermicompost	T_{12}	12.98	22.84	34.81
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	15.12	25.54	38.52
S.E.(m) ±		**0.47**	**0.70**	**0.86**
C.D. (at 5%)		**1.31**	**2.06**	**2.52**

4.2.3 Survival percentage of air layers

The perusal of the data presented in Table 4.13 and depicted in Fig. 14 indicates that all the treatments were positively affected to survival percentage of air-layers over control. However, each adjoining treatment up to T_8 was significantly at par while, rest treatments were significantly superior over each other in respect of survival percentage of air layers.

The highest survival percentage (90.17%) was obtained with T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{12} (IBA 2000ppm + soil + vermicompost) (84.13%) and T_{10} (IBA 1500ppm + soil + sand + vermicompost) (82.53%). However, there was no significant difference found between T_{13} and T_{12}; T_{12} and T_{10} whereas, comparatively lowest survival percentage (56.27%) was noted with control.

Table 4.13: Effect of IBA and rooting media on survival percentage

Treatments	Symbol	Survival percentage
IBA 0ppm + soil (Control)	T_1	56.27
IBA 500ppm + soil	T_2	61.12
IBA 500ppm + Soil + Vermicompost	T_3	63.73
IBA 500ppm + Soil + Sand + Vermicompost	T_4	66.84
IBA 1000ppm + Soil	T_5	63.78
IBA 1000ppm + Soil + Vermicompost	T_6	68.92
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	73.43
IBA 1500ppm + Soil	T_8	66.11
IBA 1500ppm + Soil + Vermicompost	T_9	73.22
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	82.53
IBA 2000ppm + Soil	T_{11}	72.11
IBA 2000ppm + Soil + Vermicompost	T_{12}	84.13
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	90.17
S.E.(m) ±		**2.17**
C.D. (at 5%)		**6.36**

4.3 ECONOMIC STUDIES

The economics of treatments was calculated and presented in Table 4.16 depicted in Fig. 17 revealed that the maximum net return (₹2017.14/-) and benefit cost ratio (5.24) was obtained with T_{13} (IBA 2000ppm + soil + sand + vermicompost). However, T_{12} (IBA 2000ppm + soil + vermicompost) was noted more net return (₹1737.47/-) as compare to T_{10} (₹1606.81/-) whereas, T_{10} (IBA 1500ppm + soil + sand + vermicompost) was noted more benefit cost ratio (4.77) as compare to T_{12} (4.73).

The minimum net return (₹734/-) was obtained by T_1 (control) whereas, minimum benefit cost ratio (3.47) was obtained under T_8 (IBA 1500ppm + soil) followed by T_5 (3.55), T_{11} (3.83), T_6 (3.89), T_2 (3.89) and control (3.99).

A. Fixed cost = ₹3200/- for 780 air layers

1. Labour cost = 2 labour for 4 days @ ₹325/- = ₹2600/-
2. Polythene = ₹300/-
3. Sutali = ₹100/-
4. Talcum powder = ₹200/-

B. Media input cost for 60 air layers

1. Vermicompost = ₹20/-
2. Sand = ₹10/-

C. IBA cost for 60 air layers

1. IBA 500ppm = ₹50/-
2. IBA 1000ppm = ₹100/-
3. IBA 1500ppm = ₹150/-
4. IBA 2000ppm = ₹200/-

D. Air layer sold @ ₹50/-

Table 4.14: Treatment wise net profit and benefit cost ratio

Treatments	Symbol	Total cost/ 60 air layers (Rs.)	Actual no. of air layer survived	Cost/ survived plant (Rs.)	Gross income (Rs.)	Net profit (Rs.)	B:C ratio
IBA 0ppm + soil (Control)	T_1	246.15	19.62	12.54	980.95	734.80	3.99
IBA 500ppm + soil	T_2	296.15	23.01	12.86	1150.58	854.43	3.89
IBA 500ppm + Soil + Vermicompost	T_3	316.15	25.28	12.50	1264.15	948.00	4.00
IBA 500ppm + Soil + Sand + Vermicompost	T_4	326.15	28.11	11.60	1405.65	1079.50	4.31
IBA 1000ppm + Soil	T_5	346.15	24.58	14.08	1228.98	882.83	3.55
IBA 1000ppm + Soil + Vermicompost	T_6	366.15	28.48	12.85	1424.16	1058.01	3.89
IBA 1000ppm + Soil + Sand + Vermicompost	T_7	376.15	33.19	11.33	1659.44	1283.29	4.41
IBA 1500ppm + Soil	T_8	396.15	27.52	14.39	1375.82	979.67	3.47
IBA 1500ppm + Soil + Vermicompost	T_9	416.15	34.32	12.12	1715.98	1299.83	4.12
IBA 1500ppm + Soil + Sand + Vermicompost	T_{10}	426.15	40.66	10.48	2032.96	1606.81	4.77
IBA 2000ppm + Soil	T_{11}	446.15	34.19	13.04	1709.66	1263.51	3.83
IBA 2000ppm + Soil + Vermicompost	T_{12}	466.15	44.07	10.57	2203.62	1737.47	4.73
IBA 2000ppm + Soil + Sand + Vermicompost	T_{13}	476.15	49.87	9.55	2493.29	2017.14	5.24

