STANDARDISATION OF MACRO AND MICRO PROPAGATION TECHNIQUES IN BOUGAINVILLEA

BY

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THESIS

submitted in partial fulfilment of

the requirement for the degree

Master of Science in Horticulture

Faculty of Agriculture

Kerala Agricultural University

Department of Pomology and Floriculture

COLLEGE OF HORTICULTURE

Vellanikkara - Trichur

KERALA — INDIA

1985

In the name of Allah, the Beneficent, the Merciful & the Owner of the Day of Judgement

DECLARATION

I hereby declare that this thesis entitled "Standardisation of macro and micropropagation techniques in bougainvillea" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Certified that this thesis entitled "Standardisation of macro and micropropagation techniques in bougainvillea" is a record of research work done independently by Miss. Aishabi, K.A. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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ACKNOWLEDGEMENTS

I would like to unveil my deepest sense of gratitude to Dr.M. Aravindakshan, Director for Centre of Advanced Studies on Tree Crops & Environmental Horticulture and also Chairman of my Advisory Committee, for his masterly guidance and encouragement, constructive criticism and logic conclusions throughout the course of this work and preparation of the thesis.

It is my previlege to thank Dr.K.M.N. Namboodiri, Professor and Head, Department of Agrl.Botany, Shri.S. Balakrishnan, Professor, Multistate Cashew Project, Madakkathara and Dr.K.Gopikumar, Assistant Professor, AICFIP, Vellanikkara, members of my Advisory Committee, for all the help rendered to me in the preparation of this manuscript.

No less is the help I received from Sri.K. Rajmohan, Assistant Professor, KADP, and I avail myself of this opportunity to place on record my heartfelt thanks, for the indispensable help, keen interest and valuable suggestions, for the successful completion of the work especially in tissue culture studies.

Grateful acknowledgements of assistance is offered to Shri.V.K.G.Unnithan, Professor, Department of Agrl.Statistics for his guidance in statistical analysis of the data.

I owe my gratitude to the staff members of the Department of Pomology and AICFIP for their sincere help throughout the conduct of the study. My profound sense of gratitude is also extended to Dr.S. Ramachandran Nair, Professor and former Head of Department of Plantation Crops and Spices, who has been generous in his support and permitted me to make use of the facilities in the tissue culture laboratory.

I express my particular thanks to Dr.P.K.Gopalakrishnan Associate Dean, College of Horticulture, for providing me prompt administrative help throughout the completion of my course.

I wish to acknowledge with gratitude the Award of research fellowship by the Kerala Agricultural University during the tenure of my M.Sc.programme.

I also place on record my great love and affection to my parents, whose whole hearted co-operation untiring support and constant encouragement helped me for the successful completion of the postgraduate course.

Aswathy

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INTRODUCTION

INTRODUCTION

Bougainvillea, named after a French botanist, Louise Antoine de Bougainville is native of South America (Pal and Vishnuswarup, 1974) and was introduced to India a century ago. With its spectacular mass of lovely and colourful sprays of bracts, bougainvillea is one among the tropical ornamental plants noted for unrivalled beauty as well as utility.

Bougainvilleas are mainly propagated through cuttings although other methods of asexual propagation such as layering and to a limited extent budding are in vogue when cuttings fail to strike roots. Even though propagation by cuttings combines the several advantages like simplicity in operation, in expensiveness and rapidity, it poses problem in the case of several varieties particularly mutants.

Horticulturists and nurserymen have resorted to the application of growth regulators for enhancing rooting of cuttings. The exact concentration and the type of growth regulators to be used, have to be decided by detailed experimentation in a particular agroclimatic situation. Such detailed investigation in bougainvillea has not been carried out in our state.

Plant propagation via tissue culture synonymously called as micropropagation is a technique which is

increasingly becoming popular for the rapid clonal multiplication of difficult-to-root plant species. As a useful technique it became available by about 1939 with the successful research work of 'Gautheret' in France and 'White' in United States. A considerable amount of information has accumulated on plant tissue and cell culture since then. At present it is being resorted to in conjunction with the conventional propagation techniques for the commercial production of clones. Where the latter method allows only a slow rate of propagation or is impossible in some plants a million fold increase in the planting stocks could be achieved through tissue culture.

Bougainvillea is an ornamental plant in great demand throughout the country. Kerala with its unique agroclimatic features, has distinct possibilities of large scale production of planting materials of this plant species. Standardisation of macro and micropropagation procedures was therefore considered necessary so as to provide the basic technical know how for such propagation works. Studies in tissue culture is in its initial stages in the tissue culture laboratory of College of Horticulture and it was also felt that bougainvillea will provide a useful material for taking up preliminary studies in tissue culture. The present studies were taken up with the following objectives.

(i) To assess the rooting potential of different varieties of bougainvillea through cuttings under field conditions and to group them as easy rooting, poor rooting and difficult rooting.

(ii) To find out the effect of different growth regulators on rooting of cuttings.

(iii) To standardise media, method and materials suited for tissue culture.

REVIEW OF LITERATUE

REVIEW OF LITERATURE

I. Macropropagation (cuttings)

Cuttings can be made from almost all parts of the plant including stem, modified stem, root and even leaves. Among these, stem cuttings are more commonly used for planting. Adventitious root formation in stem cuttings are often governed by several factors. Literature relevant to these aspects are reviewed here.

2.1. Season of collection of cuttings

Season of collection of cuttings has been reported to be one of the key factors affecting rooting of cuttings in many plants. Yadav et al. (1977) stressed the effect of season on rooting percentage, number of roots/cutting and root length in bougainvillea. They conducted a trial in West Bengal, using hard wood cuttings from 10 year old bougainvillea cv. 'Mahara'. The cuttings collected at monthly intervals from February to September, were planted in well prepared nursery beds. Maximum (90 per cent) and minimum (42.5 per cent) percentage of rooting was obtained when the cuttings were planted on 15th August and 15th September respectively. Cuttings planted during July also recorded a high percentage of rooting. They concluded that rooting response of bougainvillea cuttings was high during rainy season. Singh and Motilal (1979) on the other hand obtained maximum percentage of rooting upto 65 per cent

during February in the soft wood cuttings of bougainvillea cv. 'Thimma'.

Nambisan et al. (1977) observed better rooting in semihard wood cuttings of oleander (Nerium indicum) when planted during July-August. During this period, the number of roots and length of roots produced were also high.

Singh (1980) obtained highest rooting of cuttings in Ixora banduca during October and poorest in January, regardless of treatments with indole butyric acid (IBA), indole acetic acid (IAA) and Naphthalene acetic acid (NAA). He found that the lowest (1000 ppm) and highest (6000 ppm) concentrations of IBA and IAA tried were ineffective during all the months of planting, while NAA 1000 ppm was not effective during October, November and January, a concentration more than 1000 ppm was found beneficial in rooting of cuttings during December.

2.2. Physiological stage of cuttings

2.2.1. Type of cuttings

In several plants cuttings are either taken from vegetative or flowering shoots, although rooting varied in these two types. Hartmann and Kester (1975) however, concluded that the difference in rooting potential of these two types of shoots was more noticeable in difficult to root species. Bose et al.(1975) obtained 100 per cent

rooting when semihard wood cuttings were used for planting in the cvs. 'Mahara' and 'Million Dollars' of bougainvillea. Singh and Rathore (1977) compared the rooting efficiency of hardwood cuttings and semihard wood cuttings of bougainvillea cvs. 'Thimma' and 'Mary Palmer' and noticed that all softwood cuttings rooted but had only the lowest survival rate (48 per cent) against 83 per cent rooting and 54 per cent survival in hardwood cuttings. But singh and Motilal (1979) obtained best rooting and highest survival with softwood cuttings of bougainvillea cv.'Thimma' compared to semihard wood cuttings.

Porlinguis and Therios (1976) observed that rooting percentage of juvenile cuttings in olive remained high and relatively constant throughout the growing period, except for slight reduction during autumn season, while a wide seasonal variation occured in the rooting efficiency of adult cuttings. They also found that the juvenile cuttings rooted faster than the adult ones.

2.3. Position of the cuttings

Seetharama and Mohanaram (1972) found that the basal cuttings of bougainvillea proved superior to median and tip cuttings. They obtained 20 per cent more rooting in the basal cuttings. But, the superiority of apical cuttings in rooting was reported in lilac by Schmidt (1978); and in pelargonium by Kumar et al.(1980). Beel and Schelstraete

(1981) noticed a similar trend in two species of bougainvillea viz. B. glabra cv. 'Alexandra' and B. spectabilis. However, Shailendrarajan and Santaram (1982) obtained no rooting of terminal soft and basal hardwood cuttings in aonla and hence they suggested the use of median cuttings for its propagation.

2.4. Growth regulator treatment

2.4.1. Type of growth regulators

The discovery of naturally occurring auxin viz. indole acetic acid (IAA) and synthetic auxins viz. indole butyric acid (IBA) was a milestone in the history of propagation and was of real value in stimulating the production of adventitious roots in stem and leaf cuttings (Linder, 1939). For rooting of cuttings, most commonly used growth regulators are IAA, IBA and NAA (Audus, 1959). Besides these, auxins like 2,4-dichlorophenoxy acetic acid (2,4-D) and various commercial preparations like 'rooton', 'hormodin', 'rhizopon' and 'seradix' are also used to a lesser extent.

Mukhopadhya and Bose (1966) treated cuttings taken from one year old shoots of four varieties of bougainvillea viz. 'Partha', 'Scarlet Queen', 'Mary Palmer' and 'H.C.Buck' with IBA and NAA each at 10 and 100 ppm concentration and with a commercial product 'seradix B_3'. They found that

auxin treatment resulted in better rooting of all varieties and that IBA was significantly superior to NAA. They further noticed that varieties responded differently to growth regulator treatments. The variety 'Mary Palmer' showed no encouraging results with growth regulators other than IBA 10 ppm and NAA 100 ppm in which case also, induced rooting was only limited to few cuttings. Kale and Bhujbal (1972) emphasised the beneficial effect of IBA particularly at 500 ppm on the number and length of roots in bougainvillea cv. 'Mary Palmer'. Seetharama and Mohanram (1972) suggested that synthetic auxins like NAA and IBA were more effective in inducing rooting in bougainvillea cuttings when compared to natural auxin - IAA. They used IAA and IBA each at 10 and 20 ppm concentration and NAA at 10, 20 and 50 ppm, in five cultivars of bougainvillea viz. 'Mary Palmer', 'Partha', 'Mrs. Bhutt', 'H.C. Buck' and 'Enidlancaster'. Best results in terms of percentage of rooting were achieved with 50 ppm NAA and 20 ppm IBA. In all the varieties except 'Partha', auxin induced roots were longer.

The rooting potential and subsequent survival of four noded cuttings of bougainvillea cv. 'Mary Palmer' was studied by Gandothra et al. (1975) using IAA, NAA and IBA each at 6000, 8000 and 10,000 ppm concentrations. They found that IBA was the most efficacious, which recorded maximum percentage of rooting upto 70 per cent. All growth

regulators at higher concentrations were found toxic and suppressed rooting. Philip and Gopalakrishnan (1982) obtained maximum percentage of rooted cuttings (100 per cent) with 6000 ppm IBA treatment in bougainvillea cv. 'Mahara' followed by NAA at 2000 ppm concentration (86.7 per cent).

In Althea rosea (Hollyhock), lower concentrations of IBA and NAA (400 ppm) were found better in inducing roots in air layers, compared to higher concentrations of 2000 and 3000 ppm (Lingaraj, 1960). A series of experiments conducted by Kwak and Chung (1980) in 25 species of ornamental plants to standardise the optimum concentration of NAA and the best time for taking cuttings, showed that in many of the species, NAA was found better than IBA in promoting rooting.

2.4.2. Mode of treatment of growth regulators

Audus (1959) concluded that growth regulators could be applied in various forms such as powder, concentrated dip, dilute solution dip (soaking) or as paste and the effectiveness of these treatments often varied with plant species.

Williams (1943) experimented with the cuttings of certain broad leaved evergreens using commercial hormone preparations such as 'hormodin' and 'rooton' by soaking and dusting method to promote rooting of cuttings. The results indicated a higher percentage of rooting in solution treated

cuttings, but more number of roots were observed on dust treated cuttings. A significant but negative interaction was noticed in plum cuttings between duration of treatments and the concentration of growth regulators by Nahalawi and Howard (1972).

Leafy cuttings of Saintpaulia ionantha were treated with 1 to 1000 ppm solutions of IBA for 1 to 24 hours by Woszszynska and Borys (1976) for induction of rooting and they found that the effect of IBA increased with the concentration and maximum beneficial effect was obtained with 1000 ppm solution, when the cuttings were soaked for 15 to 24 hours. Kwack and Chung (1980) also obtained similar results in ornamental plants like rose and ligustrum. They also observed that slow dip for 24 hours was more effective than quick dip or powder treatment.

2.5. Rooting medium

Cuttings of many plant species root easily on a wide range of rooting media. Long (1932) observed that the cuttings of difficult to root plants are influenced by the rooting medium, not only in their percentage of rooting but also in the type of root system formed. The other media generally recommended are sand, peatmoss and water (Zimmerman, 1930, Smith, 1944). Vermiculite and sphagnum moss also have been found to be successful media in many plant species (Chadwick, 1949 and Creech et al. 1955).

Hartmann and Kester (1975) reported that the rooting medium affected the type of root system produced. They found that cuttings of several plant species when rooted in sand, produced only long, coarse and brittle roots but when rooted in sand plus peatmoss, the roots were slender and flexible.

According to Higaki (1981), single node cuttings of Dracaena goldeana rooted equally well in peatmoss, perlite, black cinder, or vermiculite under intermittent mist condition. Pokamy and Austin (1982) observed that softwood cuttings of blue berry rooted best in a medium containing milled pine bark alone or mixed with equal parts of perlite compared to sphagnum moss alone or mixed with perlite.

2.6. Carbohydrate : nitrogen relation

There are considerable evidences in literature that the nutrition of stock plants influences to a great extent, their root and shoot development. Kraus and Kraybill as quoted by Hartmann and Kester (1975) reported that tomato cuttings with a relatively high content of carbohydrate and low nitrogen produced many roots but only feeble shoots, while those shoots with green stem having high nitrogen and ample carbohydrate produced fewer number of roots but stronger shoots.

Exogenous application of auxins such as IAA, IBA and NAA was found to increase the rooting response of Justicea gendarussa cuttings from stock plants grown in the presence of low amount of nitrogen by Basu and Ghosh (1974). They noticed that low nitrogen supply to the stock plant was associated with a root promoting effect and that high C/N ratio increased the rooting co-factor activity in tissues of the cuttings. Rooting co-factor activity was found to be inversely related to nitrogen supply and it was highest under low nitrogen levels.

2.7. Environmental factors

The deleterious effects of extra light on rooting of cuttings in Hibiscus rosa-sinensis and H. schizopetalus was demonstrated by Kachecheba (1976). Johnson and Hamilton (1977) also confirmed this in the subsequent trials with H. rosa-sinensis cuttings.

Singh and Rathore (1977) could get no rooting in the cuttings of bougainvillea cvs. 'Thimma' and 'Mary Palmer', when planted under open condition while 91 per cent rooting was obtained under partial shade.

The advantage of less shade on better rooting in Ixora was reported by Singh (1980) under North Indian condition who found that reduced light intensity was a

limiting factor to better rooting. He attributed the poorest rooting of cuttings in January to the reduced light intensity.

Experiments conducted by Senanayake and Kirthisingh (1983) in black pepper cuttings revealed that 50 per cent shade and irrigation once in three days gave maximum number of roots with highest dry weight of shoots and roots.

II. Micropropagation

A beginning of plant propagation through tissue culture was made in early 20th Century, when Haberlandt (1902) first attempted to regenerate plants from single cells. The first prolonged culture of unorganised plant tissue was however, reported only much later by Gautheret (1939) in carrot and by White (1939) in tobacco.

Thorpe (1981) defined the term plant tissue culture as the cultivation *in vitro*, of plant parts whether a single cell, tissue or organ under aseptic conditions.

Plant propagation by tissue culture synonymously called as 'micropropagation' was stimulated by the tremendous success in orchid propagation, through this method (Holdgate, 1977). In recent years, this technique has developed into very powerful tool for propagation of

many ornamental species. Since the literature pertaining to the micropropagation of bougainvillea are scanty, those concerning with other ornamental and fruit plants are briefly reviewed here under.

Herbaceous ornamentals have been reported to be relatively easy to propagate via tissue culture and micropropagation of many herbaceous species have been successfully accomplished (Skirvin and Janick, 1976; Anderson, 1977; Hussey, 1978). But in vitro regeneration have been found difficult with certain species, particularly woody ornamentals and fruit plants (Murashige, 1974; Hughes, 1981). However recent works by Abbot and Whiteley, 1976; Zimmerman, 1978; Hammerschlag, 1980 and Krul and Meyerson, 1980 suggested that woody species are also amenable for in vitro culture and offers potential for large scale multiplication.

Murashige (1974) reviewed the works on tissue culture techniques and reported that rapid asexual proliferation could be obtained from sterile cultures of organs and tissues, in different ways viz. through induction of adventitous shoots or embryos direct on organ explant or on callus, precocious axillary shoot proliferation.

2.8. Callus formation

Callus is an undifferentiated and unorganised mass of parenchymatous cells formed from a wound. Plant cells have the potential to differentiate on division and develop organs and complete plants (Murashige, 1974). Evidence for the morphogenetic activity in callus cultures of many plant species was reported by several workers (Rao *et al.* 1976; Bott, 1980; Sutter and Laghans, 1981). The successful induction of morphogenesis in plant cells and tissue cultures has been found to be influenced by several factors, such as explant sources, media, its components and culture conditions (Murashige, 1974 and Hughes, 1981).

2.8.1. Explant sources

An explant is a piece of tissue or organ which is excised from the plant for the purpose of culture. Almost any part of a plant could be induced to produce callus or suspension culture. But, the success in culturing the explant is influenced by a number of factors inherent to the explant, such as source of the explant, its size and physiological age, which has been clearly amplified in the studies conducted by Hughes (1981).

Within any plant, the tissue differed in their ability to undergo morphogenesis. Most viable explant undergo mitosis *in vitro* and established callus from the explants consisting of shoot tips and isolated meristems.

These two sources containing mitotically active cells has been found especially successful for callus initiation and subsequent regeneration (Murashige, 1974). Establishment of callus and subsequent regeneration was reported from almost all explant sources including isolated stem pieces, leaf, flower buds, anthers and corm segments in Freesia (Bajaj and Pierik, 1974), petiole segments in begonia (Fonnesbech, 1974), young embryos in anthurium (Pierik et al. 1974), inflorescence in hyacinth (Hussey, 1975), shoot tips in chrysanthemum (Bush et al. 1976), petals in hemerocallis (Heuser and Apps, 1976) and internodal segments in English ivy (Awad and Banks, 1981).

2.8.2. Size

Size of the explant has been found to be critical in tissue culture. In their studies with carnation, Gukasyan et al.(1977) found that shoot apices measuring more than 1 mm were unable to develop, while those having 0.35 mm size produced the largest number of normal shoots. They also found that the presence of sub apical tissues again reduced the survival and shoot production.

Hughes (1981) reported that very small explants in general, had a very low survival rate when compared to large explants. Evans et al.(1981) opined that only a small

percentage of the cells in a given explant contributed to the formation of callus and the increased cell number present in large explants increased the chance for greater survival rate. According to them smaller explants were more likely to form callus, while larger explants maintained greater morphogenetic potential.

2.8.3. Physiological age

Pierik et al.(1974) reported that physiological age of the explant influenced the type and extent of morphogenesis. They found that in anthurium, callus could be produced from the youngest stem segments and embryos. In English ivy, Banks et al.(1979) found that embryos, juvenile tissues and embryo organs had a high regenerative capacity and they concluded that culture of the youngest and less differentiated tissues were successful in a wide range of species.

2.8.4. Medium and its components

The importance of the composition of the medium in growth and morphogenesis of plant tissues in culture is well established (Murashige, 1974; Conger, 1981 and Flick et al.1983). Though several media such as Murashige and Skoog (1962), White (1963), Gamborg et al.(1968) or (B_5 medium) and Nitsch (1969) are in use, the first one is the widely accepted one for plant morphogenesis.

The essential ingredients of the culture medium developed by Murashige (1974) includes inorganic and organic compounds like carbohydrates, vitamins, aminoacids, nitrogen bases and phytohormones. He also stated that other additives like endosperm fluids, fruit juices etc, though not essential, were often used when all other components failed to produce regeneration or to enhance the developmental process.

2.8.4a. Effect of phytohormones

The proportion of phytohormones like auxin and cytokinins in the culture medium has been found to be critical to the control of growth and morphogenesis by Skoog and Miller (1957) in tobacco cultures. According to them, a relatively high content of auxin favoured root initiation suppressing shoot formation. On the contrary, high concentration of cytokinin induced shoot formation and suppressed rooting. Murashige (1974) observed that a variety of auxins including IAA, NAA, IBA and 2,4-D were used either alone or in combination, but among these auxins, IAA was the weakest, but showed minimum harmful effect on explant tissue. 2,4-D was the most potent and it stimulated callus cultures. Among the various cytokinins like zeatin, kinetin, Benzyl aminopurine, the latter is more commonly used.

No universal ratio of auxin and cytokinin has so far been developed for root and shoot induction. However, Hempel (1979) concluded that in majority of cases, callus growth was best on auxin and cytokinin containing medium, though some bulbous plants like lilium and hyacinth regenerated *in vitro* without the presence of any phytohormones. Moreover, application of auxins and cytokinins hastened the development of callus. He further noticed that considerable variability exsisted between taxa and genotype in the optimum requirement of phytohormones for morphogenesis. The optimum growth regulator combination for callus development was found to be 53.7 μM NAA + 11.6 μM KIN for cyclamen (Loewenberg, 1969), 20 μM NAA + 20 μM KIN in poinsettia (De Langhe *et al.* 1974), 0.5 μM NAA + 2.2 μM BA in begonia (Fonnesbech, 1974), 5.4 μM NAA in geranium (Skirvim and Janick, 1976), 21.8 μM NAA + 9.3 μM KIN in English ivy (Banks *et al.* 1979).

Sharma *et al.* (1981) obtained good callus formation in the shoot apices of *Bougainvillea glabra* cv. 'Magnifica' when grown on MS medium supplemented with 0.4 ppm 2,4,5-T and 0.8 to 1.0 ppm kinetin (KIN). At higher concentration of both BA and KIN (1.0 ppm), the whole explant callused.

2.8.4b. Effect of organic compounds

Skoog and Tsui (1968) reported that L.glutamine and L.asparagine had beneficial effect on the cultures of tobacco. They also noticed that incorporation of adenine favoured callus growth.

According to Murashige (1974), a variety of extracts could be used in the medium including casein hydrolysate, yeast extract, coconut milk and other purine derivatives such as adenine and adenine sulphate.

Street (1979) found that in Pelargonium hortorum, addition of myo-inositol at 50 and 100 mg/l promoted growth and in Fraxinus pennsylvanica, it was absolutely essential for callus growth.

2.9. In vitro shoot multiplication

Regeneration of shoots can be either directly from the explant or through callus mediated process. As far as clonal multiplication is the aim, the former is the most efficient and rapid method of in vitro regeneration (Hussey, 1978).

Fonnesbech (1974) stressed the importance of using optimum concentration of phytohormones particularly cytokinins for getting best response. In begonia, he

observed that only higher concentrations of benzyl amino purine (BA/BAP) yielded shoots, but no shoot growth was noticed in presence of lower concentration of BA.

Thakur and Ganapathy (1977) obtained shoot differentiation from leaf explants of Begonia picta on Nitsch medium supplemented with BAP or KIN at 10^{-7} to 10^{-4} mole concentration. They also noticed that when the basal medium was supplemented with the two cytokinins, shoot differentiation occurred from both the surfaces of the laminar tissue, where as shoot buds differentiated from the adaxial surface only, on the basal medium.

Though shoot proliferation has been reported to occur in the presence of higher concentration of cytokinins, Welander (1977) found that it could occur in the presence of lower concentrations of cytokinin also. In Begonia x hiemalis, he noticed shoot formation only when NAA/BA ratio of 2:1 was maintained. A similar pattern of development was noticed in African violet by Bilkey et al.(1978), where plantlets were formed on MS medium containing NAA 0.1 mg/l and BA 0.01 mg/l.

In Bougainvillea glabra cv. 'Magnifica', Sharma et al. (1981) found that MS medium supplemented with either BAP or KIN at 0.2 to 1.0 mg/l in combination with IAA at 0.1 to 2.0 mg/l was best for initial shoot growth. Shoot apices when

grown on this medium, remained quiescent for two months. Maximum number of shoots upto 10, was produced in the presence of IAA 1.0 mg/l and BA 0.5 mg/l. Lower concentration of IAA and BA, although found to be suitable initially, favoured only slight callusing at earlier stages. Kinetin was found ineffective for shoot multliplication.

In Jasminum nudiflorum and J.officinale shoot proliferation was promoted even in the absence of cytokinin. Khoder at al. (1981) noticed shoot development on half strength MS medium from shoot apices of these two species in the presence of 1.0 mg/l GA_3 and 0.1 mg/l NAA without the addition of BA.

Silberstein et al.(1983) suggested that incorporation of various additives was beneficial for shoot proliferation in Euphorbia species. They noticed a synergistic effect on shoot proliferation, when adenine sulphate was incorporated to the medium in combination with kinetin.

2.10. In vitro rooting

Root formation under aseptic condition in various plant species was found to occur with a wide range of auxins and their concentrations (Rao et al. 1973; Anderson, 1980; Hammerschlag, 1980; Sharma et al. 1981; Hussay, 1982) or even without any growth regulators (Cooke, 1977; Krul and Meyerson, 1980).

Rao *et al.* (1973) opined that NAA was very effective for root formation in stem and leaf explants of *Petunia sp.* but IAA caused only localised root development.

There are several reports concerning the inhibitory effect of BAP on root initiation in explants cultured *in vitro*. Omission of cytokinins from the culture medium was found to favour root development in *in vitro* grown strawberry by Jones and Newton (1977); in blackberry by Broome and Zimmerman (1978). Damiano (1980) also noticed that in strawberry explants, roots were produced after 10 to 12 days of culturing, in the presence of IAA 1.0 mg/l, only when BA and GA_3 were deleted from the medium.

It has been reported that, though root formation is generally favoured by auxins in the medium, it can also occur on medium without the presence of any growth regulator. Krul and Meyerson (1980) reported that shoot explants, when cultured on White's medium devoid of any growth regulator and in presence of chelated iron, rooted readily.

Sharma *et al.* (1981) obtained 100 per cent rooting in the shoot apices of *Bougainvillea glabra* cv. 'Magnifica' when grown on MS medium containing 0.1 mg/l IBA and 0.1 mg/l 2,4,5-T (2,4,5-trichlorophenoxy acetic acid). Profuse rooting in shoot tips occurred in the presence of all the three growth regulators (IAA, IBA and 2,4,5-T) tried.

The favourable effect of lower concentration of salts and of IBA on better rooting of *in vitro* grown explants of *Prunus cerasifera* was demonstrated by Garland and Slotz (1981) and by Rugini and Fontazza (1981) in olive, where rooting of the explants occurred on half strength MS medium containing 0.2 mg/l IBA and 2.0 to 4.0 mg/l NAA respectively. Nuvak and Jujova (1983) also concluded that *in vitro* rooting of grape explants could be readily achieved, when cultured on half strength MS medium with the addition of 0.1 μM/l IBA.

Hammerschlag (1982) found that rooting response of plum explants was significantly higher on IAA containing medium, than on IBA containing one.

Rucker (1982) observed that root formation in *Digitalis purpurea* explants, decreased with low content of nitrogen in the medium compared to the one with high amount of nitrogen.

2.11. Ecological conditions

Hughes (1981) reported that environmental factors such as light, photoperiod and temperature played significant role in the growth of explants grown on a medium.

Murashige and Nakano (1968) found that light was a critical factor for shoot, but inhibited root development. The effect of photoperiod on morphogenesis was established

by Gautheret (1969) in Helianthus and by Pillai and Hildebrandt (1969) in geranium callus. They found that a 12 hour photoperiod was optimal for shoot production from Helianthus tuber sections and 15 to 16 hour photoperiod, for bud induction in geranium callus.

Ramawat and Bhansali (1978) studied the influence of light on callus growth both under light and in darkness. The light grown calli were yellowish green, while they turned soft, fragile and yellowish brown under darkness.

Hammerschlag (1982) attributed the inhibitory effect of light on root initiation of plum explants, to the photo-inactivation of IAA and rooting co-factor. He also observed a significant reduction in rooting under light, compared to that under darkness.

Studies on the influence of culture environment on growth of explants, by Fonnesbech (1974) revealed that temperature had a pronounced effect on morphogenesis. He noticed that a low temperature pretreatment of the petiole explants of Begonia x chiemantha at 15 to 18°C increased the number and size of the shoots produced.

Pierik and Steegman (1975) reported that temperature requirement of the species varied considerably, the optimum

range being at 26 to 28°C in most of the species. They noticed that in rhododendron the best temperature for rooting was 25°C. Seabrook and Cumming (1978) suggested that the temperature requirement of the intact plants might be reflected in the response of the cultured tissues from that plant.

MATERIAL AND METHODS

MATERIAL AND METHODS

The present investigations were carried out in the Department of Pomology and Floriculture, College of Horticulture during 1983-84 with the objective of standardising the propagation techniques in bougainvillea.

The studies consisted of two aspects:

(i) Macropropagation through cuttings and

(ii) Micropropagation.

The following nine varieties from the germplasm collection of bougainvillea under the All India Co-ordinated Floriculture Improvement Project were used for the study. The morphological description of these varieties are given in Table 1.

1. Scarlet Glory
2. Jayalakshmy
3. Enidlancaster
4. Lady Mary Baring
5. Thimma
6. Cherry Blossom
7. Mahara
8. Spring Festival
9. Maharaja of Mysore.

Table 1. Morphological description of bougainvillea varieties tried

Variety	Origin	Plant habit	Flowering	Colour of the bract		Nature of star
				At flowering	Change of colour from young to old stage	
1. Scarlet Glory	Bud sport	Intermediate with medium sized straight thorns	Free and profuse flowering	Tyrian-purple	Fuschia purple, & finally to crimson	prominent
2. Jeyalakshmy	Seedling of 'Meera'	Vigorous and erect with straight and large thorns	Free and profuse flowering	Fuschia purple	Fushia purple	Prominent
3. Enidlancaster	Bud sport from 'Louis Wathen'	Erect with straight large thorns	Free and profuse flowering	Orange	Yellow shaded orange	Prominent
4. Lady Mary Baring	Bud sport of 'Golden Glow'	Vigorous erect with medium sized straight thorns	Profuse flowering with large clusters	Yellowish orange	Yellowish orange	Prominent
5. Thimma	Bud sport of 'Mary Palmer'	Erect and vigorous with beautiful yellow and green variegated leaves	Winter flowering	Magenta and white	Magenta and white	Prominent

Contd...2

Contd. Table 1.

Variety	Origin	Plant habit	Flowering	Colour of the bract		Nature of star
				At flowering	Change of colour from young to old stage	
6. Cherry Blossom	Bud sport of 'Los Banos Beauty'	Erect with small straight thorns	Winter Flowering usually 1/4th from tip	Multibracted white sufficed with grenish tinge	White with mallow purple about 1/3rd from the tip and along the margin	Not prominent
7. Mahara	Bud sport	Vigorous with dropping branches and large straight thorns	Free flowering bracts appearing in large clusters	Multibracted 'rhodamine purple	'rhodamine' purple	Not prominent
8. Spring Festival	Natural hybrid of B.spectablis B.buttiana	Erect with small straight thorns	Free flowering	'Solferino' purple	'Solferino' purple	Prominent
9. Maharaja of Mysore	Natural hybrid of B.spectabilis B.glabra	Vigorous with medium sized straight thorns	Profuse flowering	Cyclamen purple	Cyclamen purple	Prominent

Source Pal & Vishnuswarup, 1974

Expt.I. **Macropropagation**

The rooting efficiency of all the nine varieties with the application of different growth regulators were studied.

3.1. **Experimental details and layout**

Design - Factorial CRD.

Treatments - 11 (2 growth regulators - IBA and NAA each at 5 concentrations and including one control).

T0	- Control	
T1	- IBA 100 ppm	Soaking the basal ends of cuttings for 6 hours.
T2	- IBA 300 ppm	
T3	- IBA 500 ppm	
T4	- IBA 750 ppm	Quick dip of the basal ends of cuttings for 5 seconds.
T5	- IBA 1000 ppm	
T6	- NAA 100 ppm	Soaking the basal ends of cuttings for 6 hours.
T7	- NAA 300 ppm	
T8	- NAA 500 ppm	
T9	- NAA 750 ppm	Quick dip of the basal ends of cuttings for 5 seconds.
T10	- NAA 1000 ppm	

Total number of treatments	-- 99
Total number of plots	-- 99
Gross plot size	-- 1.5 m x 1.5 m
Net plot size	-- 1m x 1m
Total area required for the experiment	-- 222.75 sq. m (5.56 cent).

3.2. Preparation of nursery beds

The area was dug thoroughly to a depth of about 30 cm and all the weeds and stubbles were removed. Thus the soil was brought to a fine tilth. Raised beds of 1.5m x 1.5m x 25.0cm were prepared at a spacing of 30 cm between beds. A fine mixture of sand and well rotten cattle manure (3:1) was evenly spread (5 cm) over these beds before planting.

3.3. Preparation of the cuttings

Hardwood cuttings of about 12 to 15 cm length and 2.0 to 2.5 cm in diameter were collected from the middle portion of mature shoots of the nine varieties of bougainvillea during June 1983 (rainy season) as well as in February 1984 (summer season). Cuttings were prepared in such a way that a flat cut on the top and a slanting cut of 1 to 2 cm long, were made just below a node. The cuttings were tied into bundles of 50 for treating with the growth regulator.

3.4. Preparation of growth regulators

In the preparation of stock solution precipitation was noticed which was over come by the addition of few drops of N/10 Na OH (sodium hydroxide) as advocated by Miller (1963). Chemically pure crystalline salts of IBA and NAA were used for the preparation of growth regulator

solution. The growth regulators were first dissolved in minimum quantity of 0.1 N alkali. Stock solutions of 1000 ppm concentrations of both IBA and NAA were thus prepared by dissolving 1 g of the chemical in 0.1 N Na OH solution and then slowly adding distilled water to make up the volume to one litre. Treatment solutions of the required concentrations were prepared from the stock solution by proper dilution with distilled water. These solutions were used within two days of preparation and kept in a refrigerator whenever not in use.

3.5. Treatment of the cuttings and planting

The cuttings were treated by prolonged dip and quick dip methods. For prolonged dip, the basal 3 to 4 cm of the cuttings were soaked in dilute solutions (100, 300 and 500 ppm) of IBA and NAA for a period of six hours and for quick dip, they were dipped in 750 ppm and 1000 ppm solutions of IBA and NAA for five seconds. Fifty cuttings were used for each treatment. The cuttings were planted on the prepared beds immediately after treatment at a spacing of 10 cm.