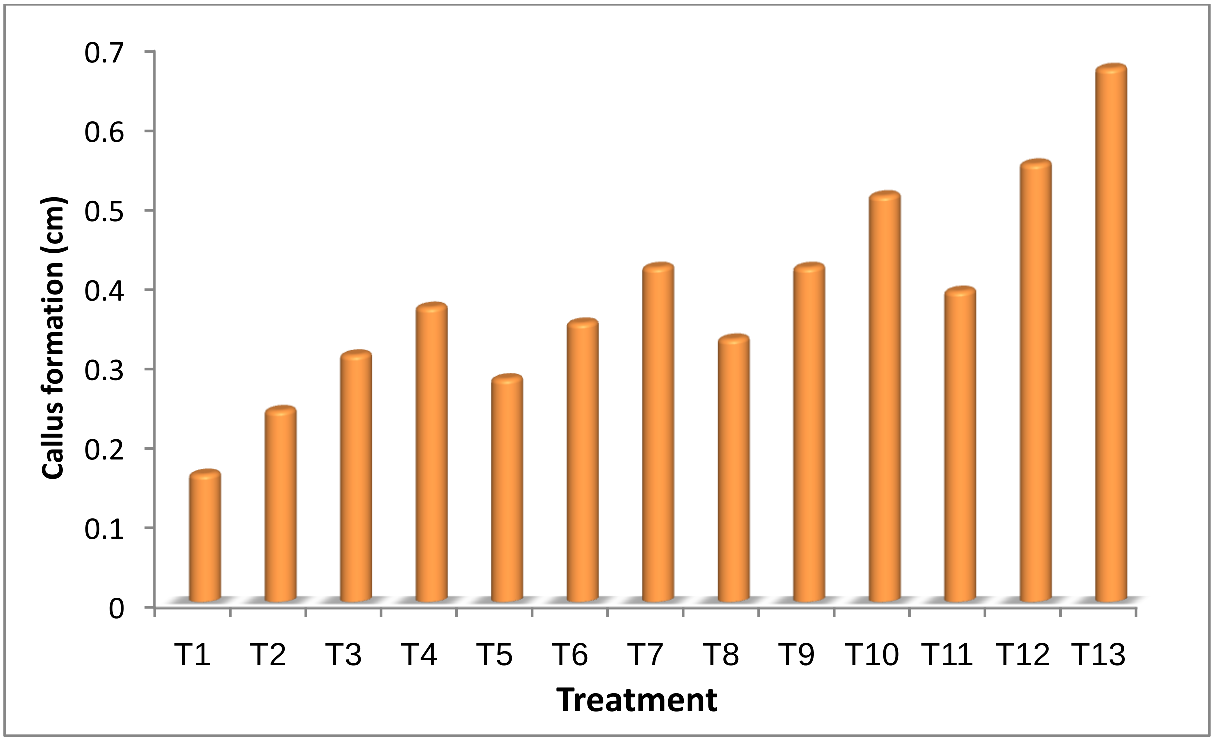

Fig 2: Effect of IBA and rooting media on callus formation

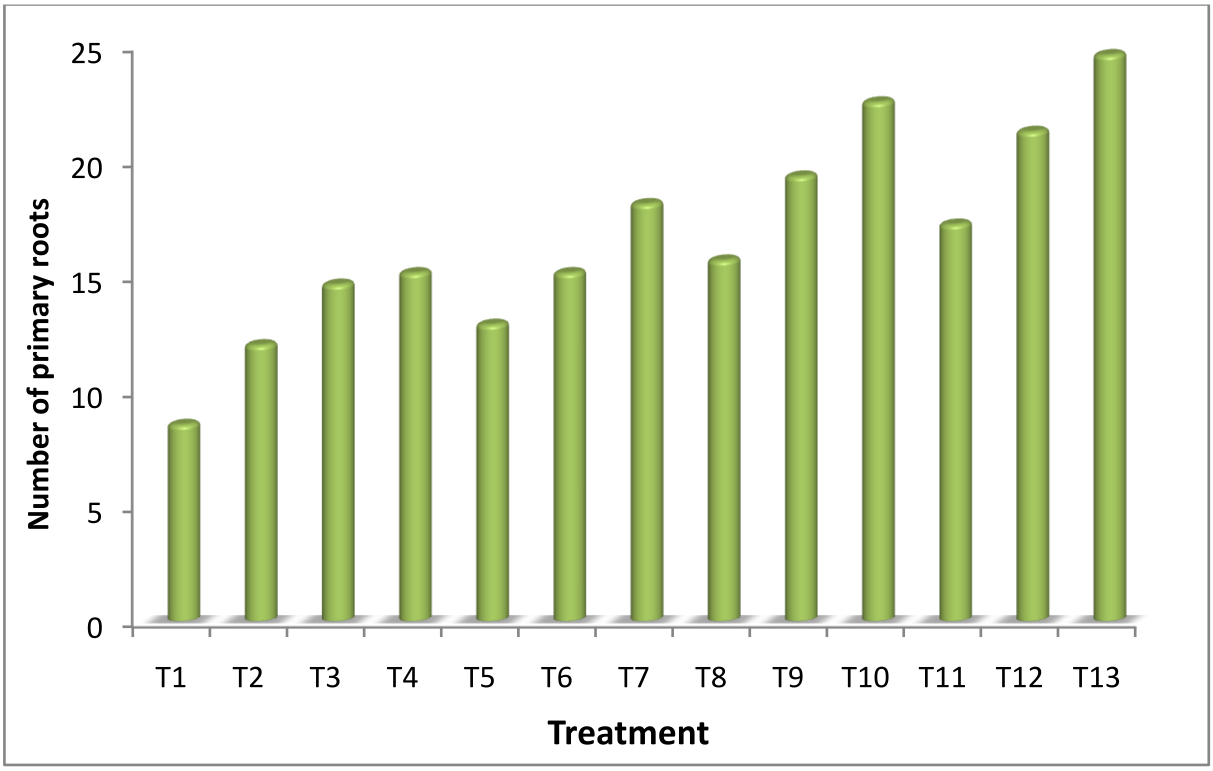

Fig 3: Effect of IBA and rooting media on number of primary roots

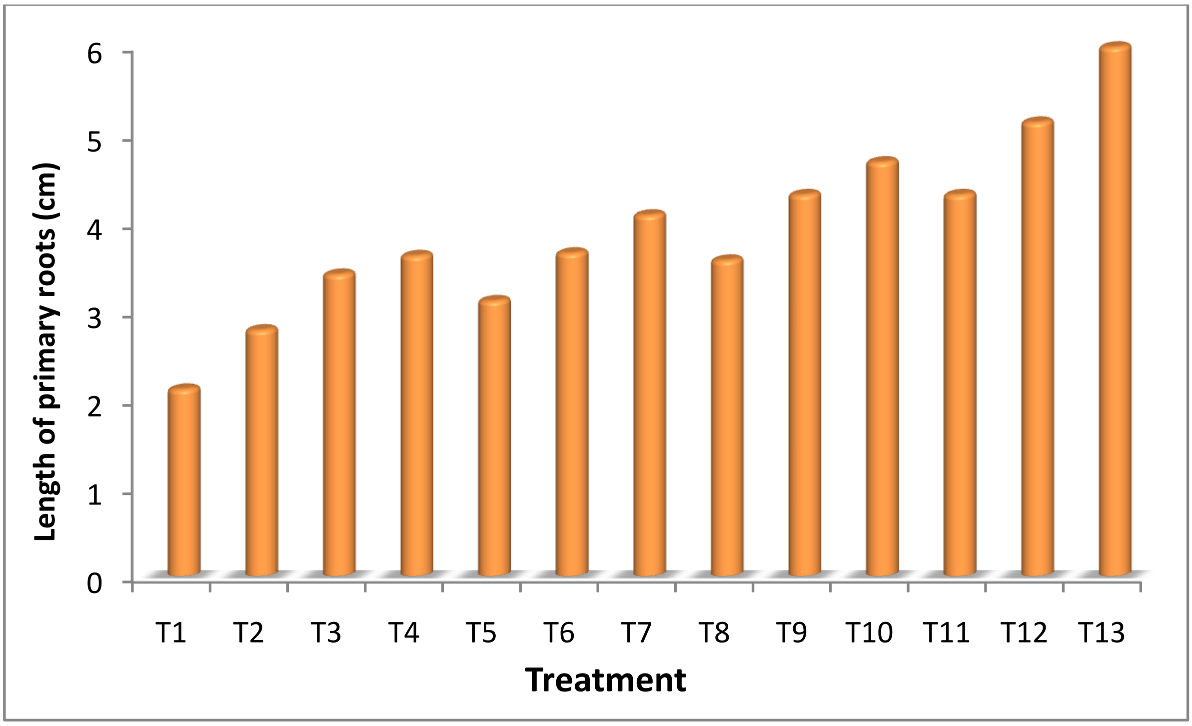

Fig 4: Effect of IBA and rooting media on length of primary roots

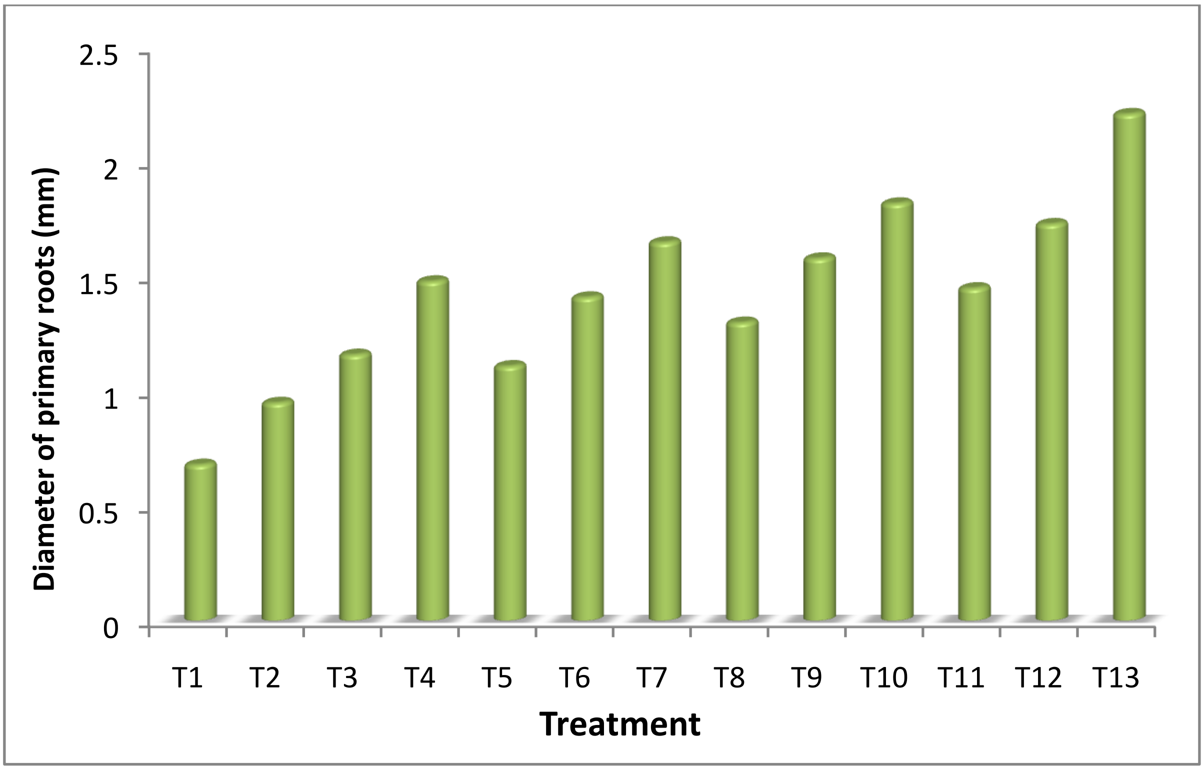

Fig 5: Effect of IBA and rooting media on diameter of primary roots

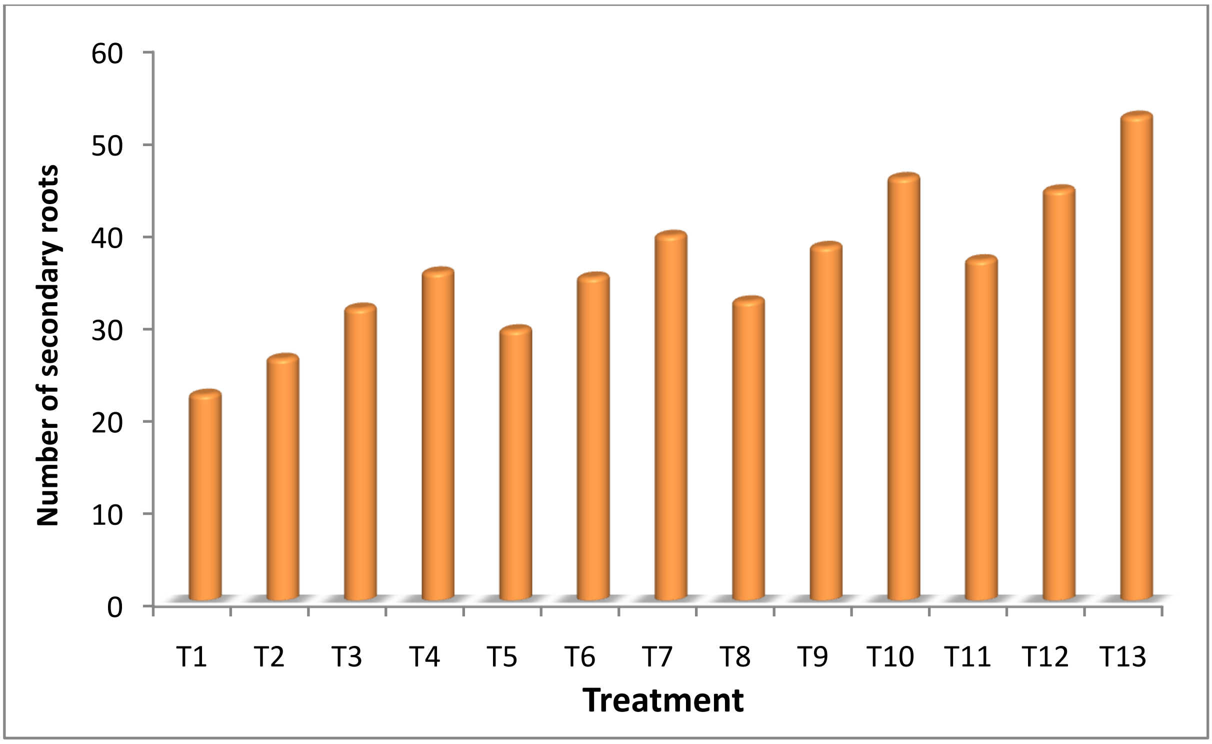

Fig 6: Effect of IBA and rooting media on number of secondary roots

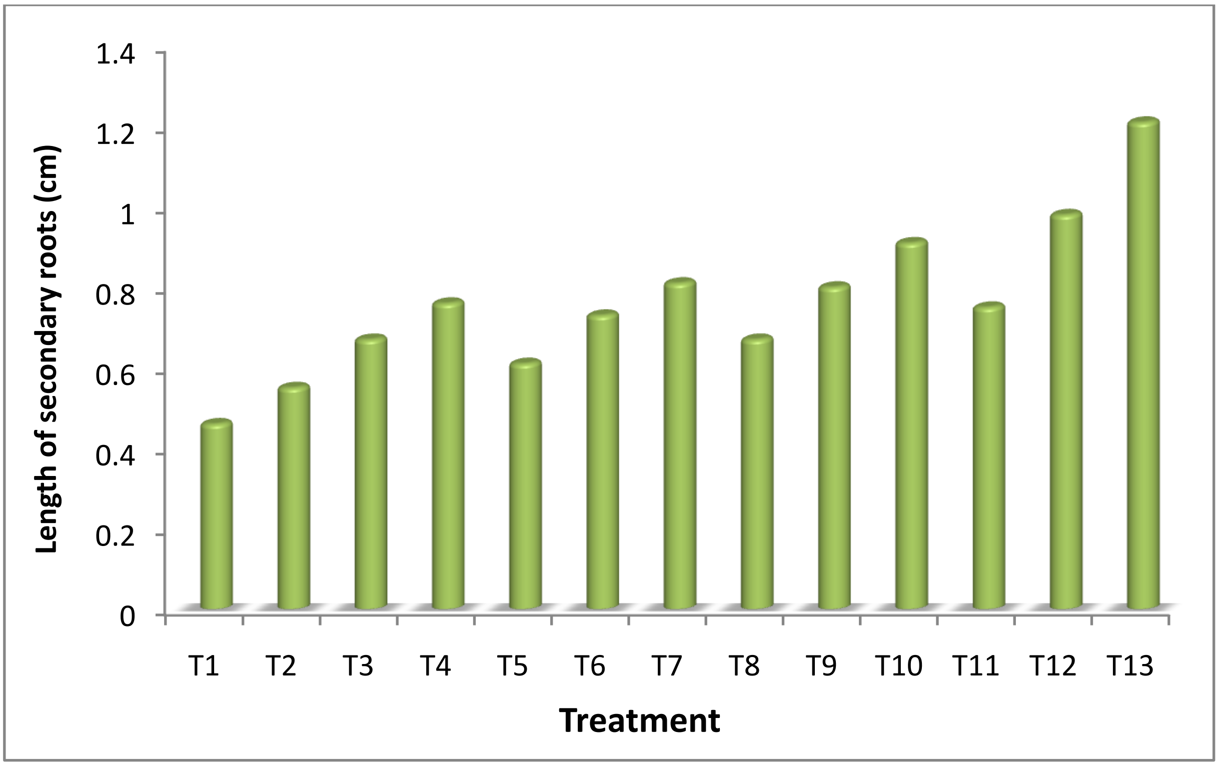

Fig 7: Effect of IBA and rooting media on length of secondary roots

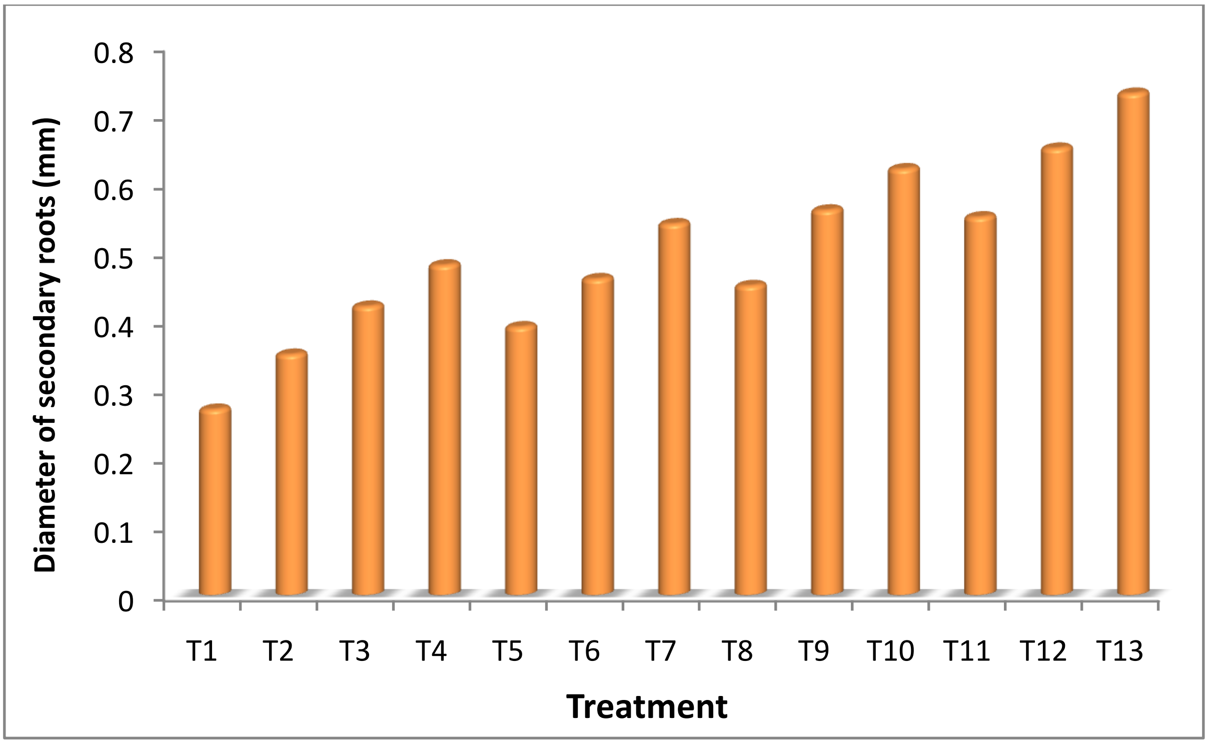

Fig 8: Effect of IBA and rooting media on diameter of secondary roots

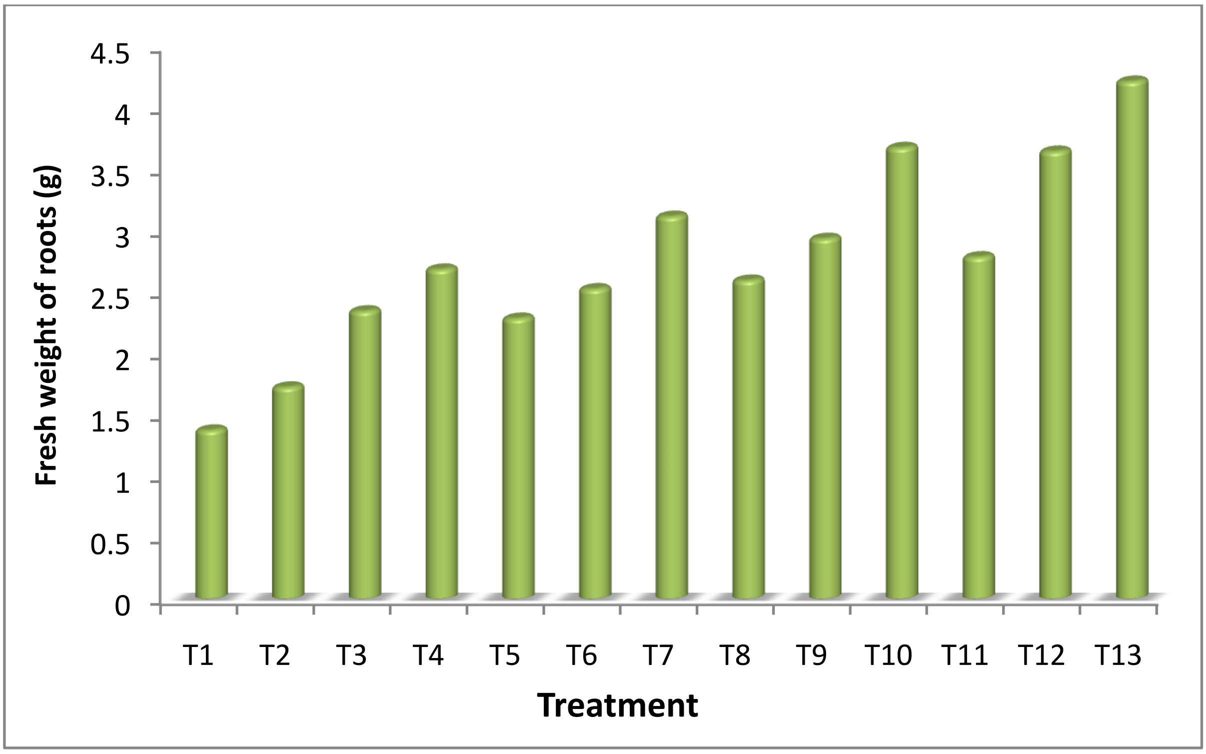

Fig 9: Effect of IBA and rooting media on fresh weight of roots

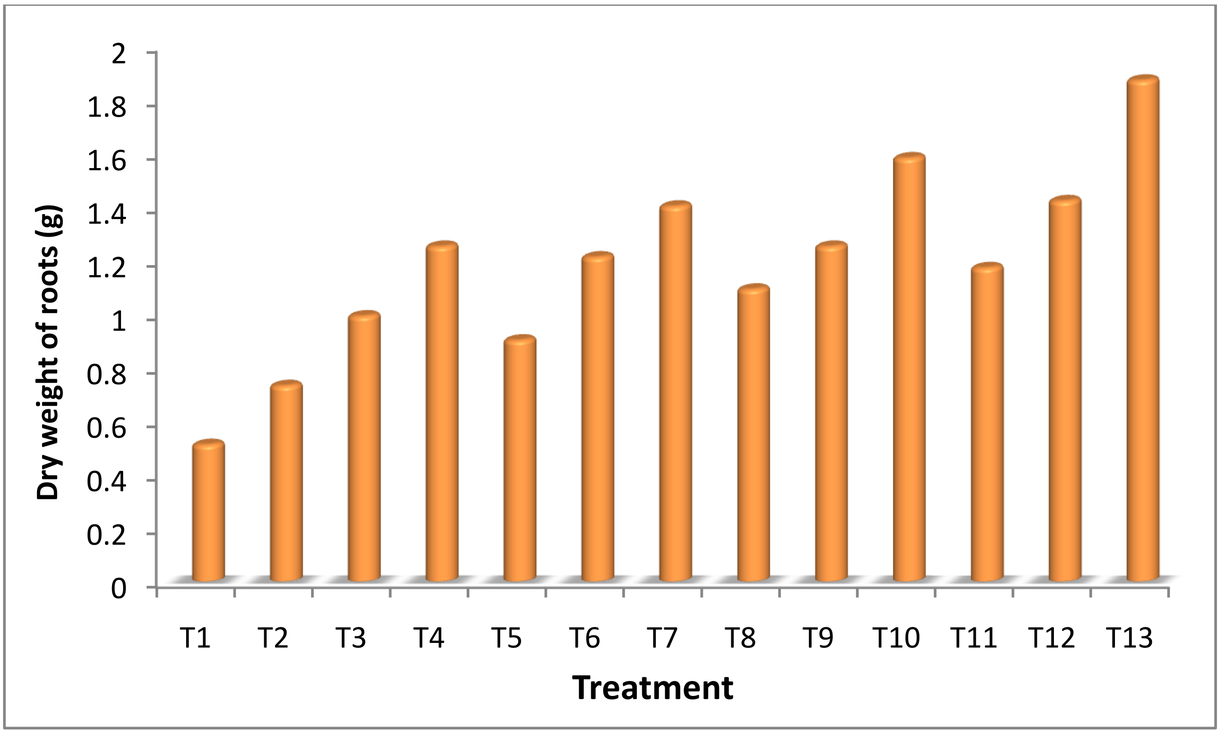

Fig 10: Effect of IBA and rooting media on dry weight of roots

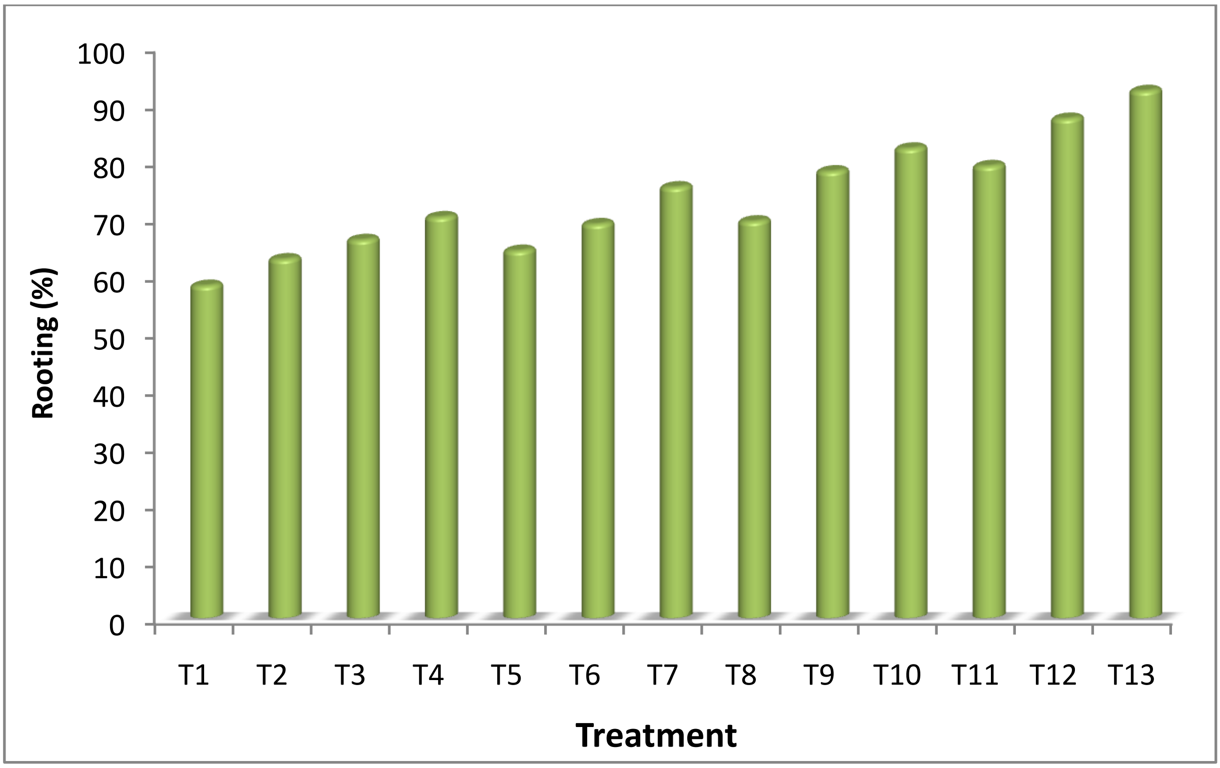

Fig 11: Effect of IBA and rooting media on rooting percentage

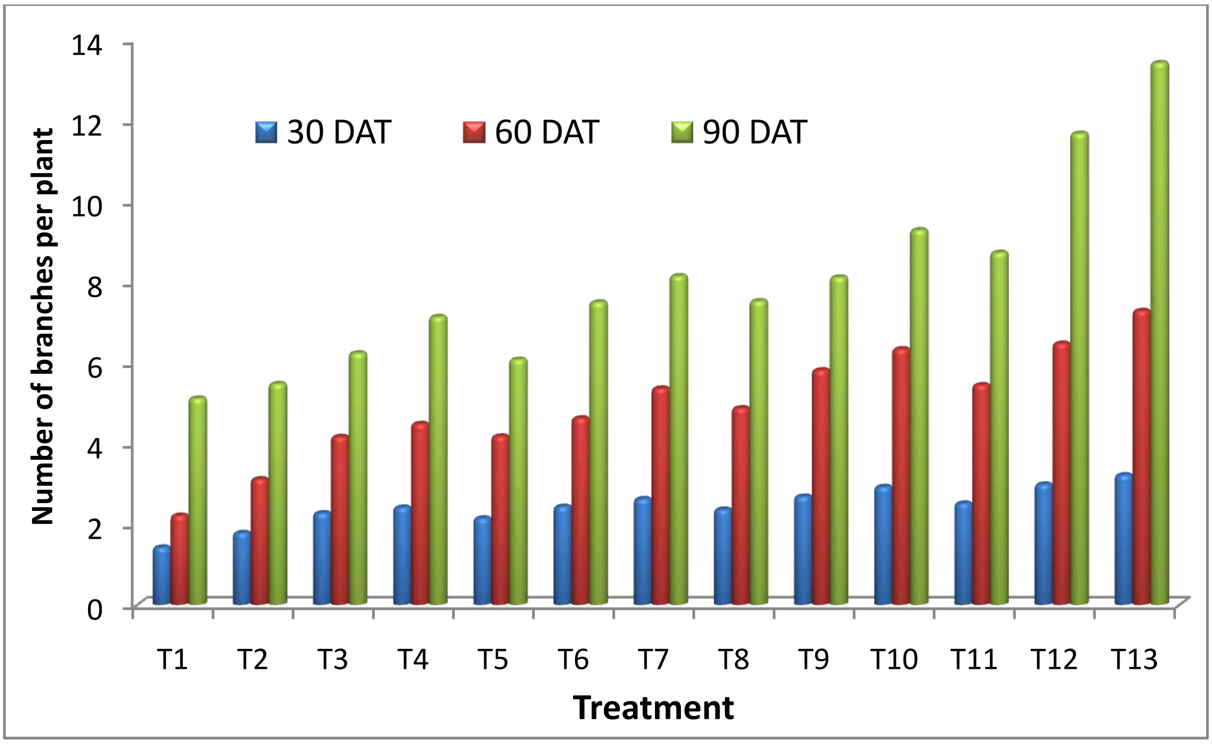

Fig 12: Effect of IBA and rooting media on number of branches

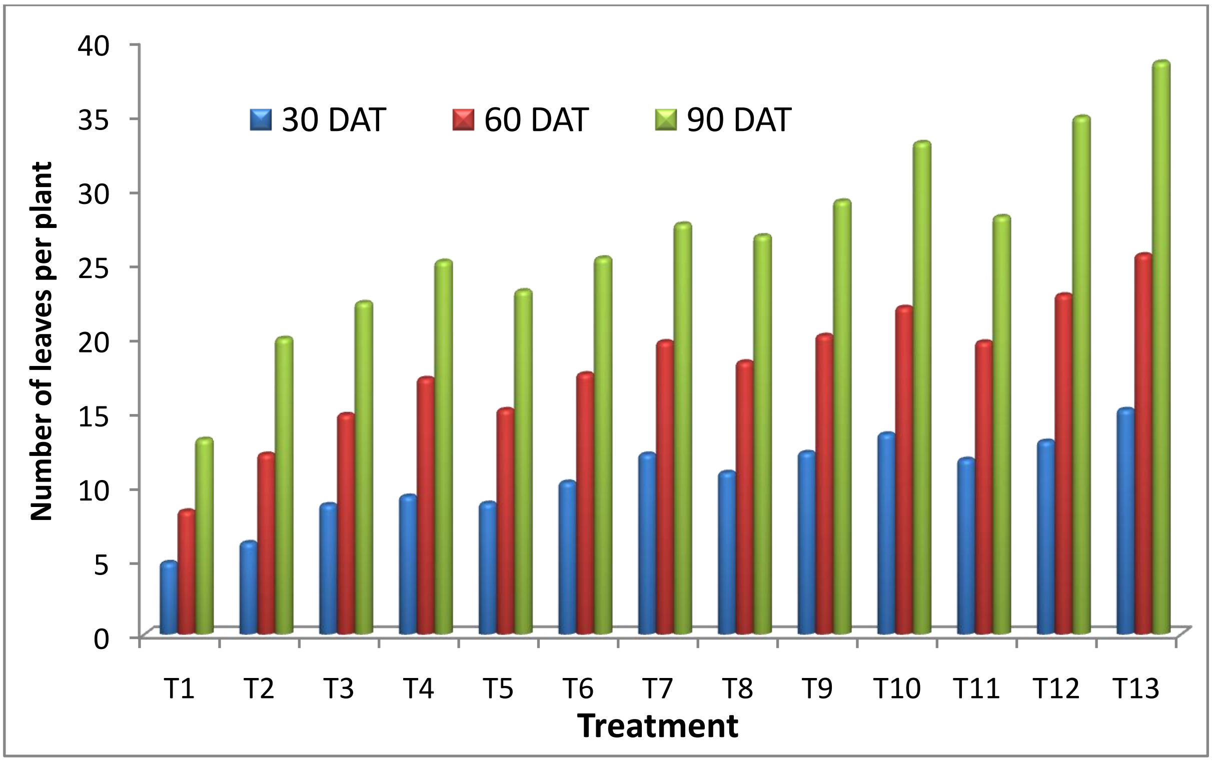

Fig 13: Effect of IBA and rooting media on number of leaves

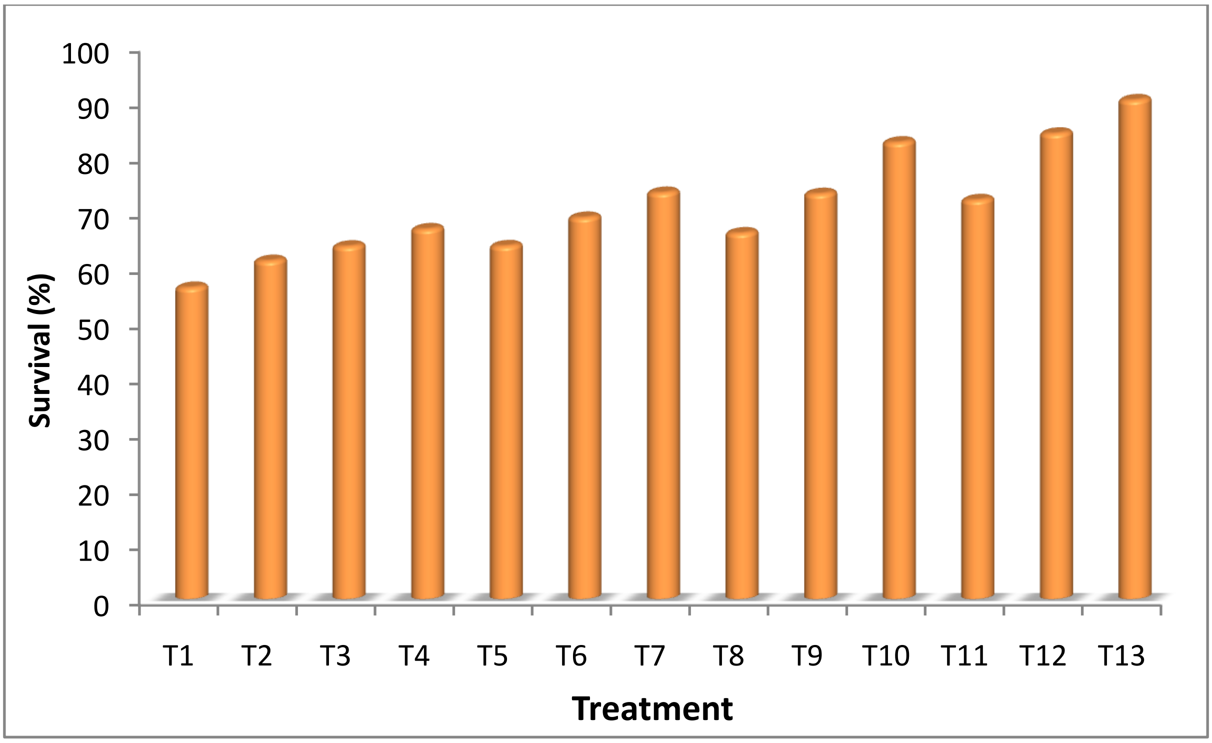

Fig 14: Effect of IBA and rooting media on survival percentage

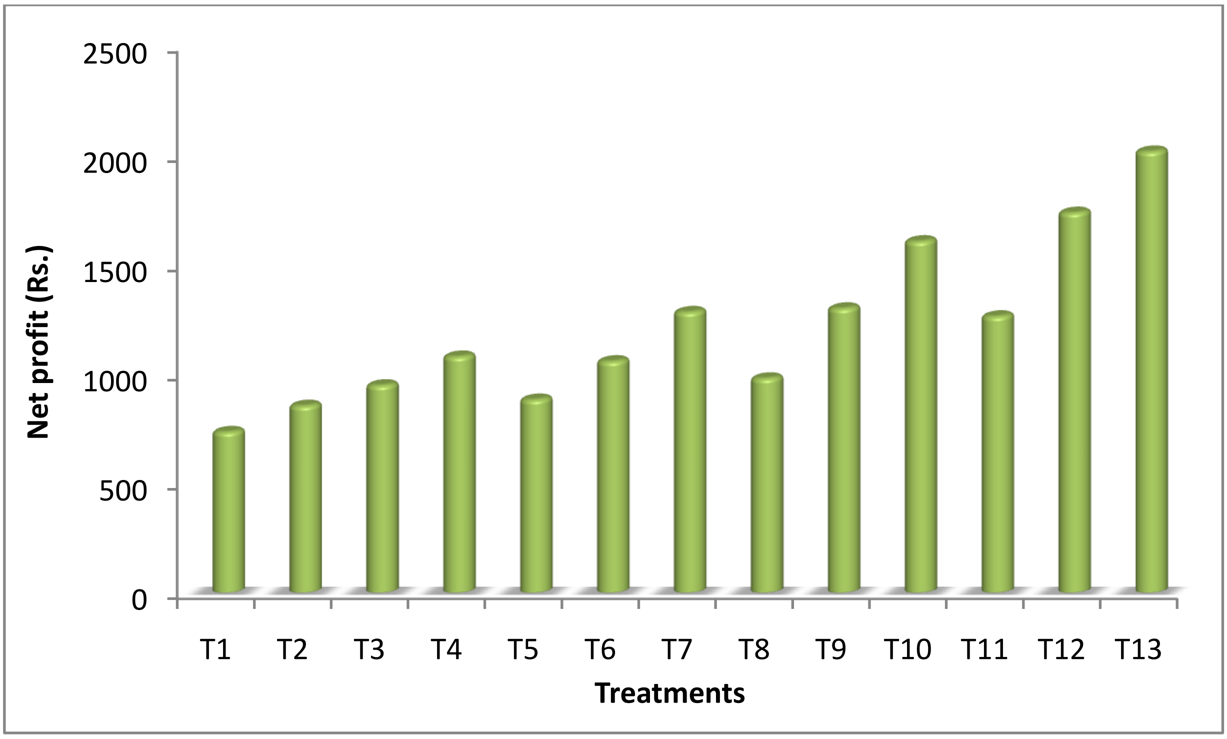

Fig 15: Treatment wise net profit per 60 air layers

CHAPTER – V

DISCUSSION

The present investigation entitled **"Effect of different concentration of IBA and rooting media on callusing and survival of air layering in acid lime (*Citrus aurantifolia* Swingle)"** was carried out during 2018-19 at University orchard, Department of Horticulture, College of Agriculture, RVSKVV, Gwalior. The experiment was laid out in Randomized Block Design (RBD) with three replications. There were thirteen treatments viz. T_1 (IBA 0ppm + soil), T_2 (IBA 500ppm + soil), T_3 (IBA 500ppm + soil + vermicompost), T_4 (IBA 500ppm + soil + sand + vermicompost), T_5 (IBA 1000ppm + soil), T_6 (IBA 1000ppm + soil + vermicompost), T_7 (IBA 1000ppm + soil + sand + vermicompost), T_8 (IBA 1500ppm + soil), T_9 (IBA 1500ppm + soil + vermicompost), T_{10} (IBA 1500ppm + soil + sand + vermicompost), T_{11} (IBA 2000ppm + soil), T_{12} (IBA 2000ppm + soil + vermicompost) and T_{13} (IBA 2000ppm + soil + sand + vermicompost) tried to study their effect on rooting and growth of air layers in *Kagzi* lime. The effect of different treatments on different rooting parameters (callus formation, number of primary and secondary roots, length of primary and secondary roots, diameter of primary roots and secondary roots, fresh and dry weight of roots and rooting percentage), growth parameters (number of branches and leaves per air