To find out the effect of season on rooting of cuttings, the experiment was conducted in two seasons viz. during June, 1983 and during February 1984 with a duration of three months in both cases. During June-September

(Rainy season) the experiment was carried out under open field condition while, during February-May (Summer season) it was conducted in pots in partial shade under irrigated condition. The details of the medium used and method of planting in pots are given under section 3.6.

3.6. Preparation of potting mixture

A rooting medium consisting of one part of red soil, two parts of sand and 1 part of well rotten powdered cattle manure, was prepared by thorough mixing. The pots of size 25 cm x 15 cm were filled with this mixture leaving a space of 10 cm on the top. A layer of sand (5 cm) was evenly spread on the surface, before planting the cuttings collected during February, 1984. Pots were kept under partial shade and watered daily during the course of the experiment which lasted for three months.

3.7. Nursery management

Regular weeding was done during both the seasons without disturbing the cuttings. Being rainy season, no watering was needed during June-September in the case of cuttings planted in nursery beds. The cuttings planted in potswere irrigated daily during summer. BHC 10 per cent dust was applied to the beds/pots against the possible attack of white ants.

3.8. Sampling technique for observations on rooting

Random samples consisting of five cuttings were uprooted from each treatment at fortnightly intervals. The following observations were recorded.

3.9. Percentage of rooting

The number of cuttings rooted were recorded under each treatment and the final rooting percentage was computed.

3.10. Number of roots

The mean number of roots produced by cuttings were recorded at fortnightly intervals.

3.11. Length of roots

The length of all the roots produced on a cutting was measured from the point of emergence and upto the extreme tip. The mean length of roots per cutting was then worked out.

3.12. Fresh weight of shoots

The shoots produced in the cuttings were separated carefully and the mean fresh weight was found out by a chemical balance.

3.13. Number of shoots

The mean number of shoots produced in each of the sprouted cutting was recorded at fortnightly intervals.

3.14. Fresh weight of roots

Roots were removed carefully and washed thoroughly to remove the dirt and soil particles. They were then weighed in a chemical balance to get the fresh weight and the mean value was calculated.

3.15. Shoot/root ratio

The mean shoot/root ratio was computed from the fresh weight of shoots and that of roots, at fortnightly intervals for a period of three months.

3.16. Chemical analysis

3.16.1. Preparation of samples

Representative samples of five to six cuttings were taken from the whole lot uprooted at fortnightly intervals during both the seasons. Cuttings were first washed in tapwater and then in distilled water and dried thoroughly in a hot air oven, at 80°C till constant weights were obtained. The dried material was powdered in a Wiley mill. Samples were analysed for total carbon and nitrogen as per procedures given below.

3.16.2. Estimation of organic carbon

The total carbon content of the mother plant and that of the cuttings at fortnightly intervals, was estimated as per the method suggested by Blackley and Walk's (Jackson, 1958).

3.16.3. Estimation of total nitrogen

Total nitrogen content of the cuttings as well as that of the mother plant, was estimated by Kjeldahl digestion and distillation method (Jackson, 1958).

3.17. Statistical analysis

The data recorded were statistically analysed using standard procedures suggested by Panse and Sukhatme (1978).

The effect of various growth regulators and varieties on rooting percentage was tested for significance using the following formula.

$$\chi^2 = \frac{1}{n1\ n2}\left\{\frac{(an2 - bn1)^2}{a+b}\right.$$

b = number of cuttings not rooted in each of the treatments/varieties

where χ^2 = Chi-square value

a = number of cuttings rooted in each of the treatments/variety

n1 = total number of cuttings rooted in all the treatments/varieties.

n2 = total number of cuttings not rooted in all the treatments/varieties.

Significance between a pair of treatments/varieties was calculated as follows:-

$$\chi^2 = \left\{\frac{(\,|ad - bc|\,) - n/2}{(a+b)\ (a+c)\ (b+d)\ (d+c)}\right\}^2 n$$

where

a & c are the number of cuttings rooted in two treatments/varieties.

b & d = number of cuttings not rooted in two treatments/varieties.

n = a+b+c+d.

The difference among various growth regulator treatments and varieties in both seasons, with respect to quantitative characters such as number of roots, length of roots, number of shoots, fresh weight of shoots and roots per cuttings and shoot/root ratio were tested for significance using analysis of variance.

Correlation coefficient among the various growth parameters excepting for shoot/root ratio with percentage of rooting was computed from the following equation.

$$r = \frac{E\,x\,y - Ex \cdot Ey}{\sqrt{(Ex^2 - Ex \cdot \bar{x})\ (Ex^2 - \frac{Ey \cdot Ey}{n})}}$$

Correlation coefficient (r) was calculated among the organic carbon content, nitrogen content, C/N ratio and percentage of rooting and tested for significance.

Expt.2. Micropropagation studies

The multibracted mutant variety of bougainvillea viz. 'Mahara', which recorded a lower percentage of rooting in the conventional propagation method was mainly used for the experiment. Callus formation was studied in two other poor rooting varieties viz. 'Cherry Blossom' and 'Spring Festival'.

The tissue culture studies carried out were aimed at standardising a suitable technique for the propagation of 'Shy' rooting varieties. The main aspects of the study consisted of (i) Callus formation and regeneration (ii) multiple shoot formation (iii) rooting of shoot.

The details of the medium and the explants used are presented in the following sections.

I. Culture medium

In order to study the morphogenetic response of bougainvillea explants in culture, the most widely used MS medium (Murashige and Skoog, 1962) was tried. The composition of the medium is given in Table 2.

3.1. Preparation of the nutrient solutions

Stock solutions of major and minor nutrients were prepared first, by dissolving the required quantity of the chemicals in double glass distilled water and stored under refrigerated condition, in amber coloured bottles. Due to the problem of decreased activity during storage, the stocks solutions of nutrients were prepared fresh in every four weeks and that of vitamins, amino acids and phytohormones were prepared fresh in every week.

Table 2. Composition of Murashige and Skoog (1962) medium

Components	Quantity (mg/l)
Macro elements	
Ammonium nitrate, $NH_4 NO_3$	1650
Potassium nitrate, KNO_3	1900
Calcium Chloride, $Cacl_2 . 2H_2O$	440
Magnesium Sulphate, $Mg SO_4 . 7H_2O$	370
Potassium dihydrogen phosphate, $KH_2 PO_4$	170
Iron Chelate, Na_2 EDTA	37.30
Ferrous Sulphate, $Fe SO_4 . 7H_2O$	27.80
Micro elements	
Boric acid, $H_3 BO_3$	6.20
Manganese Sulphate, $Mn SO_4 . 7H_2O$	22.30
Zinc Chloride, $Zncl_2$	3.93
Potassium Iodide, KI	0.83
Sodium molybdate, $Na_2 Mo) . 2H_2O$	0.25
Copper Sulphate, $Ca SO_4 . 5H_2O$	0.025
Cobalt Chloride, $Co C/2 . 5H_2O$	0.025
Sucrose	30 g
Organic constituents	
Inositol	100 mg
Nicotinic acid	0.5
Pyridoxine Hcl	0.1
Thyamine Hcl	0.1
Glycine	2.0
Agar	7.0 g

3.1.1. Growth regulators in the medium

In order to standardise the optimum requirement of growth regulators for callus formation, multiple shoot formation and rooting, the basal medium of Murashige and Skoog (MS) was supplemented with various growth regulators. Two major groups of growth regulators viz. auxins, including natural and synthetic and cytokinins were utilised for the present study.

Stocks solutions of 200 ppm concentrations of auxins such as IAA, NAA, IBA and 2,4-D were prepared. 20 mg of the auxins was dissolved in minimum quantity of 95 per cent ethanol. The final volume was made upto 100 ml in a standard flask using double glass distilled water. From the stock solutions, the required quantity was pipetted out into the medium. To prepare stocks solutions of kinetin (KIN) and 6-benzyl amino purine (BAP/BA), 0.1 N Hcl was used instead of ethanol.

3.1.2. Other additives

Optional additives such as adenine and adenine sulphate were used in the medium, to study their effect on callus formation and multiple shoot formation respectively. A stock solution of 1500 ppm concentration of both these were prepared by dissolving 150 mg of chemical in water and making up the volume to 100 ml using double glass distilled water. Adequate quantity of these solutions were pipetted into the medium.

3.2. Preparation of the culture medium

Specific quantities of the stock solutions of the chemicals, as given in table 2, were pipetted out into a 1000 ml beaker. Sucrose and inositol were added fresh and dissolved. Then the volume was made upto about 1000 ml by adding double glass distilled water.

The pH of the solution was adjusted between 5.6 to 5.8, with an ELICO Electronic pH meter, using 0.1 N Hcl/NaOH. Agar (7 gm) was added to the medium and the final volume was made up exactly to one litre. The solution was melted at 90-95°C by keeping in a water bath. The medium was poured to the culture vessels after rinsing with double glass distilled water. The containers were filled upto 1/3rd volume and tightly closed with sterile cotton plugs.

In order to ensure aseptic condition of the medium, the containers plugged with cotton were autoclaved. Sterilisation was done in a horizontal type steam sterilizer, for 15 to 20 minutes at 15 psi and 121°C temperature. After sterilisation, the culture vessels were stored under refrigerated condition.

3.3. Explant

3.3.1. Source of explants

The excised plant part used for *in vitro* culturing is called an explant. In the present study, shoot apices, immature and mature axillary stem pieces, very immature

leaf (embryonic leaf) and mature leaf discs, formed the explant (excised plant) sources to study their potential for callus initiation. For multiple shoot development shoot apices alone were used.

3.3.2. Collection of explants

Explants meant for culturing were collected from active young sprouts of field grown plants, subjected to pruning. Such sprouts were separated from the mother plants using a sharp knife and brought immediately to the laboratory for surface sterilisation.

3.3.3. Surface sterilisation of explants

The culture medium provides a very congenial condition for the growth of fungi, bacteria and other microbial organisms. They may overgrow the explant and produce some toxic substances which may inhibit the growth of the explant. So the explant has to be surface sterilised. A separate experiment was conducted to standardise the optimum concentration of the chemical and duration of the treatment, so that the explants were made free of fungal and bacterial contamination, without injuring the tissues. The details of the methods of surface sterilisation tried are given in Table 3.

Surface sterilisation was carried out using any of the sterilants such as sodium hypochlorite and/or mercuric

Table 3. Standardisation of surface sterilisation of bougainvillea explants

	Explant	Sterilant	Concentration (%)	Time (min.)
1	Shoot apices	Mercuric chloride	0.05	12-13
2	Immature axillary stem piece	Mercuric chloride	0.1	8-10
3	Mature axillary buds	Absolute alcohol	95.00	10 sec.
		Sodium hypochlorite	2.5	15 min.
		Mercuric chloride	0.2	12-13
4	Immature leaf	Sodium hypochlorite	2.5	20-25
5	Mature leaf	Sodium hypochlorite	2.5	10
		Mercuric chloride	0.05	9-10

chloride, depending on the maturity of the explant tissue. To begin with, the collected shoots were defoliated without injuring the buds at the apex/axils, washed thoroughly with distilled water containing a few drops of surfactant teepol, followed by rinsing with distilled water. Further procedures were carried under the aseptic condition of the inoculation chamber. The tiny leaves covering the apical buds were separated out using a needle and were treated with the sterilant. After the end of the required time, the solution was drained off completely and rinsed thoroughly with sterile double glass distilled water four to five times. These explants were cautiously transferred to sterile petri dish and cut into 1 cm pieces using sharp knife. Needles, forceps and knife used for transferring the tissues to the medium were first autoclaved and repeatedly sterilised by dipping in 95 per cent ethanol for 10 seconds followed by flaming.

In the case of leaf explants, after the surface sterilisation, leaf discs of about 1 sq.cm size were separated from the intact leaves and cultured. Embryonic leaves were used as such. To prepare the axillary stem pieces for inoculation, 1 cm long immature shoot pieces containing an intact axillary buds were excised using a sterile knife.

3.4. Inoculation process

The transfer of sterile explants to the culture medium is termed as inoculation. All the operations starting from the surface sterilisation of explants upto the completion of inoculation were conducted under perfect aseptic condition. For this a 'Thermadyne' laminar air flow cabinet (inoculation chamber) in which a steady flow of filtered air, devoid of fungal spores and bacteria is maintained, was used. Before the inoculation process, the chamber was sterilised by ultra violet rays. Then the table of the chamber, and hands of the operator were swabbed with alcohol. Then cotton plug of the culture vessels were removed, and the neck was flamed over a gas burner kept in the chamber. The sterile explant was quickly transferred to the container with the help of forceps and slightly plunged into the medium. The neck of the culture tubes were once again flamed over the burner and quickly replaced the cotton plug.

3.5. Culture conditions

After inoculation, the flasks/tubes were properly labelled and incubated under $27\pm3^{\circ}C$ temperature. Artificial illumination was provided using cool white flourescent lamps at about 2000 lux intensity for a photoperiod of 16 hours per day. Extreme care was taken to keep the culture room and its premises free from dust and dirt.

The floor was sprayed with 3 per cent solution of formalin.

3.6. Studies on callus formation

Callus formation was studied using different explant sources on MS medium supplemented with various concentrations and combinations of auxins like NAA, IAA, 2,4-D and cytokinins like BAP and KIN. Treatments tried are given below.

i) NAA - 0.5, 1.0, 1.5, 2.0 mg/l + BA - 0.2, 0.5, 0.8 1.0 mg/l

ii) NAA - 1.0, 2.0 mg/l + KIN 0.2, 0.5, 0.8, 1.0 mg/l

iii) 2,4-D - 0.1, 0.5, 1.0 and 2.0 mg/l + BAP and KIN at 0.5 mg/l each.

iv) IAA - 0.5, 1.0, 2.0, 3.0, 5.0 + BA 0.5 mg/l

3.6.1. Effect of explants on callus formation

Explant used

Shoot apex

Immature axillary stem piece

Mature dormant buds

Immature and mature leaf tissue

These explants were grown on MS medium supplemented with an auxin and cytokinin for a period of five weeks.

Shoot apices were collected from three varieties of bougainvillea viz. 'Mahara', 'Cherry Blossom' and 'Spring Festival' and cultured in presence of NAA at 1.0, 1.5 and 2.0 mg/l + BAP at 0.5 and 1.0 mg/l.

The shoot apices were inoculated into the medium containing the above growth regulators with or without the presence of adenine 15 ppm. Following observations were recorded at weekly intervals.

Observations

1. Number of days taken for initiation of callus
2. Number of cultures forming callus
3. Percentage cultures forming callus

Growth rate of the callus

a) Mean length of callus

b) Mean width of callus

3.6.1. Subculturing for callus proliferation

The callus was allowed to grow on the primary medium for a period of four weeks. Then it was subcultured to MS solid medium. The same hormonal combination of the primary medium which gave maximum growth of the callus was used as the proliferation medium. The callus mass was cut into small pieces using a sterile knife and aseptically transferred to the proliferation medium. These cultures were maintained under similar conditions as mentioned earlier.

3.6.2. Regeneration from callus

Trial was conducted to obtain regeneration from callus tissue by transferring the tissue mass on to solid MS medium supplemented with IAA at the rate of 0.01, 0.1

and 0.4 mg/l and BA at 0.2, 0.8 and 1.6 mg/l, together with 0.01 mg/l GA_3. Calcium pantothenate and biotin each at 0.05 mg/l. The culture tubes/flasks were labelled and incubated under 27±3°C and 2000 lux light intensity for 16 hours photoperiod.

3.7. Multiple shoot formation

The explant used for studying the multiple shoot development was shoot apices from 'Mahara' variety of bougainvillea. The treatments included the following combinations of growth regulators-0, 0.5, 1.0 and 2.0 mg/l of BAP and 0, 0.5, 1.0, and 2.0 mg/l KIN.; 1.0, 3.0, 9.0 and 16.0 mg/l of BAP alone.

Solid MS medium supplemented with the above hormonal combination were utilised for culturing the shoot apices and they were maintained under the same conditions as mentioned for callus formation for a period of 45 to 60 days. They were subcultured to the same medium after this period, so as to avoid the risk of nutrient depletion from the medium. The following observations were recorded after the development of shoots.

Observations

i. Number of cultures that developed shoots
ii. Elongation of shoots (cm)
iii. Number of shoots/culture
iv. Number of leaves developed/culture

Effect of adenine sulphate on multiple shoot development was studied by incorporating 50 mg/l of the substance in the culture medium before autoclaving.

3.8. In vitro rooting of bougainvillea explants

For rooting of apical shoots of bougainvillea, 1 cm explants were cultured on Murashige and Skoog solid medium containing both full and half strength of all major and minor salts along with various combinations of auxins. Treatments given were as follows.

Full strength of MS salt + NAA at 2.0 and 4.0 mg/l

Full strength of MS salt + IAA - 3.0, 4.0 and 6.0 mg/l

Full strength of MS salt + IBA 0.8 and 1.6 mg/l

Full strength of MS salt + IBA 0.1, 0.2 and 0.4 mg/l+ IAA 0.1, 0.2 and 0.4 mg/l.

Half strength MS salt (½ MS) + NAA 2.0 and 4.0 mg/l

½ MS + IAA 3.0, 4.0 and 6.0 mg/l

½ MS + IBA 0.8 and 1.6 mg/l

½ MS + IBA 0.1, 0.2, 0.4 mg/l+ IAA 0.1, 0.2 and 0.4 mg/l.

Sucrose — 30 g

Agar — 7.0 g

The shoot apices after surface sterilisation were transferred to the above media and kept both under light

and dark conditions for initiation of roots. Both fresh shoot apices from field grown plants and precallused shoots were utilised for rooting.

Observations

1. Number of days taken for root initiation
2. Percentage of cultures rooted
3. Number of roots/culture
4. Mean length of the longest roots.

3.9. Planting out

Rooted plantlets were transferred to small plastic containers filled with equal parts of sterilised sand and soil. The plantlets were taken out from the culture vessels with the help of forceps and needles. Then the agar adhering to the roots was completely removed by washing with sterile distilled water and planted out in pots. Then they were covered with glass beakers to maintain high humidity and these pots were regularly watered.

Statistical analysis

The data pertaining to callus formation, multiple shoot formation and rooting, were analysed statistically using chi-square test of analysis.

RESULT

RESULT

The results of the present studies on standardisation of macro and micropropagation in bougainvillea are presented hereunder.

4.1. Effect of growth regulators on rooting

Effect of rooting hormones like IBA and NAA on adventitious root formation was studied during two seasons viz. June to September 1983 (rainy season) and February to May 1984 (Summer season) and the results are presented in Table 4. A perusal of the data indicated that all concentrations of IBA and NAA had pronounced effects on rooting of cuttings compared to control. However, IBA 500 ppm gave maximum rooting percentage of 63.1 during rainy season and 66.2 during summer. The percentage of rooting was minimum in control 26.4 and 31.0 respectively during these seasons. Statistical comparison of different treatment effects, carried out using chi-square test (Appendix I & II) also proved the significant superiority of IBA 500 ppm treatment over others. The effect of IBA and NAA on rooting is illustrated in Fig.1. An overall analysis of the data showed that IBA was more effective in inducing rooting of cuttings in bougainvillea compared to NAA. Further it was evident that, of the two methods, soaking the basal ends in

Table 4. Effect of growth regulators on rooting of cuttings (Values in percentage)

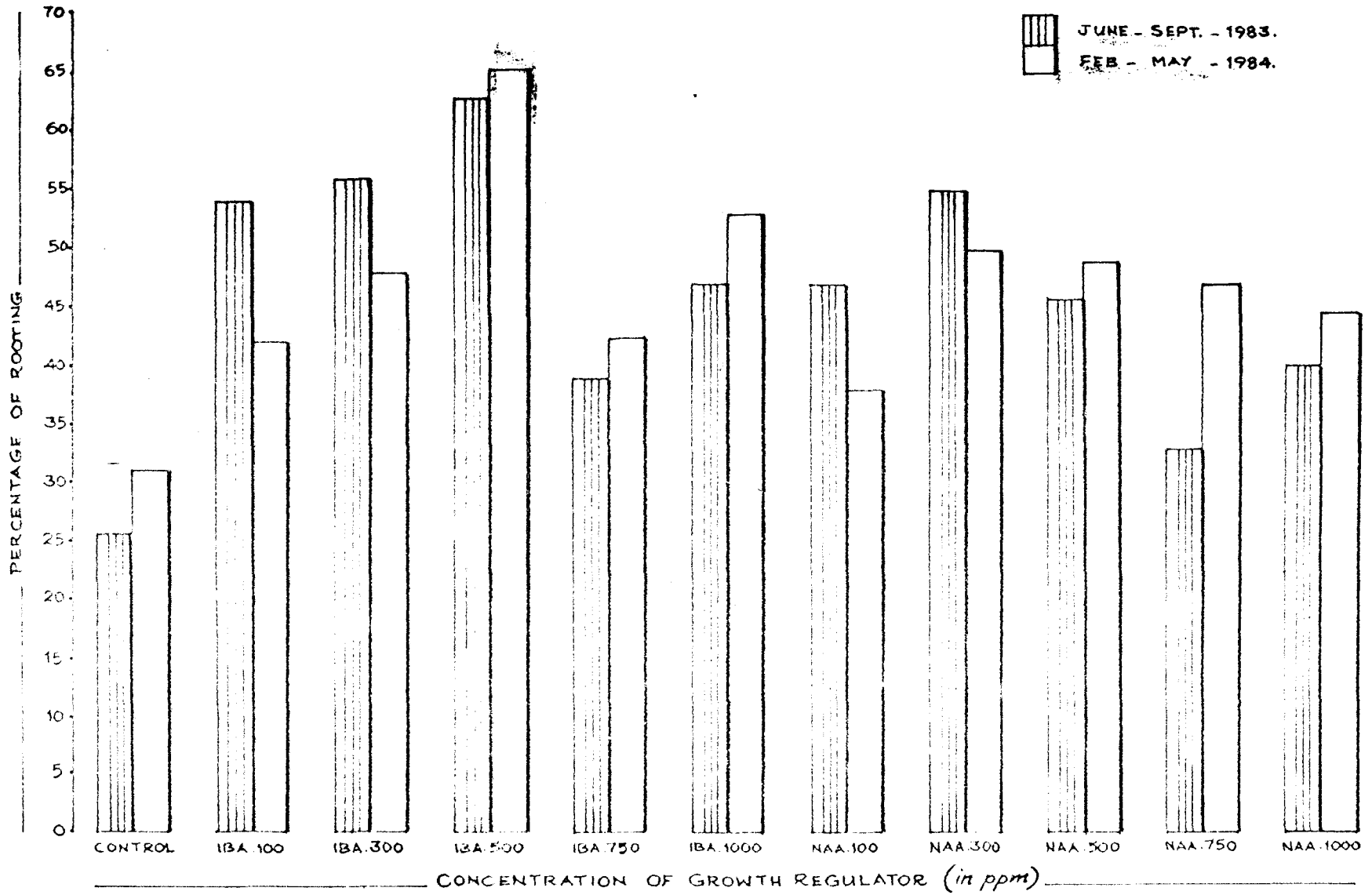
Variety	Season	No.of cuttings per treatment	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean
Scarlet Glory	Rainy[1]	50	52.0	82.0	86.0	88.0	76.0	76.0	82.0	82.0	78.0	54.0	70.0	76.9
	Summer[2]	50	50.0	64.0	58.0	88.0	62.0	86.0	54.0	56.0	64.0	58.0	80.0	65.4
Jayalakshmy	Rainy	50	64.0	84.0	88.0	92.0	72.0	76.0	80.0	86.0	84.0	72.0	78.0	79.4
	Summer	50	58.0	68.0	70.0	80.0	56.0	82.0	58.0	60.0	74.0	80.0	66.0	68.5
Enidlancaster	Rainy	50	24.0	66.0	68.0	78.0	60.0	70.0	74.0	78.0	48.0	42.0	42.0	59.0
	Summer	50	24.0	32.0	40.0	48.0	24.0	24.0	26.0	30.0	30.0	24.0	24.0	29.6
Lady Mary Baring	Rainy	50	18.0	56.0	62.0	66.0	26.0	32.0	26.0	26.0	32.0	20.0	28.0	36.3
	Summer	50	26.0	34.0	48.0	70.0	40.0	62.0	24.0	54.0	44.0	30.0	20.0	40.0
Thimma	Rainy	50	14.0	30.0	24.0	42.0	24.0	34.0	31.0	40.0	32.0	20.0	20.0	32.5
	Summer	50	20.0	34.0	50.0	64.0	36.0	46.0	46.0	62.0	60.0	54.0	36.0	48.5
Cherry Blossom	Rainy	50	20.0	32.0	32.0	46.0	24.0	26.0	24.0	38.0	28.0	28.0	28.0	30.0
	Summer	50	30.0	36.0	42.0	66.0	30.0	54.0	26.0	30.0	54.0	60.0	34.0	40.1
Mahara	Rainy	50	16.0	46.0	50.0	56.0	20.0	32.0	32.0	50.0	38.0	20.0	28.0	37.2
	Summer	50	22.0	40.0	50.0	68.0	40.0	52.0	22.0	58.0	60.0	44.0	22.0	45.4
Spring Festival	Rainy	50	12.0	36.0	38.0	42.0	26.0	32.0	22.0	40.0	32.0	22.0	22.0	30.5
	Summer	50	24.0	42.0	36.0	58.0	50.0	48.0	42.0	44.0	24.0	50.0	50.0	42.5
Maharaja of Symore	Rainy	50	18.0	56.0	56.0	58.0	24.0	50.0	52.0	56.0	44.0	26.0	46.0	44.3
	Summer	50	26.0	34.0	42.0	64.0	28.0	48.0	30.0	66.0	42.0	44.0	38.0	42.3
			26.4	54.2	56.9	63.1	39.1	47.5	47.0	55.1	46.2	33.7	40.2	
			31.0	42.6	49.2	66.2	43.5	53.1	38.2	50.8	49.5	47.3	44.8	

1 - June-September 1983
2 - February-May 1984

Chi-square values : 197.6**
92.2**

** Significance at 1 per cent level of probability.

Fig. 1 - EFFECT OF GROWTH REGULATORS ON THE PERCENTAGE OF ROOTING

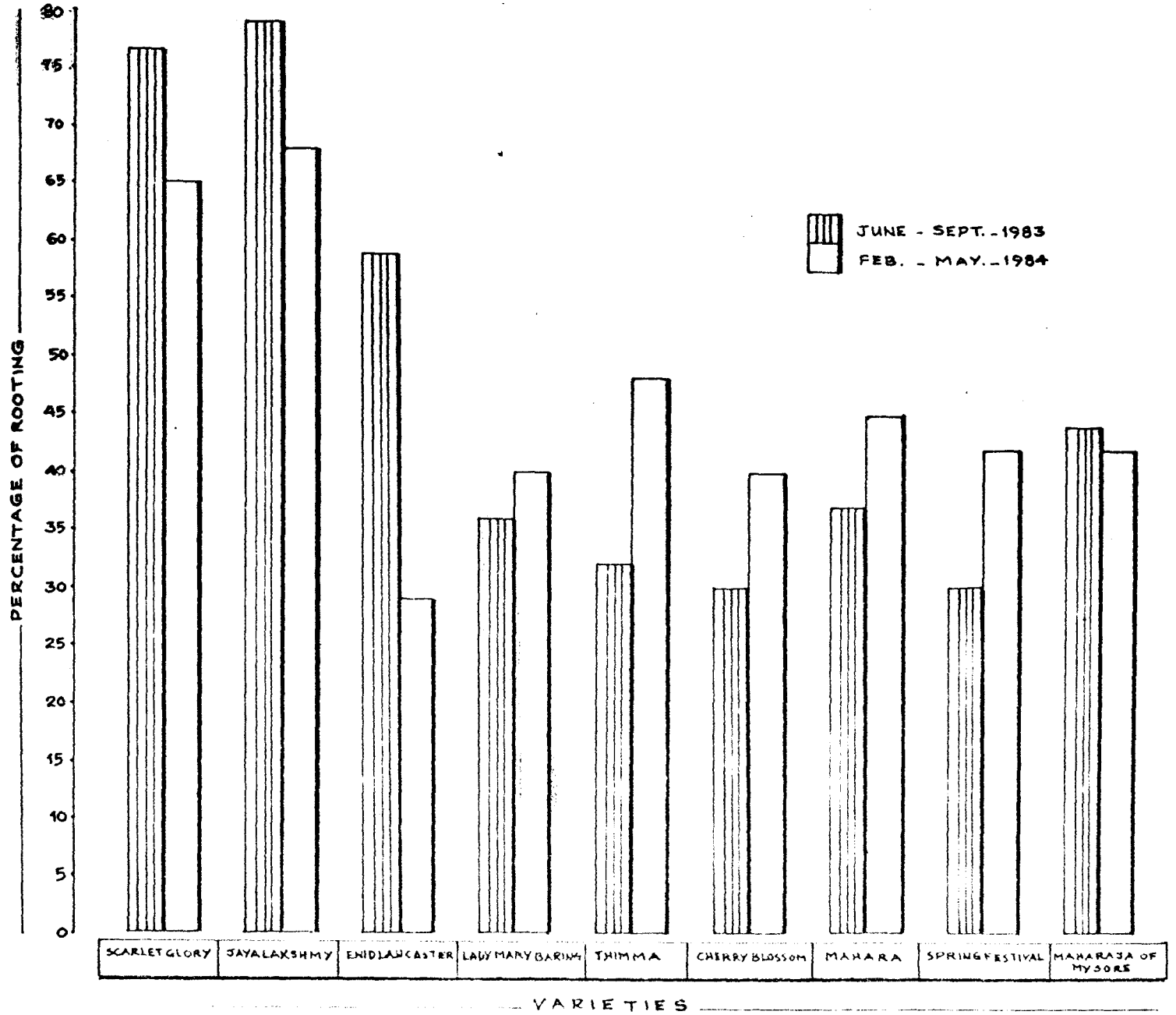


dilute solutions (100, 300 and 500 ppm) of IBA and NAA for a period of six hours was found to be more effective than quick dip for five seconds in concentrated solutions of 750 or 1000 ppm.

The response of nine varieties of bougainvillea with respect to rooting during rainy as well as summer seasons is presented in Fig.2. Response of these varieties varied during both seasons. During rainy season the number of cuttings rooted were more in the varieties 'Scarlet Glory', 'Jayalakshmy', 'Enidlancaster' and 'Maharaja of Mysore' compared to that in summer, while the percentage of rooting was more during summer in other varieties like 'Lady Mary Baring', 'Thimma', 'Cherry Blossom', 'Mahara' and 'Spring Festival' (Table 4). The variety 'Jayalakshmy' recorded maximum rooting of 79.4 per cent and 68.5 per cent respectively during rainy season and summer season, followed by variety 'Scarlet Glory' 76.9 and 65.4 per cent respectively during these seasons. Minimum rooting was observed in the varieties 'Spring Festival' (30.0 per cent) and 'Cherry Blossom' (29.6 per cent) during summer season. Comparison of different varieties using chi-square test also showed a significant variation in rooting among different varieties (Appendix III & IV).

The effect of weather parameters like maximum and minimum temperature, relative humidity, total rainfall and

Fig. 2 - VARIETAL RESPONSE ON PERCENTAGE OF ROOTING OF CUTTINGS



the number of rainy days on rooting of cuttings is given in Appendix V. The weather parameters seemed to influence the rooting of cuttings. From the present study it was observed that a higher percentage of rooting was obtained when the rainfall was maximum and during this period variation in temperature was also less.

4.2. Number of roots

The consolidated data on the effect of IBA and NAA at various concentrations on number of roots produced at monthly intervals are presented in Table 5a, 5b and 5c. It was observed that treating the cuttings with IBA and NAA significantly increased the number of roots over the control (Appendix VI) during rainy as well as in summer seasons. Highest number of roots per cutting was observed with IBA 300 ppm (26.5 and 13.9 respectively during rainy season and summer season) followed by IBA 500 ppm (22.38 and 12.5) two months after planting for rainy and summer periods (Table 5b). However, there was no significant difference between these two treatments. Minimum number of roots was recorded in the control (5.23 & 2.6 for the above two seasons). The effect of IBA and NAA on root production is depicted in Plates I to IV. It could be concluded that soaking the basal ends of the cuttings in dilute solutions of IBA and NAA was more effective in producing more number

Table 5a. Effect of growth regulators on number of roots/cuttings one month after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy[1]	7.0	18.5	42.5	21.0	15.0	15.0	19.0	20.0	21.0	18.5	19.0	19.77	
	Summer[2]	3.0	5.5	15.5	14.5	4.0	3.5	13.5	9.0	11.5	10.0	11.5	9.22	
Jayalakshmy	Rainy	6.0	12.5	22.0	24.5	9.5	5.5	17.5	20.0	19.0	10.5	11.5	13.72	
	Summer	4.0	7.0	26.5	23.5	11.0	8.0	13.0	19.0	19.0	13.5	16.0	15.22	
Enidlancaster	Rainy	1.5	16.0	35.5	25.5	9.0	12.0	12.5	31.0	24.0	8.5	12.5	17.09	CD
	Summer	3.0	4.5	18.5	18.5	3.5	3.0	9.0	5.0	9.5	5.5	6.5	7.86	3.26
Lady Mary Baring	Rainy	3.0	7.5	14.5	12.0	4.0	6.0	3.0	2.5	3.0	3.5	3.5	5.54	2.91
	Summer	1.0	2.5	12.5	9.0	1.0	1.5	8.5	1.0	5.5	3.0	4.5	4.68	
Thimma	Rainy	1.0	2.0	14.0	11.5	3.5	6.5	3.0	4.5	14.0	1.5	4.0	5.04	SEM
	Summer	2.0	2.5	12.0	11.0	3.5	3.0	9.5	3.5	9.5	6.0	9.0	6.31	1.16
Cherry Blossom	Rainy	1.5	2.0	12.5	7.0	3.0	4.0	1.0	7.0	3.0	2.0	2.5	3.36	1.03
	Summer	1.0	2.5	4.0	10.5	1.5	2.5	8.5	3.0	7.0	3.0	3.5	5.05	
Mahara	Rainy	2.5	6.0	16.0	12.0	2.5	9.5	2.5	8.0	14.0	3.5	3.0	7.09	
	Summer	1.0	2.0	20.0	11.5	1.5	2.0	11.0	6.5	3.5	5.5	8.0	6.73	
Spring Festival	Rainy	3.5	4.0	13.0	6.5	5.5	5.0	6.5	8.5	6.5	5.0	2.5	4.72	
	Summer	1.0	2.5	6.0	12.5	3.5	3.5	10.0	4.5	5.0	5.5	7.0	6.90	
Maharaja of Mysore	Rainy	2.5	7.0	16.5	10.5	5.0	9.0	1.0	9.0	7.0	4.5	6.0	6.36	
	Summer	1.0	5.5	10.0	9.0	3.5	4.5	9.5	9.5	7.0	7.5	7.5	7.55	
		3.16	8.38	20.76	14.50	6.33	8.05	7.33	12.27	12.38	6.38	7.16		
		1.88	13.85	13.80	13.30	3.60	3.50	10.94	6.11	8.61	6.61	8.16		