layer at 30, 60 and 90 days after transplanting and survival percentage of air layers) were studied. The salient findings of this research work have been presented in the chapter and discussed in detail as under. In this chapter an attempt has been made to evaluate and compare the results obtained with the findings of earlier workers.

Since the discovery of auxins various workers have used them to initiate roots in air layers or cuttings of different fruit trees with varying degree of success. In the present trial IBA in four concentrations along with three rooting media level was tried to study their effect on the rooting and growth behavior of lime air layers. Results presented in previous chapter was clearly indicated that all the roots and growth characters increased with the increase in concentration of IBA and higher concentration of IBA gave better results as compare to lower concentrations. This could be because of the adequate quantity of auxin for cambial activity is necessary for initiation of root primordial, therefore the highest performance was seen at higher concentration of IBA (2000ppm), which is supported by Eganathan *et al.*(2000) .The enhanced expression of root primordial and profuse development of root system as observed in the present investigation could be due to synergistic mode of action between auxins and other constituents in plant tissues control organ formation (Cooper, 1940). Besides the application of hormone, the other reason which resulted in present observations could be an accumulation of certain chemical substances (carbohydrates, rooting co-factors etc.) at the base of cut which might have stimulated the meristem to divide quickly and produce roots (Kumar and Syamal, 2005). The mobilization and utilization of carbohydrate and nitrogen fraction in the presence of cofactor at wounding portion might have helped in better root initiation, root number and root length (Pandey *et al.*, 2003). The higher percentage of callus formation may be due to synergistic effect of the plant growth regulators controlling the initiation of root primordial and development of profuse root system as it is a well-known phenomenon that callus formation in air-layered twigs is the first apparent system of the auxin adenine balance. It arises from cells in the region of the vascular cambium and adjustment phloem. Increased formation of more developed roots and vigorous growth in treated air layers may be because of increased accumulation of auxin at the base as opined by Cooper (1940). It seems that endogenous auxin content of the branches was insufficient to differentiate the rooting primordial and the exogenous application of growth regulators enhanced the development of callus cells and thereafter root initiation which ultimately helped in better root development as well as growth reported by Gautheret (1969), Oliman *et al.* (1971) and Haising (1971).