CD = 3.60 / 3.21

SEM = 1.28 / 1.15

Table 5b. Effect of growth regulators on number of roots/cuttings two months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	15.0	22.5	67.5	41.5	19.5	19.0	32.0	41.5	41.5	28.5	38.0	32.09	
	Summer	7.0	10.0	29.0	17.5	8.5	9.5	16.0	11.0	12.5	11.5	11.5	13.09	
Jayalakshmy	Rainy	11.0	15.0	28.5	26.5	14.5	12.0	15.5	20.5	22.5	19.0	19.5	18.22	
	Summer	5.0	10.5	27.5	22.5	8.0	10.0	26.0	11.5	20.0	15.0	17.0	16.09	
Enidlancaster	Rainy	5.0	20.5	44.5	41.0	17.0	10.0	22.0	25.5	34.0	25.5	33.0	25.22	CD= 5.88 3.03
	Summer	2.0	8.5	15.5	14.5	17.5	5.0	13.5	8.0	12.5	9.5	9.5	9.59	
Lady Mary Baring	Rainy	2.5	9.0	22.0	16.5	6.5	3.0	7.5	10.5	15.5	9.0	10.0	10.18	
	Summer	1.0	2.5	15.5	10.5	1.5	2.0	6.0	2.5	5.5	2.5	3.0	4.72	
Thimma	Rainy	4.0	10.0	26.5	22.0	6.5	5.5	14.5	20.0	22.0	10.5	20.0	14.68	
	Summer	2.5	6.5	18.0	13.5	5.0	6.5	10.0	6.5	9.0	7.0	8.5	8.46	SEM= 2.1 1.08
Cherry Blossom	Rainy	2.5	5.0	18.5	12.5	3.0	2.5	5.5	9.0	9.0	3.0	7.0	7.04	
	Summer	1.0	2.5	11.0	8.0	1.5	1.5	7.0	3.5	5.0	3.5	4.0	4.46	
Mahara	Rainy	2.5	5.5	23.5	15.5	2.5	3.5	6.0	10.5	11.5	6.0	8.5	8.64	
	Summer	1.5	3.5	16.5	10.0	2.0	3.5	6.0	3.5	5.0	4.5	5.0	5.50	
Spring Festival	Rainy	1.0	3.5	11.5	7.0	2.0	2.5	4.0	4.5	5.0	4.0	4.5	4.50	
	Summer	1.5	3.0	11.0	7.5	2.0	2.5	7.0	3.5	5.0	5.0	4.5	4.77	
Maharaja of Mysore	Rainy	4.5	8.5	21.0	19.0	4.5	6.5	13.5	18.0	18.0	9.0	10.5	12.64	
	Summer	2.5	4.5	9.5	8.5	3.5	3.5	8.0	5.0	5.0	5.5	7.5	6.00	
		5.33	10.66	29.27	22.38	8.44	7.16	13.39	17.77	19.88	12.72	17.44		
		2.61	5.72	17.05	12.50	5.55	4.88	11.05	6.11	8.83	7.11	8.11		

CD = 6.51
3.35

SEM = 2.32
1.19

Table 5c. Effect of growth regulators on number of roots/cuttings three months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Searlet Glory	Rainy	11.0	22.5	63.5	32.0	13.5	21.0	29.0	25.0	27.0	22.0	24.5	26.68	
	Summer	7.5	8.5	17.0	15.0	8.0	8.5	11.5	11.0	14.0	10.5	10.5	11.09	
Jayalakshmy	Rainy	7.0	16.0	23.5	20.0	10.0	13.5	25.0	19.5	15.5	13.5	15.5	16.36	CD = 4.63
	Summer	3.5	12.5	25.0	20.0	10.5	10.0	18.5	12.0	18.5	14.0	16.5	14.63	2.22
Enidlancaster	Rainy	6.0	7.0	46.5	34.0	14.5	22.5	11.0	30.0	27.5	14.5	18.5	21.09	
	Summer	2.0	4.5	18.5	9.0	3.5	4.5	5.5	5.0	7.0	5.0	5.5	6.36	
Lady Mary Baring	Rainy	2.5	6.0	18.0	14.5	6.5	6.0	6.5	6.5	12.5	6.5	9.0	9.04	
	Summer	1.5	5.0	15.0	13.0	3.5	3.5	10.0	4.5	11.0	6.0	8.5	7.36	
Thimma	Rainy	3.0	5.5	22.5	15.0	5.0	3.0	4.5	6.5	10.5	9.5	8.0	8.45	SEM = 1.65
	Summer	2.0	4.0	13.5	8.0	2.0	2.5	6.5	4.5	8.0	5.0	5.5	5.59	0.75
Cherry Blossom	Rainy	2.5	2.0	10.0	10.0	3.0	3.0	3.5	6.0	7.0	6.0	2.0	5.04	
	Summer	2.0	3.0	10.0	9.5	2.5	2.5	5.5	3.5	5.5	4.5	3.0	4.86	
Mahara	Rainy	3.5	9.0	18.5	14.0	5.0	6.5	9.5	9.0	15.0	10.0	10.5	10.60	
	Summer	2.5	4.0	11.5	7.5	3.5	3.5	5.5	4.0	7.0	6.0	5.0	5.50	
Spring Festival	Rainy	1.0	3.0	11.5	7.5	2.0	1.0	6.0	3.0	7.0	4.5	5.5	4.72	
	Summer	2.0	3.5	7.5	7.0	2.0	2.0	6.0	4.0	6.5	4.0	5.5	4.41	
Maharaja of Mysore	Rainy	4.0	7.0	24.5	23.0	4.5	4.5	11.5	5.5	20.0	9.0	9.0	11.18	
	Summer	1.0	3.0	7.5	7.5	2.0	2.5	6.0	3.5	7.0	4.0	5.0	4.14	
		4.70	10.33	26.50	18.88	7.11	9.00	11.83	12.33	15.77	10.61	11.38		
		2.66	5.33	13.94	10.72	4.16	4.38	8.33	5.77	9.38	6.55	7.22		

CD = 5.119
2.45

SEM = 1.82
0.87

Plate I. Effect of IBA on root production in the var. 'Scarlet Glory'

1. Control
2. IBA 100 ppm
3. IBA 300 ppm
4. IBA 500 ppm
5. IBA 750 ppm
6. IBA 1000 ppm

Plate II. Effect of NAA on root production in the var. 'Scarlet Glory'

7. NAA 100 ppm
8. NAA 300 ppm
9. NAA 500 ppm
10. NAA 750 ppm
11. NAA 1000 ppm


1
2
3
4
5
6


7
8
9
10
11

Plate III. Effect of IBA on root production in the var. 'Spring Festival'

1. Control
2. IBA 100 ppm
3. IBA 300 ppm
4. IBA 500 ppm
5. IBA 750 ppm
6. IBA 1000 ppm

Plate IV. Effect of NAA on root production in the var. 'Spring Festival'

7. NAA 100 ppm
8. NAA 300 ppm
9. NAA 500 ppm
10. NAA 750 ppm
11. NAA 1000 ppm

15cm

15cm

Fig-3. EFFECT OF IBA ON NUMBER OF ROOTS DURING THE SEASON JUNE-SEPT. 1983.

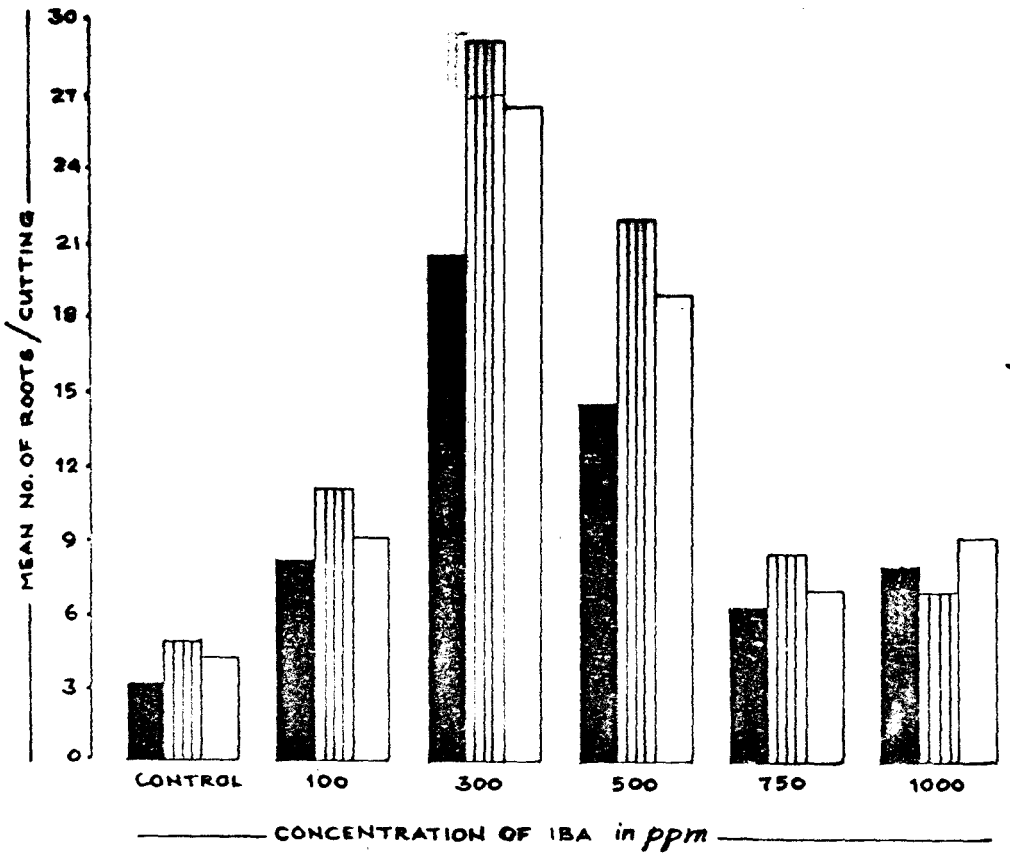


Fig-4. EFFECT OF NAA ON NUMBER OF ROOTS DURING THE SEASON JUNE-SEPT. 1983.

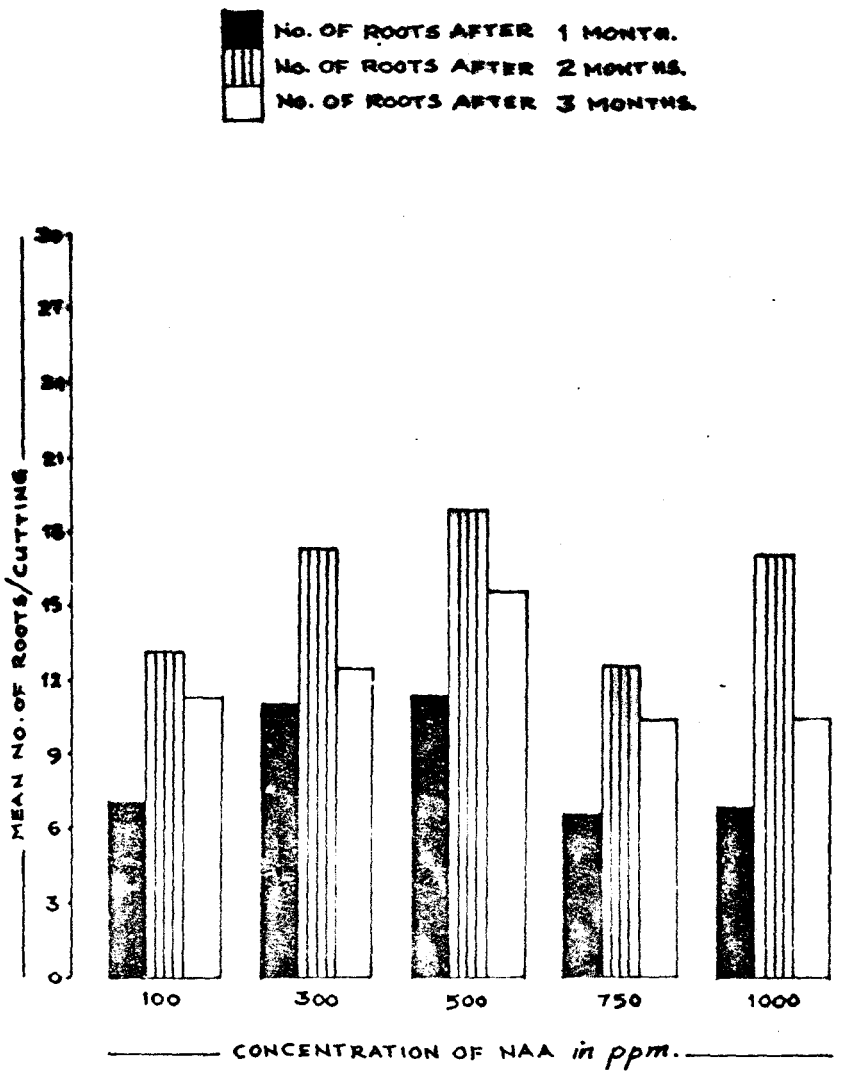


Fig-5. EFFECT OF IBA ON NUMBER OF ROOTS DURING THE SEASON FEB. - MAY. 1984.

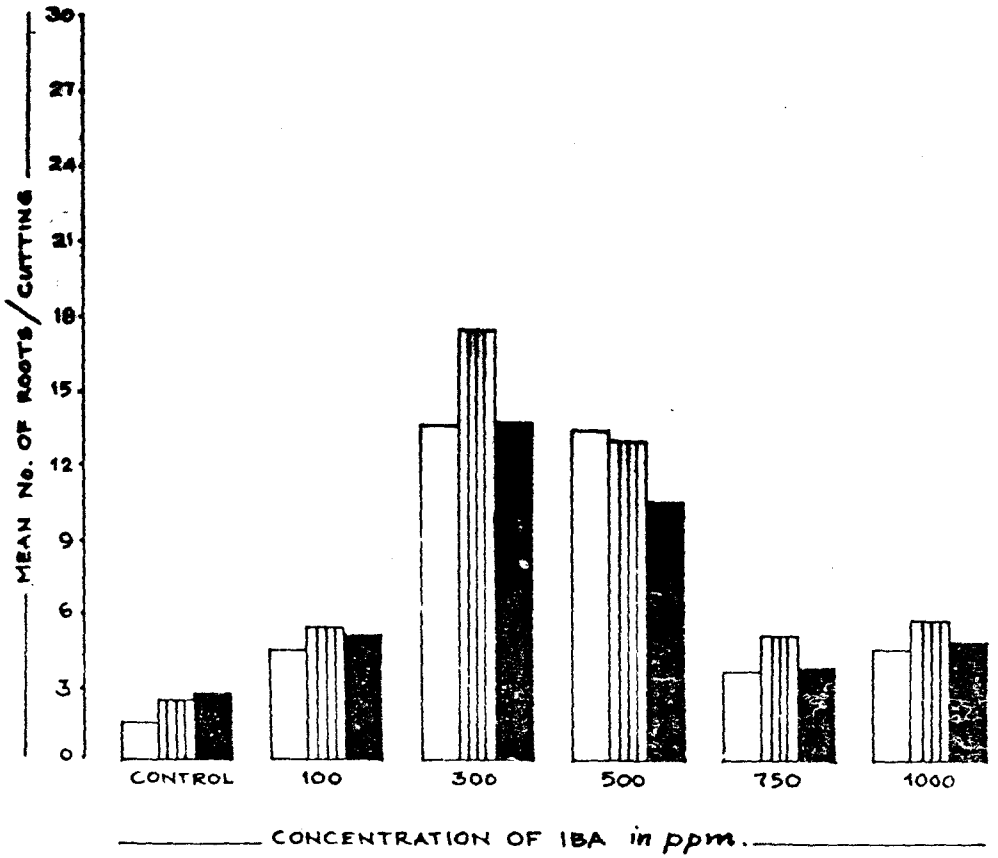


Fig-6. EFFECT OF NAA ON NUMBER OF ROOTS DURING THE SEASON FEB.-MAY-1984.

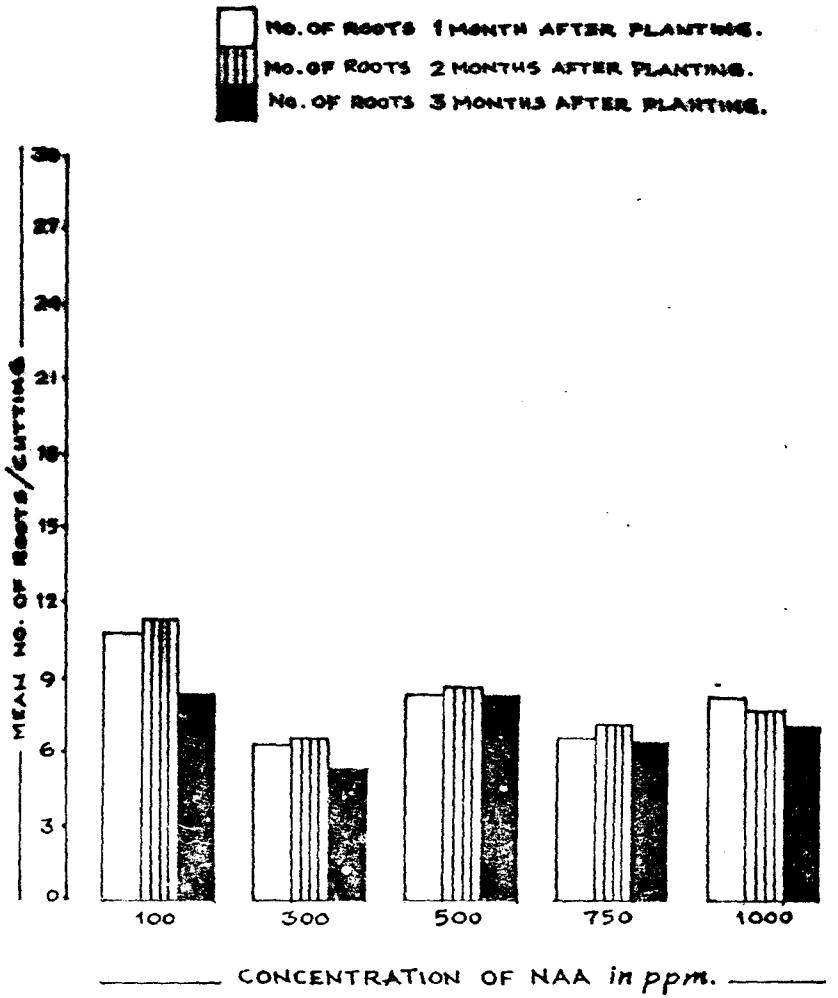
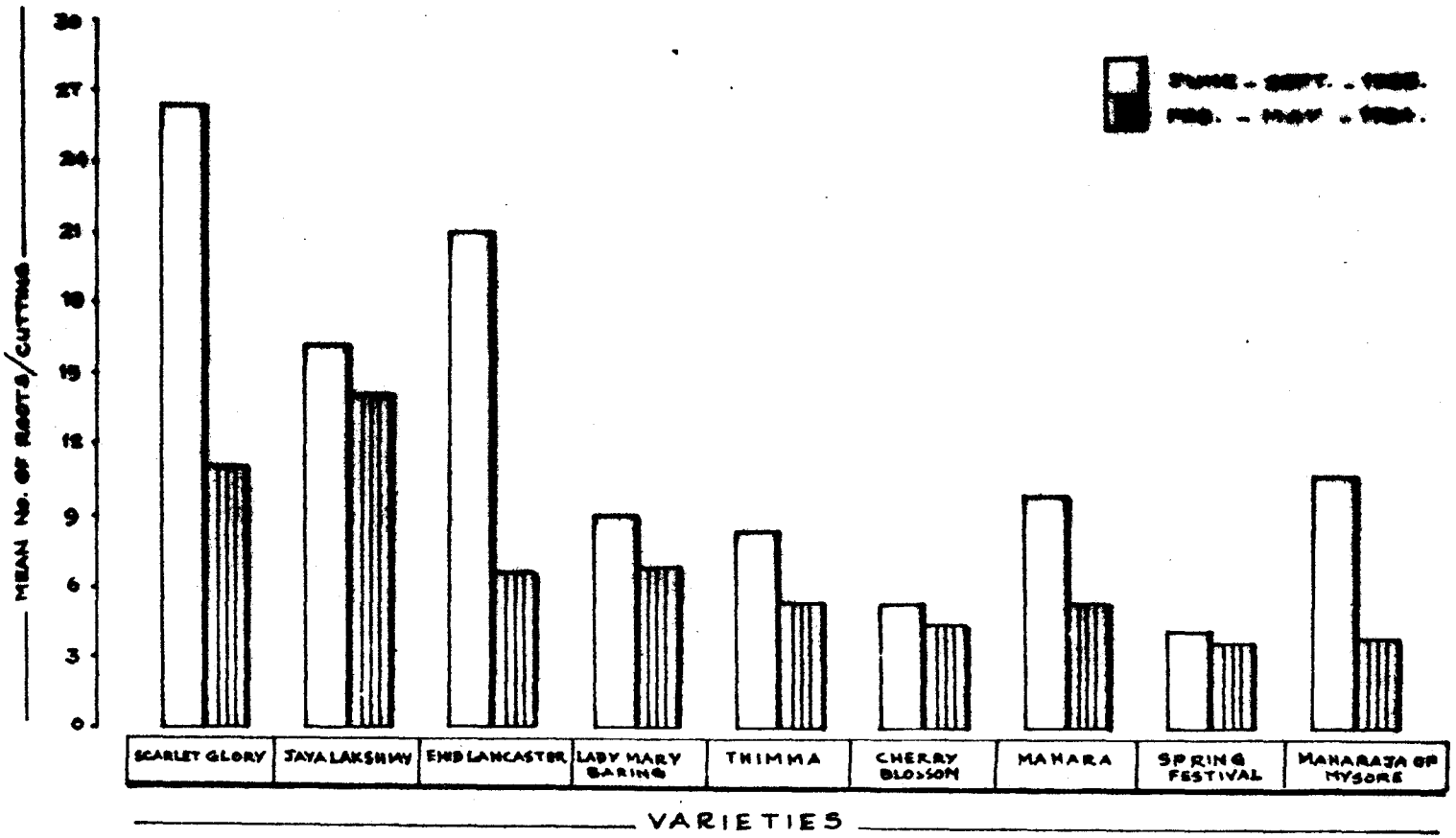


Fig. 7. VARIETAL RESPONSE ON NUMBER OF ROOTS.



of roots than quick dip in concentrated solution. The number of roots produced was maximum two months after planting which decreased slightly after three months in almost all treatments. The seasonal influence on root production with IBA and NAA treatment is illustrated in Fig.3 to 6. The number of roots produced in all the treatments and varieties were more during rainy season than during summer season.

The data on monthly production of roots of different varieties of bougainvillea are also furnished in Table 5a, 5b and 5c. The statistical analysis of the data showed significant difference between the varieties (Appendix VI). Maximum number of roots were produced in the variety 'Scarlet Glory' (32.07) followed by the varieties 'Enidlancaster' (25.22) and 'Jayalakshmy' (18.22) and the least in 'Spring Festival' (4.5). A graphical representation of this is given in Fig.7. Root production in varieties like 'Lady Mary Baring', 'Thimma', 'Cherry Blossom', 'Mahara' and 'Maharaja of Mysore' was significantly lower than the three varieties mentioned above.

4.3. Length of roots

Pooled data on the mean length of roots at monthly intervals as affected by IBA and NAA treatment are presented in Table 6a, 6b and 6c. Irrespective of the seasons, treating the cuttings with NAA 300 ppm produced the longest

Table 6a. Effect of growth regulators on length of roots (cm)/cutting one month after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	1.06	1.27	1.20	1.02	1.10	1.36	0.78	2.28	0.81	0.91	1.01	1.163	
	Summer	0.38	1.05	1.05	1.10	0.89	0.80	0.78	1.50	1.05	0.89	0.87	0.937	
Jayalakshmy	Rainy	0.95	1.09	1.02	0.94	0.97	1.31	0.44	1.52	0.75	0.83	0.88	0.97	
	Summer	0.50	1.37	1.33	1.40	0.93	0.99	1.02	1.43	1.08	1.06	1.06	1.10	
Enidlancaster	Rainy	0.78	1.15	1.04	0.75	0.98	1.38	0.55	2.10	0.65	0.75	0.75	0.98	
	Summer	0.55	1.03	1.03	1.05	0.75	0.56	0.89	1.05	0.99	0.98	0.93	0.89	CD = 0.252
Lady Mary Baring	Rainy	0.82	1.09	1.02	0.74	1.00	1.13	0.38	1.89	0.66	0.84	0.70	0.93	0.154
	Summer	0.80	1.18	1.17	1.21	0.87	0.87	0.90	1.28	1.10	1.10	0.98	1.04	
Thimma	Rainy	0.94	1.12	1.07	0.88	1.00	1.27	0.43	1.58	0.54	0.55	0.64	0.90	
	Summer	0.60	0.89	0.82	0.98	0.65	0.65	0.70	0.77	0.76	0.75	0.75	0.78	SEM =
Cherry Blossom	Rainy	0.67	1.17	1.08	0.81	1.04	1.28	0.26	1.78	0.63	0.69	0.77	0.94	0.252
	Summer	0.36	0.86	0.67	0.96	0.46	0.38	0.60	1.03	0.62	0.53	0.53	0.64	0.05
Mahara	Rainy	0.73	0.91	0.95	0.59	0.73	7.29	0.33	1.34	0.63	0.48	0.53	0.850	
	Summer	0.52	0.92	0.86	1.28	0.59	0.53	0.63	1.35	0.62	0.83	0.75	0.83	
Spring Festival	Rainy	0.85	1.02	0.89	0.82	0.87	1.03	0.50	1.42	0.41	0.60	0.69	0.841	
	Summer	0.43	0.96	0.85	1.01	0.62	0.57	0.71	1.17	0.83	0.77	0.74	0.77	
Maharaja of Mysore	Rainy	1.10	1.34	1.26	0.92	1.13	1.37	0.71	1.55	0.76	0.90	0.91	1.08	
	Summer	0.53	1.11	1.08	1.17	0.83	0.62	0.87	0.91	0.95	0.92	0.91	0.93	
		0.897	1.12	1.05	0.82	0.97	1.26	0.45	1.83	0.64	0.73	0.76		
		0.52	1.04	0.98	1.13	0.73	0.66	0.78	1.16	0.91	0.87	0.84		

CD = 0.279
0.171

SEM = 0.09
0.06

Table 6b. Effect of growth regulators onlength of roots (cm)/cutting two months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	3.86	5.27	4.44	3.34	4.12	5.88	1.41	5.94	1.95	2.53	3.05	3.80	
	Summer	1.17	2.82	2.65	3.32	1.39	1.25	1.53	5.21	2.04	1.94	1.65	2.72	
Jayalakshmy	Rainy	2.33	2.93	2.68	2.28	2.36	3.85	1.23	3.90	1.67	1.89	2.00	2.46	
	Summer	0.95	2.42	2.34	2.83	1.47	1.30	1.34	2.87	1.95	1.90	1.89	1.98	
Enidlancaster	Rainy	1.95	2.59	2.01	1.93	1.96	2.70	1.45	3.08	1.51	1.69	1.86	2.07	CD = 0.58 0.642
	Summer	1.03	2.53	2.28	2.85	1.28	1.26	1.39	2.89	1.98	1.63	1.40	1.87	
Lady Mary Bering	Rainy	1.45	1.57	1.52	1.33	1.51	1.83	0.82	2.06	1.21	1.26	1.29	1.44	
	Summer	1.18	2.42	2.04	2.85	1.41	1.52	1.60	2.90	2.00	1.78	1.62	1.90	
Thimma	Rainy	1.82	2.14	2.05	1.79	1.90	2.44	0.89	3.80	1.35	1.48	1.70	1.94	
	Summer	1.30	3.21	3.10	3.65	2.00	1.64	2.13	4.43	2.43	2.39	2.09	2.58	
Cherry Blossom	Rainy	1.57	2.71	1.83	1.56	1.65	2.82	0.84	2.89	1.64	1.08	1.56	1.78	SEM 0.185 0.23
	Summer	1.95	3.03	3.00	3.05	2.15	2.44	2.65	3.51	2.82	2.54	2.65	2.71	
Mahara	Rainy	1.56	1.75	1.61	1.52	1.60	1.77	1.08	2.4	1.26	1.31	1.40	1.52	
	Summer	1.57	2.54	2.47	2.54	1.83	1.65	2.30	2.60	2.26	2.20	2.12	2.19	
Spring Festival	Rainy	1.54	1.72	1.58	1.51	1.55	1.84	0.80	2.32	1.42	1.43	1.45	1.56	
	Summer	1.36	3.22	2.90	3.25	1.70	1.48	2.30	4.61	2.80	2.26	2.20	2.53	
Maharaja of Mysore	Rainy	2.68	3.52	2.81	2.42	2.72	4.34	1.33	4.37	1.44	1.85	1.94	2.68	
	Summer	1.80	3.32	2.84	3.40	1.69	1.48	2.00	4.20	2.59	2.50	2.44	2.61	
		3.10	2.68	2.28	1.96	2.15	3.05	1.09	3.35	1.42	1.62	1.80		
		1.37	2.83	2.62	3.08	1.65	1.56	1.92	3.69	2.29	2.13	2.13		

CD = 0.572
0.71

SEM = 0.204
0.253

Table 6c. Effect of growth regulators on length of roots (cm)/cutting three months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	5.66	3.55	7.17	7.30	3.05	8.65	5.47	4.82	5.20	5.57	4.73	5.78	
	Summer	2.42	4.79	9.16	10.67	3.25	4.97	5.32	6.77	6.02	6.97	4.97	5.75	
Jayalakshmy	Rainy	5.80	4.51	3.76	2.76	3.74	3.87	3.02	2.71	3.49	2.90	4.02	4.94	
	Summer	2.70	3.49	5.16	5.96	5.87	4.27	3.78	9.01	5.46	3.25	5.80	3.69	
Enidlancaster	Rainy	3.08	4.02	3.44	3.41	3.49	2.24	3.21	4.05	2.37	2.19	3.75	3.87	CD = 1.11 1.008
	Summer	2.08	4.60	3.45	3.57	5.96	6.07	3.54	4.25	2.92	2.03	4.42	3.23	
Lady Mary Baring	Rainy	2.25	5.99	5.44	3.65	2.81	3.99	3.10	3.75	3.30	1.98	2.01	3.94	
	Summer	3.16	5.14	4.69	5.10	3.03	7.55	4.61	5.67	5.66	5.39	4.92	4.54	
Thimma	Rainy	2.26	2.73	3.22	3.20	1.61	2.30	2.44	4.92	2.81	2.15	1.65	2.78	SEM 0.399 0.36
	Summer	3.54	4.92	4.35	4.60	3.37	4.48	3.47	5.78	5.20	6.53	2.50	4.33	
Cherry Blossom	Rainy	1.58	2.49	3.32	3.49	2.83	3.35	2.04	2.50	2.13	1.94	3.00	2.72	
	Summer	1.95	5.90	3.64	5.67	3.95	3.56	2.74	4.52	3.19	2.16	3.00	3.55	
Mahara	Rainy	2.00	2.02	2.00	3.01	2.76	1.96	2.72	1.82	2.62	2.67	3.24	2.90	
	Summer	3.67	5.27	4.56	3.96	4.38	2.61	4.94	2.42	3.44	3.79	4.85	3.60	
Spring Festival	Rainy	2.32	4.60	3.42	2.20	2.35	3.71	2.21	3.00	2.17	2.53	2.66	3.70	
	Summer	3.30	3.08	5.17	7.10	2.50	5.76	2.32	4.56	2.35	3.25	3.20	3.06	
Maharaja of Mysore	Rainy	3.38	2.78	3.74	4.06	3.53	2.18	2.55	5.96	5.02	5.65	4.60	5.93	
	Summer	1.37	11.57	4.51	1.87	2.75	7.33	8.30	8.33	6.54	6.67	9.06	4.24	
		3.14	4.73	4.66	4.38	3.46	4.80	3.40	4.94	3.52	3.64	3.70		
		2.69	4.44	4.42	4.49	3.59	3.42	3.71	4.92	4.18	4.16	3.98		