The beneficial effect on root growth parameters due to improved soil texture, structure, porosity, water holding capacity, activity of useful soil micro flora and fauna, maintained soil temperature and improved soil health and nutrient status of medium (Bhojvaid and Negi, 2003). Further the rooting media of vermicompost also provides close contact between air layer and media increases steady moisture supply facilitates root respiration and encourages overall root growth; the best choice for rooting is a media with low capacity of water retention (Tyagi and Kumar, 2006).

Among the different rooting media, combination of soil + sand + vermicompost was superior over soil + vermicompst and soil alone. The promising effect of soil + sand + vermicompost (1:1:1) on rooting and growth parameters might be due to perfect combination of soil, sand and vermicompost because, vemicompost is a main source of organic matter, supplying essential nutrients and minerals needed by the plants. Soil has basic requirements of plants having nutrients retention and good water holding capacity. Sand increased the porosity of the media, so that permitting adequate moisture and proper aeration to the roots, it helps to rapid callus formation and rooting (Kumar and Sharma, 2007; Bisen, 2010; Rymbai and Reddy, 2010; Kumar *et al.*, 2015).

A critical examination of data on root and growth characters (Table 4.1 to 4.15) revealed that different doses of IBA and rooting media significantly affect the rooting and growth parameters of air layer of lime cv. Kagzi Lime. The T_{13} (IBA 2000ppm + soil + sand + vermicompost) was recorded maximum callus formation (0.97 cm), number of primary roots (24.58) and secondary roots (52.31), length of primary roots (5.98 cm) and secondary roots (1.21 cm), diameter of primary roots (2.21 mm) and secondary roots (0.73 mm), fresh weight of roots (4.21g) and dry weight of roots (1.87g), rooting percentage (92.17%); number of branches (3.21, 7.28 and 13.42) and number of leaves (15.21, 25.54 and 38.52), at 30, 60 and 90 days after transplanting and survival percentage (90.17) followed by T_{12} (IBA 2000ppm + soil + vermicopmost) for callus formation, length of primary and secondary roots, diameter of secondary roots, rooting and survival percentage, number of branches at 30,60 and 90 DAT and number of leaves at 60 and 90 DAT; whereas, followed by T_{10} (IBA 1500ppm + soil + sand + vermicompost) for number of primary and secondary roots, diameter of primary roots, fresh and dry weight of roots, and number of leaves at 30 DAT. Similar results were obtained by Kumar *et al.* (2001) in custard apple, Kanpure *et al.* (2015) in lime, Kumar *et al.* (2015) and Singh *et al.* (2015) in lemon, Parmar *et al.* (2018) in guava.

However, no significant difference was found between both follower treatments (T_{12} and T_{10}) for all rooting and growth characters except length of secondary roots and dry weight of roots. Results also indicated that T_{13} was significantly at par with T_{12} and T_{12} also at par with T_{10} for success in rooting and survival percentage.

The different concentrations of IBA and various rooting media gave an advantageous response in rooting and growth of air layers in lime. Such types of observation might be due to activation of auxin in vegetative parts by using auxin like IBA. Optimum level of IBA accelerated cell elongation, cell division and proliferation of callus resulted highly rooting and growth (kumar, 1992; Jan *et al.*, 2003). Similarly, the media like soil + sand + vermicompost (1:1:1) provided good congenial condition for penetration of roots in rooting media helps early callusing and rooting as well as excellent growth of roots in air layers. Similarly, vermicompost favoured the vegetative growth due to it has major and micro nutrients in rooting media. So that it favoured number of shoots and number of leaves per cutting (Tyagi and Kumar, 2006; Kumar and Sharma, 2007 and Kumar *et al.*, 2015).

Economics of the treatments

The T_{13} (IBA 2000ppm + soil + sand + vermicompost) was secured the highest net return (₹2493) and benefit cost ratio (5.24) followed by T_{12} (IBA 2000ppm + soil + vermicompost) for net return (₹1737.47/-) and T_{10} (IBA 1500ppm + soil + sand + vermicompost) for benefit cost ratio (4.77) due to higher success percentage of air layers under these treatments as compared to others. It might be due to higher concentration of IBA along with suitable rooting media increased the healthy and more root initiation leads to higher rooting percentage, vigorous growth of plant and more survival percentage. Similar results also reported by Reddy *et al.* (2014), Mali *et al.* (2015) and Soni *et al.* (2015).

CHAPTER – VI

SUMMARY, CONCLUSION AND SUGGESTIONS FOR FURTHER WORK

The present investigation entitled **"Effect of different concentration of IBA and rooting media on callusing and survival of air layering in acid lime (*Citrus aurantifolia* Swingle)"** was carried out at University orchard, Department of Horticulture, College of Agriculture, RVSKVV, Gwalior during the rainy season of 2018-19 under Gird agro-climatic and soil conditions of Northern Madhya Pradesh. The experiment was laid out in Randomized Block Design with three replications. There were thirteen treatments *viz.*, T_1 (IBA 0ppm + soil), T_2 (IBA 500ppm + soil), T_3 (IBA 500ppm + soil + vermicompost), T_4 (IBA 500ppm + soil + sand + vermicompost), T_5 (IBA 1000ppm + soil), T_6 (IBA 1000ppm + soil + vermicompost), T_7 (IBA 1000ppm + soil + sand + vermicompost), T_8 (IBA 1500ppm + soil), T_9 (IBA 1500ppm + soil + vermicompost), T_{10} (IBA 1500ppm + soil + sand + vermicompost), T_{11} (IBA 2000ppm + soil), T_{12} (IBA 2000ppm + soil + vermicompost) and T_{13} (IBA 2000ppm + soil + sand + vermicompost) tried to study their effect on rooting and growth of air layering in Kagzi lime. The 7 years old 39 plants of lime cv. Kagzi Lime (13 plants under each replication) of uniform size and vigour were selected for air layering. 20 well-matured and healthy branches having pencil's size thickness were selected on every plant. 260 branches were layered on 13 lime plants under each replication. Thus the total 780 branches were layered under the 3 replications. The observations were recorded mainly on rooting characters *viz.* callus formation (cm), number of primary and secondary roots, length of primary and secondary roots, diameter of primary and secondary roots, fresh and dry weight of roots and success in rooting percentage; and growth characters *viz.* number of branches and leaves and survival percentage. The data of final observations of root and growth characters were statistically analyzed. The salient findings of the investigation are summarized below:

1. Mean sum of squares due to treatments were found significant for all rooting and growth characters over control.
2. All the rooting and growth characters increased with the increase in concentration of IBA.
3. Higher concentration of IBA (2000ppm) performed better in respect of rooting and growth characters over lower concentrations.
4. Rooting media consisting soil + sand + vermicompost was found most suitable for rooting and growth over rooting media consist soil + vermicompost as well as soil alone.
5. The maximum callus formation (0.67 cm), number of primary (24.58) and secondary (52.31) roots, length of primary (5.98 cm) and secondary (1.21 cm) roots, diameter of primary (2.21 mm) and secondary (0.73 mm) roots, fresh (4.21g) and dry (1.87g) weight of roots per air layer, success in rooting (92.17), number of branches (3.21, 7.28 and 13.42) and number of leaves (15.12, 25.54 and 38.52) at 30, 60 and 90 days after transplanting and survival percentage of air layers (90.17) were recorded with T_{13} (IBA 2000ppm + soil + sand + vermicompost) followed by T_{12} and T_{10}.
6. There was no significant difference found between both follower treatments (T_{12} and T_{10}) for all rooting and growth characters except length of secondary roots and dry weight of roots.
7. Results also indicated that T_{13} was significantly at par with T_{12} and T_{12} also at par with T_{10} for success in rooting and survival percentage.

Economics of the treatments

Application of IBA @ 2000ppm + soil + sand + vermicompost secured the highest net profit along with benefit cost ratio (₹2453/- and 5.24) followed by T_{12}

(IBA 2000ppm + soil + vermicompost) for net return (₹1737.47/-) and T_{10} (IBA 1500ppm + soil + sand + vermicompost) for benefit cost ratio (4.77).

Conclusion

Amongst the treatments used, treatment combination of IBA 2000ppm + soil + sand + vermicompost was found to be the most suitable for increasing the callusing, rooting, growth and survival of air layers of lime and ultimately the higher net profit and benefit cost ratio.

Suggestions for further work

1. In order to confirm the validity of results the experiment must be repeated over years and location.
2. Higher concentration of the growth regulator gave the better results for rooting and growth characters. Thus, the increased dose of the plant growth regulator should also be tested during further investigation.
3. Study on more rooting media combinations should be included.
4. The different coloured polythene may also be tested as wrappers

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APPENDIX – I

Analysis of variance for different characters in lemon air layers (mean sum of squares)

Source of variance	D.F.	Callus formation (cm)	Number of primary roots	Length of primary roots (cm)
Replications	2	0.001	3.395	0.015
Treatment	8	0.050**	60.054**	3.097**
Error	16	0.001	1.490	0.082

Source of variance	D.F.	Diameter of primary roots (mm)	Number of secondary roots	Length of secondary roots (cm)
Replications	2	0.005	0.850	0.005
Treatment	8	0.477**	203.723**	0.112**
Error	16	0.001	4.189	0.001

Source of variance	D.F.	Diameter of secondary roots (mm)	Fresh weight of roots (g)	Dry weight of roots (g)
Replications	2	0.001	0.005	0.020
Treatment	8	0.043**	1.842**	0.382**
Error	16	2.430	0.023	0.008

Source of variance	D.F.	Success in rooting (%)	Survival percentage
Replications	2	4.795	35.005
Treatment	8	302.799**	287.493**
Error	16	14.495	14.066

APPENDIX – II

Analysis of variance for different characters in lemon air layers (mean sum of squares)

Source of variance	D.F.	Average number of branches		
		30 DAT	60 DAT	90 DAT
Replications	2	0.010	0.065	1.080
Treatment	8	0.703**	5.813**	16.970**
Error	16	0.015	0.007	0.314

Source of variance	D.F.	Average number of leaves		
		30 DAT	60 DAT	90 DAT
Replications	2	0.230	1.670	5.065
Treatment	8	25.639**	63.784**	130.664**
Error	16	0.597	1.481	2.205

VITA

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