CD = 1.236
1.114

SEM = 0.44
0.398

Fig-8. EFFECT OF IBA ON LENGTH OF ROOTS

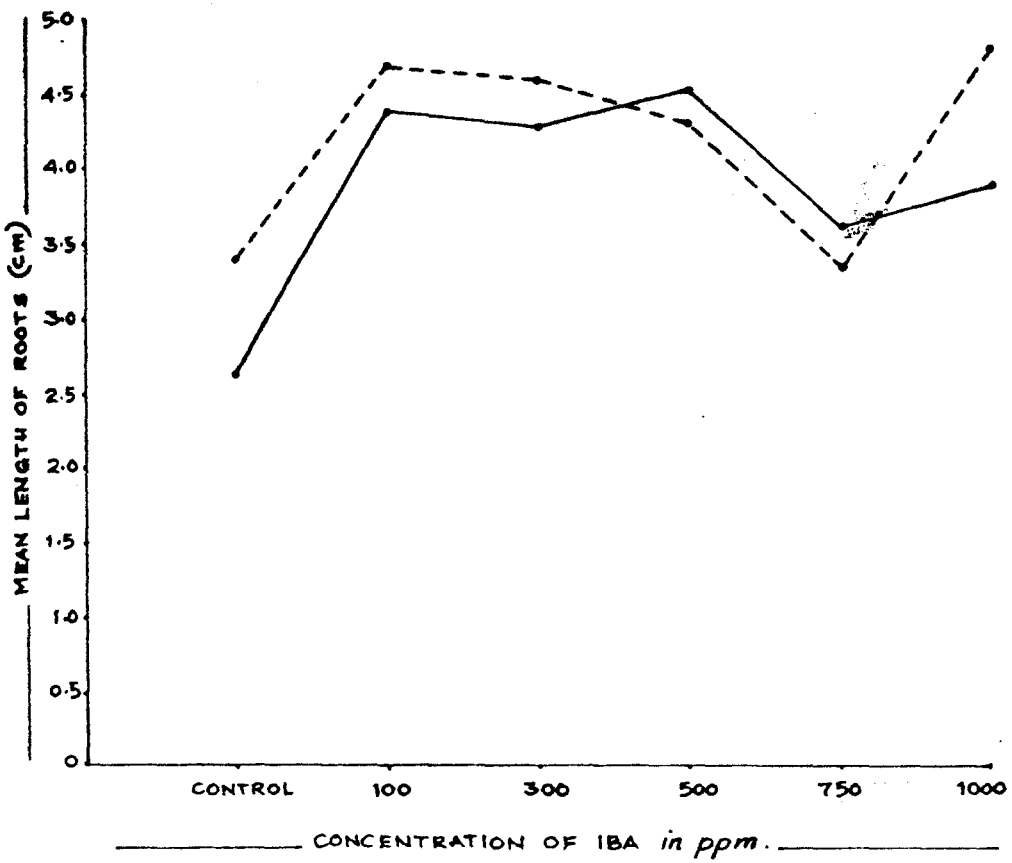


Fig-9. EFFECT OF NAA ON LENGTH OF ROOTS

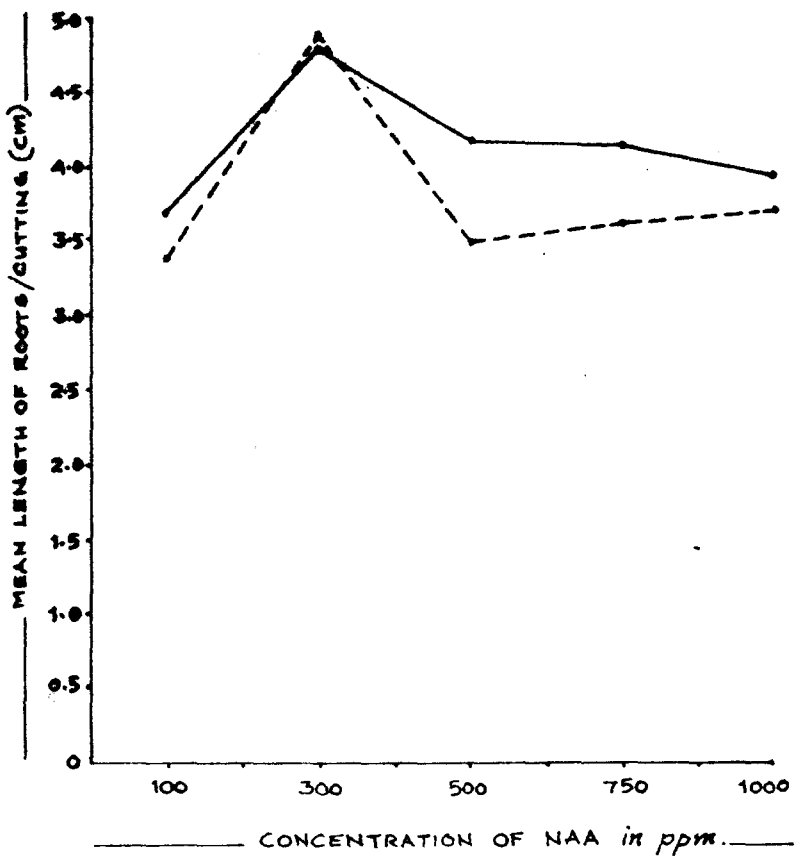
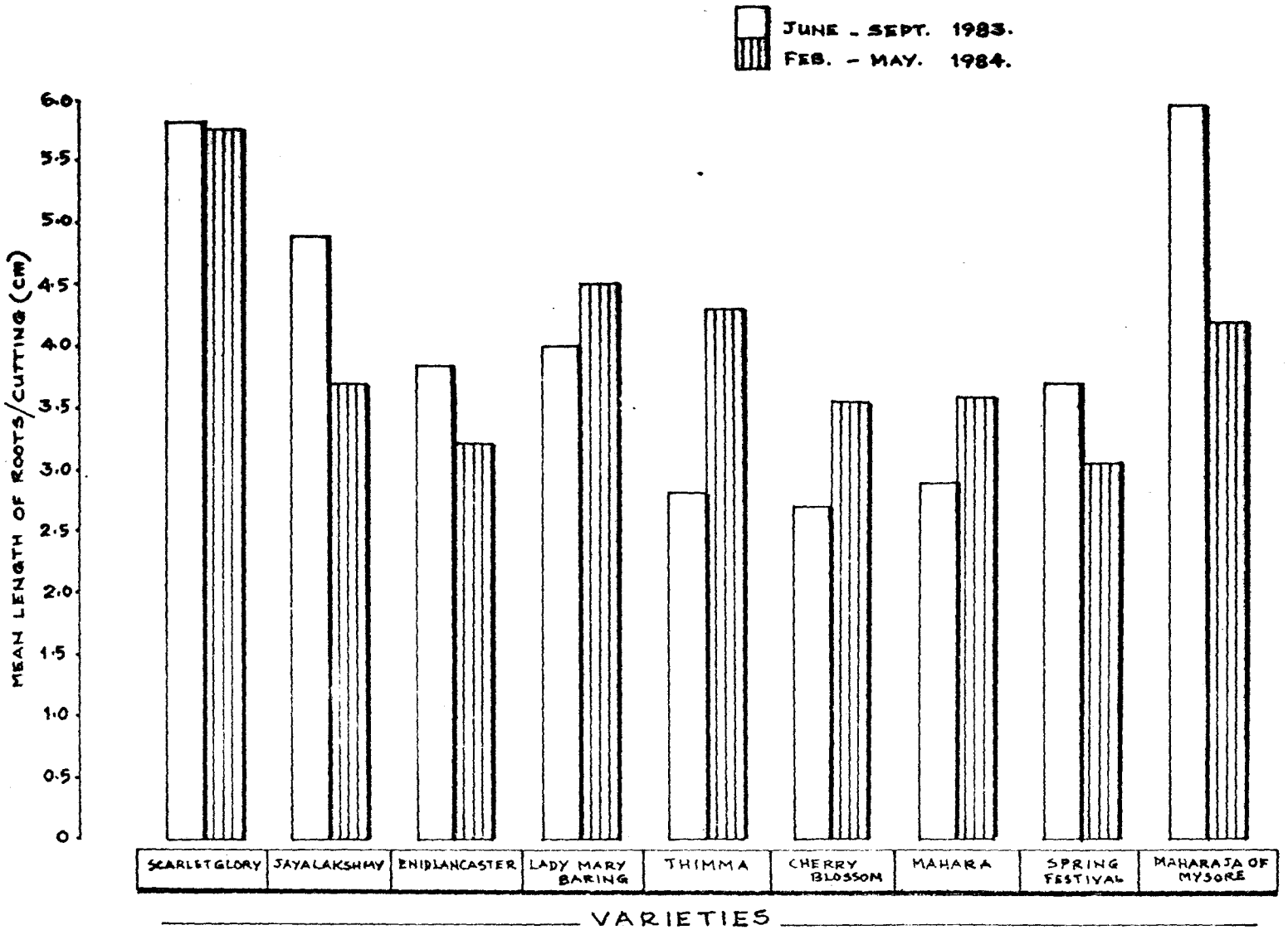


Fig - 10. VARIETAL RESPONSE ON LENGTH OF ROOTS



(4.94 cm) roots. Length of roots was more in all the auxin treated cutting than in the control. Statistical analysis of the pooled data to compare various treatment effects confirmed the significant difference between them (Appendix VII). A graphical representation of mean length of roots produced per cutting as influenced by IBA and NAA treatment may be seen in Fig.8 and 9. It could be observed that there was not much variation in length of roots between seasons.

The present study also indicated that the root length differed significantly between the varieties (Fig.10). Maximum length of roots was recorded by the varieties 'Maharaja of Mysore' (5.93 and 4.24 cm) and 'Scarlet Glory' (5.78 and 5.75 cm) during the two seasons. The results further indicated that in general, root length was less during summer season than in rainy season in cvs 'Scarlet Glory', 'Jayalakshmy', 'Enidlancaster' and 'Maharaja of Mysore', while, it was more during summer in varieties like 'Lady Mary Baring', 'Thimma', 'Cherry Blossom', 'Mahara' and 'Spring Festival'.

4.4. Number of shoots

The mean number of shoots produced per cutting of different varieties during the two seasons is presented in Tables 7a, 7b and 7c. The results showed that there was no significant change in the number of shoots produced with the

Table 7a. Effect of growth regulators on number of shoots/cutting one month after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	2.5	3.5	2.5	2.5	3.0	2.0	3.0	3.0	3.0	3.0	1.5	2.68	
	Summer	1.5	3.5	2.5	1.5	3.0	3.0	2.5	3.0	1.5	2.5	3.5	2.45	
Jayalakshmy	Rainy	2.0	2.0	3.0	2.5	3.5	2.5	2.0	2.5	2.0	2.0	1.5	2.31	
	Summer	1.5	3.0	1.5	1.5	2.5	1.0	2.5	1.5	3.0	1.0	2.5	1.95	CD = 1.43
Enidlancaster	Rainy	1.5	2.5	4.0	2.5	2.5	3.5	3.0	3.0	2.5	2.5	2.0	2.68	0.64
	Summer	2.0	2.0	2.5	2.5	2.0	3.0	2.0	2.5	2.5	2.5	4.0	2.50	
Lady Mary Baring	Rainy	3.0	3.5	3.0	1.5	1.5	2.5	3.5	2.0	1.5	1.5	2.0	3.95	
	Summer	2.5	2.0	2.5	1.5	2.0	2.5	1.5	2.5	3.0	1.0	2.0	2.00	
Thimma	Rainy	1.0	1.5	2.5	2.5	2.5	3.0	1.5	2.0	2.0	1.0	1.0	1.86	
	Summer	1.5	2.0	2.0	2.0	1.5	2.0	1.5	3.5	1.5	1.0	1.0	1.77	SEM 0.511
Cherry Blossom	Rainy	1.5	3.5	3.0	2.5	2.0	2.5	2.5	3.0	2.5	2.5	2.5	2.54	0.226
	Summer	1.0	2.0	4.0	2.5	3.5	3.5	2.0	2.0	1.5	3.0	1.0	2.40	
Mahara	Rainy	1.5	3.0	2.0	3.5	3.0	3.0	3.0	2.5	2.5	2.0	2.5	2.59	
	Summer	2.0	2.5	4.0	4.0	1.5	5.5	1.5	1.0	4.5	2.5	1.5	2.81	
Spring Festival	Rainy	1.5	2.0	1.5	3.0	1.0	2.0	1.0	2.0	2.0	1.0	1.0	1.63	
		3.5	5.5	4.5	3.0	2.5	3.5	3.5	2.5	2.5	2.5	5.0	3.59	
Maharaja of Mysore	Rainy	2.5	3.5	3.5	4.0	1.5	2.0	2.5	3.0	3.0	1.0	1.5	2.59	
	Summer	2.0	2.5	2.5	3.0	2.5	3.5	1.0	2.0	2.5	2.5	2.0	2.36	
		1.88	2.77	2.77	2.72	2.27	2.55	2.44	2.61	2.05	1.83	1.72		
		1.94	2.77	2.94	2.44	2.22	3.05	2.00	2.38	2.5	2.06	2.38		

CD = 1.58 / 0.70 SEM = 0.565 / 0.250

Table 7b. Effect of growth regulators on number of shoots/cutting two months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	2.5	3.5	2.5	2.5	3.5	2.5	4.0	3.0	2.0	2.5	3.0	2.95	
	Summer	1.5	2.5	3.0	2.0	2.0	2.5	3.0	1.5	2.0	2.0	2.5	2.22	
Jayalakshmy	Rainy	2.5	2.5	3.0	3.0	2.5	3.5	2.0	3.0	2.0	3.5	3.5	2.81	
	Summer	1.5	3.5	1.5	2.0	2.0	1.0	3.0	1.5	2.0	3.0	1.0	2.00	CD = 0.807
Enidlancaster	Rainy	1.0	1.5	2.0	2.0	3.0	2.5	2.5	2.5	2.5	2.5	2.0	2.18	0.492
	Summer	1.0	4.0	2.0	2.5	2.0	2.0	2.5	2.0	2.0	1.0	2.5	2.31	
Lady Mary Baring	Rainy	2.0	1.5	2.5	2.0	2.5	3.5	2.5	3.5	3.5	2.5	3.5	2.54	
	Summer	1.5	2.0	2.5	3.0	3.5	2.0	1.5	2.5	3.0	3.0	2.0	2.22	
Thimma	Rainy	2.0	2.5	1.5	1.5	1.5	3.5	3.5	2.5	2.5	2.0	2.5	2.31	SEM
	Summer	1.0	1.5	2.0	1.5	2.0	2.0	2.0	1.5	2.0	1.5	2.0	1.72	0.286
Cherry Blossom	Rainy	2.0	3.0	3.5	3.0	3.0	3.5	5.0	3.0	3.5	3.0	3.0	3.22	0.175
	Summer	3.0	4.5	4.0	2.5	3.0	1.5	1.5	2.0	3.0	2.0	2.5	2.77	
Mahara	Rainy	3.5	1.5	5.0	2.0	4.0	2.5	1.5	4.5	3.5	7.0	2.0	3.36	
	Summer	2.5	3.0	2.5	2.0	3.0	2.5	1.5	2.0	2.5	1.5	2.5	2.31	
Spring Festival	Rainy	1.0	2.5	2.5	5.5	4.5	1.5	1.5	2.0	2.5	4.0	2.5	2.72	
	Summer	2.5	3.0	3.5	4.5	2.5	4.5	2.5	1.5	2.0	3.0	2.0	2.86	
Maharaja of Mysore	Rainy	2.0	3.0	1.5	3.0	2.0	4.5	2.5	2.5	2.0	2.0	3.5	2.81	
	Summer	2.0	3.0	1.5	3.0	2.0	4.5	2.5	3.5	2.5	2.0	3.5	2.72	
		2.27	2.38	2.83	2.72	3.00	2.88	2.77	2.94	2.62	3.27	2.77		
		1.83	3.00	2.50	2.55	2.44	2.50	2.22	2.00	2.33	2.11	2.38		

CD = 0.892
0.544

SEM = 0.318
0.194

Table 7c. Effect of growth regulators on number of shoots/cutting three months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	2.5	4.0	3.5	3.5	2.5	3.5	3.5	2.5	2.5	2.5	3.5	3.04	
	Summer	2.5	2.0	2.0	2.5	2.5	2.5	2.5	2.0	1.5	2.5	1.5	2.18	
Jayalakshmy	Rainy	3.0	2.5	3.0	2.0	3.5	2.5	3.5	3.5	2.5	2.5	3.5	2.90	
	Summer	2.0	1.5	2.0	2.0	1.5	2.5	2.5	1.5	2.0	1.5	2.5	1.95	CD = 0.659
Enidlancaster	Rainy	4.0	4.0	3.0	3.5	3.0	4.0	3.5	2.5	4.0	2.0	2.5	3.27	0.39
	Summer	3.5	1.5	2.5	1.0	1.5	2.0	2.0	3.0	2.0	2.0	3.0	2.18	
Lady Mary Baring	Rainy	3.5	4.0	2.5	3.0	3.0	3.0	3.0	3.5	2.5	3.0	2.5	3.00	
	Summer	1.0	2.0	1.5	1.0	1.5	2.0	3.0	3.5	2.0	1.5	2.0	1.90	
Thimma	Rainy	2.5	3.0	2.0	3.0	1.0	2.5	3.0	2.5	3.5	2.5	1.5	2.45	SEM
	Summer	1.0	1.0	1.5	2.0	1.5	1.5	1.5	2.0	1.5	2.0	2.0	1.63	0.23
Cherry Blossom	Rainy	3.0	2.0	3.5	3.0	1.5	3.5	3.0	3.0	3.0	1.5	3.0	2.72	0.142
	Summer	2.5	2.5	2.0	3.0	2.5	1.5	3.0	1.5	1.5	3.0	2.5	2.36	
Mahara	Rainy	2.5	4.0	2.5	2.0	3.5	2.5	3.0	3.0	4.0	2.0	3.0	2.90	
	Summer	2.0	2.0	2.0	1.5	2.5	2.0	2.5	1.5	2.5	1.5	1.0	1.90	
Spring Festival	Rainy	1.0	3.0	2.0	1.5	2.5	2.5	3.0	2.0	2.5	2.5	2.5	2.27	
	Summer	3.0	2.0	2.5	1.5	2.5	3.5	2.0	1.5	3.0	2.5	2.5	2.45	
Maharaja of Mysore	Rainy	3.0	3.5	4.5	1.5	2.5	2.5	2.5	1.5	3.0	4.0	2.0	2.81	
	Summer	1.0	1.0	1.5	3.5	2.5	2.0	2.0	3.5	3.0	2.5	3.0	2.31	
		2.77	3.33	2.94	2.50	2.55	2.94	3.11	2.66	2.83	2.72	2.66		
		2.05	1.72	2.05	2.0	2.05	2.22	2.05	2.33	2.33	2.05	2.22		

CD = 0.729
0.440

SEM = 0.26
0.157

various concentration of IBA and NAA during rainy season (Appendix VIII). But during summer, the treatment difference in number of shoots was significant in the early period. The number of shoots produced per cutting was more during rainy season than in summer in almost all the treatments(Table 7c). The mean number of shoots was maximum (3.33) in IBA 100 ppm treatment three months after planting during rainy season, while it was maximum (2.33) in NAA 300 ppm treatment in summer. The number of shoots was not found to be influenced by the method of treatments. Shoot production was confined to the top most two to three nodes.

The data on the number of shoots per cutting in different varieties indicated significant variation between season. Maximum number of shoots were produced in the variety 'Enidlancaster' (3.95) during rainy season, while it was maximum in 'Spring Festival' (3.59) during summer, one month after planting.

4.5. Fresh weight of the shoots

The data on the mean fresh weight of shoots produced per cutting in different treatments at monthly intervals are presented in Tables 8a, 8b and 8c and the results of the statistical analysis in Appendix IX. The results revealed the significant influence of growth regulators on the fresh

Table 8a. Effect of growth regulators on fresh weight of shoots (g)/cutting one month after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	0.37	0.12	0.62	0.12	1.40	1.47	1.26	0.46	0.92	0.61	0.59	0.819	CD =
	Summer	0.149	1.16	1.315	2.01	4.25	2.62	1.70	1.45	1.53	0.62	1.53	1.583	346.5
Jayalakshmy	Rainy	0.65	1.10	1.12	1.37	0.875	1.28	1.29	1.41	0.43	0.80	0.350	1.386	304.3
	Summer	1.19	1.61	1.252	1.47	1.35	1.93	1.32	1.45	1.59	2.68	2.14	1.570	SEM
Enidlaneaster	Rainy	0.37	1.55	0.74	0.89	0.59	0.98	0.55	0.94	0.41	0.24	0.485	0.769	123.65
	Summer	0.57	1.78	2.22	2.29	0.900	1.18	0.90	1.28	0.55	0.94	1.150	1.199	108.56
Lady Mary Baring	Rainy	0.38	0.44	1.30	1.54	0.58	0.85	0.107	0.15	0.89	0.71	0.157	0.859	
	Summer	0.64	0.85	2.25	1.24	0.80	1.10	0.49	0.78	1.32	0.73	0.457	0.896	
Thimma	Rainy	0.19	0.73	0.33	0.63	0.91	0.38	0.36	0.32	0.48	0.35	0.12	0.424	
	Summer	0.36	0.79	1.25	0.73	1.06	1.47	1.13	1.22	1.00	0.56	1.12	0.969	
Cherry Blossom	Rainy	0.12	0.45	0.81	0.71	1.03	0.24	0.13	0.45	0.13	0.17	0.28	0.342	
	Summer	0.28	0.53	0.91	0.80	1.25	1.29	0.28	0.70	0.53	0.85	0.802	0.615	
Mahara	Rainy	0.16	0.62	0.68	0.27	0.88	1.07	0.47	0.23	1.19	0.45	0.162	0.510	
	Summer	0.30	1.01	1.49	1.35	0.99	1.12	0.53	1.12	0.14	1.14	0.83	1.004	
Spring Festival	Rainy	0.24	0.35	0.43	0.40	0.22	0.29	0.30	0.24	0.15	0.19	0.26	0.320	
	Summer	0.27	0.36	0.55	0.50	0.43	0.32	0.19	0.51	0.49	0.30	0.33	0.350	
Maharaja of Mysore	Rainy	0.15	0.51	0.86	0.69	0.18	0.21	0.61	0.27	0.77	0.16	0.51	0.530	
	Summer	0.10	1.22	1.77	0.92	1.72	1.20	1.70	1.02	1.04	1.45	0.48	1.060	
		0.25	0.84	0.88	0.16	0.59	0.75	0.76	0.57	1.19	0.41	0.32		
		0.43	0.84	1.33	1.23	1.27	1.42	0.83	0.99	0.89	1.08	0.98		

CD = 383.17
336.42

SEM = 136.7
120.02

Table 8b. Effect of growth regulators on fresh weight of shoots (g)/cutting two months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean
Scarlet Glory	Rainy	4.45	4.51	4.40	0.75	2.82	4.77	3.97	4.00	2.44	2.06	2.60	3.624
	Summer	1.88	3.05	3.36	3.43	4.58	6.30	2.70	4.56	7.45	4.34	5.05	4.59
Jayalakshmy	Rainy	2.97	3.21	3.74	2.90	3.03	3.95	2.33	2.55	1.59	2.33	2.02	2.916
	Summer	2.37	2.42	1.81	4.34	6.81	3.03	3.11	2.39	2.25	3.09	3.00	2.988
Enidlancaster	Rainy	0.56	1.50	1.91	1.93	1.65	3.27	3.30	2.11	3.18	1.44	2.06	2.132
	Summer	2.00	4.56	2.72	3.05	3.09	5.41	2.61	1.63	3.31	1.87	2.68	2.900
Lady Mery Baring	Rainy	1.36	3.13	4.40	2.33	2.39	2.91	0.96	1.00	2.20	2.28	2.64	2.50
	Summer	1.59	2.36	1.19	2.57	4.59	1.37	3.48	2.84	5.46	2.02	3.59	2.65
Thimma	Rainy	2.49	2.18	1.45	2.08	1.09	1.79	1.48	1.98	1.64	1.96	1.66	1.86
	Summer	2.88	2.98	1.71	2.42	4.68	2.37	2.15	4.23	1.76	2.10	2.21	2.66
Cherry Blossom	Rainy	0.83	2.10	2.15	4.01	0.66	1.25	2.16	1.28	1.23	1.32	0.76	1.62
	Summer	2.89	2.38	5.24	4.71	3.57	2.75	1.32	3.01	2.67	2.24	2.19	2.99
Mahara	Rainy	0.35	1.86	2.56	1.08	1.50	1.85	2.24	1.91	1.89	1.18	2.89	1.84
	Summer	1.21	1.23	3.13	6.99	3.56	2.71	0.99	2.01	2.38	2.10	2.91	2.57
Spring Festival	Rainy	0.55	0.50	0.56	0.88	0.64	0.83	0.49	0.51	0.89	1.01	1.03	0.79
	Summer	0.64	1.09	0.78	3.29	1.68	1.68	1.97	1.33	0.86	2.33	1.47	1.48
Maharaja of Mysore	Rainy	1.90	5.50	2.75	2.72	1.25	3.60	1.26	2.86	2.55	3.80	1.77	2.72
	Summer	1.26	3.57	4.45	5.12	1.13	6.70	2.79	3.16	6.62	4.55	4.61	4.00
		1.71	2.76	2.66	2.38	1.67	2.73	2.02	2.34	1.97	2.18	2.05	
		1.86	2.63	2.71	4.44	3.71	3.53	2.35	2.49	3.62	2.43	3.02	

CD = 0.796, 0.283

SEM = 284.1, 101.01

CD = 1487.17, 949.2

SEM = 314.13, 111.67

Table 8c. Effect of growth regulators on fresh weight of shoots (g)/cutting three months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	5.95	4.76	8.00	8.85	5.59	8.15	5.75	6.07	4.66	3.51	5.17	5.86	CD =
	Summer	6.70	8.25	11.47	10.35	7.45	7.40	6.67	6.80	6.80	7.49	7.53	7.90	1.34
Jayalakshmy	Rainy	6.48	4.45	13.49	5.12	4.53	6.12	4.61	7.55	4.50	5.93	5.57	6.21	0.86
	Summer	2.68	4.35	5.80	4.46	5.79	4.25	4.83	4.22	4.82	4.44	1.84	4.32	
Enidlancaster	Rainy	1.94	4.11	3.14	3.15	3.49	3.69	4.07	5.75	3.34	2.18	2.89	3.43	SEM
	Summer	2.78	5.65	3.02	5.57	4.61	5.42	6.75	3.21	5.50	2.15	3.12	4.34	0.479
Lady Mary Baring	Rainy	2.61	6.50	7.66	2.30	2.77	4.16	5.11	3.49	5.29	3.02	2.31	4.11	0.306
	Summer	2.40	8.33	7.26	5.22	5.80	3.54	4.00	5.45	5.84	2.89	5.17	5.08	
Thimma	Rainy	2.60	2.73	3.00	3.04	2.27	2.92	2.33	2.76	2.02	2.64	2.05	2.58	
	Summer	1.74	3.37	4.69	5.10	5.45	4.71	3.78	5.38	4.95	3.99	2.75	4.12	
Cherry Blossom	Rainy	1.95	3.06	2.32	3.45	2.74	2.15	2.84	2.50	2.64	1.92	2.50	2.55	
	Summer	4.05	3.80	7.26	4.80	4.30	7.12	4.49	3.24	3.90	3.17	3.23	4.28	
Mahara	Rainy	1.02	3.55	2.63	2.73	3.49	2.38	2.65	2.46	2.26	2.43	3.83	2.68	
	Summer	3.01	7.35	7.87	7.82	6.13	6.98	1.71	3.90	4.48	4.69	3.99	5.27	
Spring Festival	Rainy	0.90	1.89	1.44	1.29	1.96	1.43	1.31	1.62	1.31	1.41	1.34	1.50	
	Summer	1.74	2.15	1.95	2.55	2.85	2.43	3.63	1.44	1.93	3.16	3.51	2.49	
Maharaja of Mysore	Rainy	4.79	7.49	5.82	4.40	2.58	7.37	3.30	3.78	3.60	5.28	2.55	4.63	
	Summer	2.58	4.51	5.08	5.33	6.29	6.34	5.55	6.48	8.82	4.71	5.07	5.69	
		3.13	4.28	5.29	3.81	3.26	4.04	3.55	4.00	3.00	3.14	3.13		
		3.08	5.31	6.04	5.68	5.40	5.34	4.60	4.46	5.16	4.08	3.97		

CD = 1.49
0.95

SEM = 0.479
0.530

weight of shoots. The shoot weight was minimum for the control (3.12 g, 3.07 g respectively during rainy season and summer season) while it was maximum (5.28 g and 6.04 g respectively for the corresponding periods) in the case of IBA 300 ppm (Table 8c). The results further indicated that of the two growth regulators used, IBA at all concentrations produced higher shoot weight compared to the corresponding levels of NAA. The method of treatment did not seem to influence the shoot weight.

The data on the effect of growth regulators on shoot weight of different varieties are also furnished in Tables 8a, 8b and 8c. In all the varieties, the shoot weight was more during summer season, except in the variety 'Jayalakshmy' which produced maximum fresh weight of shoots/cutting 6.2 g during rainy season followed by 'Scarlet Glory' (5.86 g). But the variety 'Scarlet Glory' ranked first during summer with a shoot weight of 7.9 g. It was minimum for 'Spring Festival' (1.49 g and 2.48 g) irrespective of the seasons.

4.6. Fresh weight of roots

The consolidated data pertaining to the fresh weight of roots produced per cutting in different treatments and varieties at monthly intervals are tabulated in Tables 9a, 9b and 9c and the results of the statistical analysis are furnished in Appendix X. From the results it was found that

Table 9a. **Effect of growth regulators on fresh weight of roots (mg)/cutting one month after planting**

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	82.5	137.5	345.0	170.0	100.0	120.0	275.0	130.0	340.0	117.5	77.5	172.27	
	Summer	62.5	129.0	92.5	125.0	71.5	110.0	91.5	126.0	111.5	80.0	98.5	99.81	
Jayalakshmy	Rainy	57.5	102.5	100.0	435.0	85.0	225.0	102.5	160.0	131.0	175.0	126.5	154.54	CD=
	Summer	41.5	136.5	250.0	334.0	110.0	106.5	260.0	140.0	180.0	105.0	122.5	162.36	32.36
Enidlancaster	Rainy	17.0	150.0	200.0	170.0	95.0	117.5	325.0	285.0	297.5	85.0	90.0	165.63	10.58
	Summer	21.5	90.5	97.5	163.0	77.0	102.5	70.0	93.5	118.0	50.0	60.0	85.77	
Lady Mary Baring	Rainy	27.5	42.5	150.0	127.5	80.0	67.5	45.5	14.5	161.5	32.5	40.5	71.77	SEM
	Summer	16.5	42.0	41.5	52.0	25.5	34.5	18.0	26.5	51.5	17.0	18.0	31.18	11.54
Thimma	Rainy	13.5	44.0	61.5	81.5	52.5	92.5	70.0	95.0	125.0	29.0	27.5	62.90	3.77
	Summer	20.5	34.5	37.5	40.5	21.5	18.0	17.0	27.0	51.5	22.5	38.5	29.90	
Cherry Blossom	Rainy	17.5	30.0	47.50	39.0	27.5	26.5	21.0	47.5	22.5	19.0	42.5	30.95	
	Summer	15.0	21.0	26.5	28.5	16.5	18.0	17.0	28.5	20.0	16.0	14.5	20.13	
Mahara	Rainy	19.0	97.5	47.0	37.5	19.0	102.0	41.5	57.5	110.0	47.5	22.5	54.68	
	Summer	17.0	31.0	20.0	38.5	20.0	30.5	26.0	30.5	38.0	19.5	20.0	26.45	
Spring Festival	Rainy	13.0	16.0	26.0	29.5	14.5	22.5	16.0	27.5	35.5	20.0	22.5	22.09	
	Summer	14.5	22.0	35.0	43.0	22.0	32.0	31.5	43.5	37.5	26.0	29.0	30.54	
Maharaja of Mysore	Rainy	10.0	61.5	42.5	57.5	77.5	107.5	49.5	21.0	61.5	47.5	24.0	50.90	
	Summer	29.5	35.5	56.0	65.0	45.0	36.5	40.0	32.5	37.0	23.0	30.5	39.13	
		27.5	75.72	113.27	129.7	61.22	97.44	105.05	93.16	142.72	63.66	52.11		
		25.3	60.22	72.9	98.83	45.38	55.55	63.44	59.27	71.06	39.88	48.88		

CD = 35.78
11.70

SEM = 12.76
4.17

Table 9b. Effect of growth regulators on fresh weight of roots (mg)/cutting two months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean
Scarlet Glory	Rainy	275.0	587.5	1400.0	322.5	320.0	775.0	1465.0	1070.0	1075.0	305.0	192.5	707.95
	Summer	134.5	185.0	177.5	400.0	265.0	165.0	241.5	229.0	484.0	201.5	269.0	250.18
Jayalakshmy	Rainy	110.0	264.0	147.5	182.5	190.0	132.5	172.5	212.5	187.5	210.0	156.5	178.68
	Summer	105.0	175.0	437.5	440.0	344.0	127.5	270.0	380.0	450.0	265.0	220.0	292.18
Enidlancaster	Rainy	80.0	242.5	234.0	345.0	170.0	220.0	432.5	462.5	302.5	118.5	309.0	265.13
	Summer	90.0	160.0	119.5	220.0	115.0	127.5	115.0	135.0	144.5	110.0	109.0	131.40
Lady Mary Baring	Rainy	80.0	157.5	195.0	142.5	105.0	135.0	136.5	155.0	141.5	127.5	150.0	138.68
	Summer	75.0	102.5	117.5	115.0	156.5	126.0	115.0	106.0	132.0	117.5	97.5	114.27
Thimma	Rainy	76.5	127.5	115.0	382.5	102.5	152.5	147.5	157.5	277.5	140.0	80.0	159.90
	Summer	75.0	160.0	195.0	390.0	99.0	111.0	87.5	126.0	197.0	132.5	117.5	154.50
Cherry Blossom	Rainy	82.5	92.5	137.5	170.0	70.0	89.0	115.0	85.0	90.0	87.5	107.5	70.68
	Summer	55.0	71.5	66.5	110.0	44.0	78.0	89.0	62.5	87.5	67.5	42.5	102.40
Mahara	Rainy	87.5	117.5	110.0	122.5	80.0	105.0	122.5	150.0	135.0	87.5	97.5	97.45
	Summer	61.5	125.5	115.0	132.0	107.5	100.0	79.0	87.5	115.0	80.0	70.0	110.54
Spring Festival	Rainy	55.0	62.5	85.0	117.0	62.5	103.5	47.5	110.0	112.5	121.5	117.5	88.95
	Summer	66.5	87.5	70.0	120.5	90.0	101.5	77.5	106.5	110.5	75.0	72.5	90.45
Maharaja of Mysore	Rainy	112.5	587.5	405.0	175.0	77.5	387.5	127.5	377.5	375.0	170.0	177.5	123.68
	Summer	75.0	92.5	127.5	237.5	80.5	77.5	112.5	150.0	205.0	87.5	115.0	270.22
		106.55	248.77	314.33	217.77	130.8	233.3	307.8	308.88	299.6	151.9	154.2	
		65.83	128.33	158.44	240.54	145.2	112.7	132.3	153.61	213.9	126.3	123.7	

CD = 109.88, 32.07, 99.39, 29.01

SEM = 39.20, 11.44, 35.46, 10.35

Table 9c. Effect of growth regulators on fresh weight of roots (mg)/cutting three months after planting.

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	1567.5	1655.0	1812.5	1675.0	1000.0	1900.0	1300.0	1680.0	2527.5	200.0	400.0	1428.86	
	Summer	335.0	530.0	687.5	912.5	362.5	430.0	296.5	640.0	770.0	537.0	477.5	543.50	
Jayalakshmy	Rainy	507.5	370.0	800.0	578.5	460.0	306.0	460.0	935.0	725.0	770.0	522.5	584.95	
	Summer	324.5	469.5	669.0	660.0	614.0	367.5	450.6	501.5	882.5	349.5	385.0	515.77	
Enidlancaster	Rainy	197.5	492.5	285.0	422.5	301.5	377.5	407.5	565.0	337.5	157.5	340.0	353.09	CD
	Summer	135.0	200.0	412.5	249.0	150.0	120.0	462.5	164.5	253.0	119.5	128.5	217.22	206.61
Lady Mary	Rainy	142.5	362.5	312.5	127.5	130.0	222.5	185.0	281.5	302.5	192.5	240.0	227.18	91.56
Baring	Summer	150.0	340.0	377.5	382.5	200.0	275.0	157.5	394.0	420.0	135.0	265.0	218.5	
Thimma	Rainy	167.5	277.5	282.5	575.0	245.0	287.5	512.5	347.5	220.0	162.5	142.5	292.72	SEM
	Summer	124.5	247.5	285.0	605.0	155.0	387.5	205.0	532.5	467.5	405.0	137.5	322.86	73.71
Cherry	Rainy	92.5	220.0	227.5	168.0	633.0	182.5	162.0	153.5	192.5	120.0	165.0	210.59	32.66
Blossom	Summer	142.5	335.0	284.5	599.5	264.0	210.0	185.0	188.0	214.0	181.5	162.5	251.5	
Mahara	Rainy	132.5	220.0	242.5	233.0	173.0	182.5	137.5	158.0	147.5	220.0	142.5	180.81	
	Summer	127.5	284.0	237.5	375.0	265.0	151.5	142.5	225.0	200.0	150.0	145.0	209.36	
Spring	Rainy	132.5	172.5	196.5	242.5	902.5	140.5	115.0	199.0	155.0	132.5	117.5	227.81	
Festival	Summer	127.5	150.0	122.5	175.0	141.0	205.0	155.0	185.0	292.5	200.0	107.5	169.18	
Maharaja of	Rainy	316.5	789.0	272.0	277.0	254.0	845.0	266.5	414.0	295.0	845.0	327.0	445.91	
Mysore	Summer	109.0	119.0	290.0	314.5	137.5	266.5	352.5	587.5	560.5	200.0	212.5	286.31	
		261.83	506.56	492.33	477.67	455.44	493.77	394.05	525.88	544.77	311.11	366.33		
		175.05	297.16	374.00	474.33	254.33	268.11	267.38	379.77	451.11	253.05	224.05		

CD = 228.42
101.22

SEM = 81.49
36.11

Fig-11. EFFECT OF IBA ON WEIGHT OF ROOTS

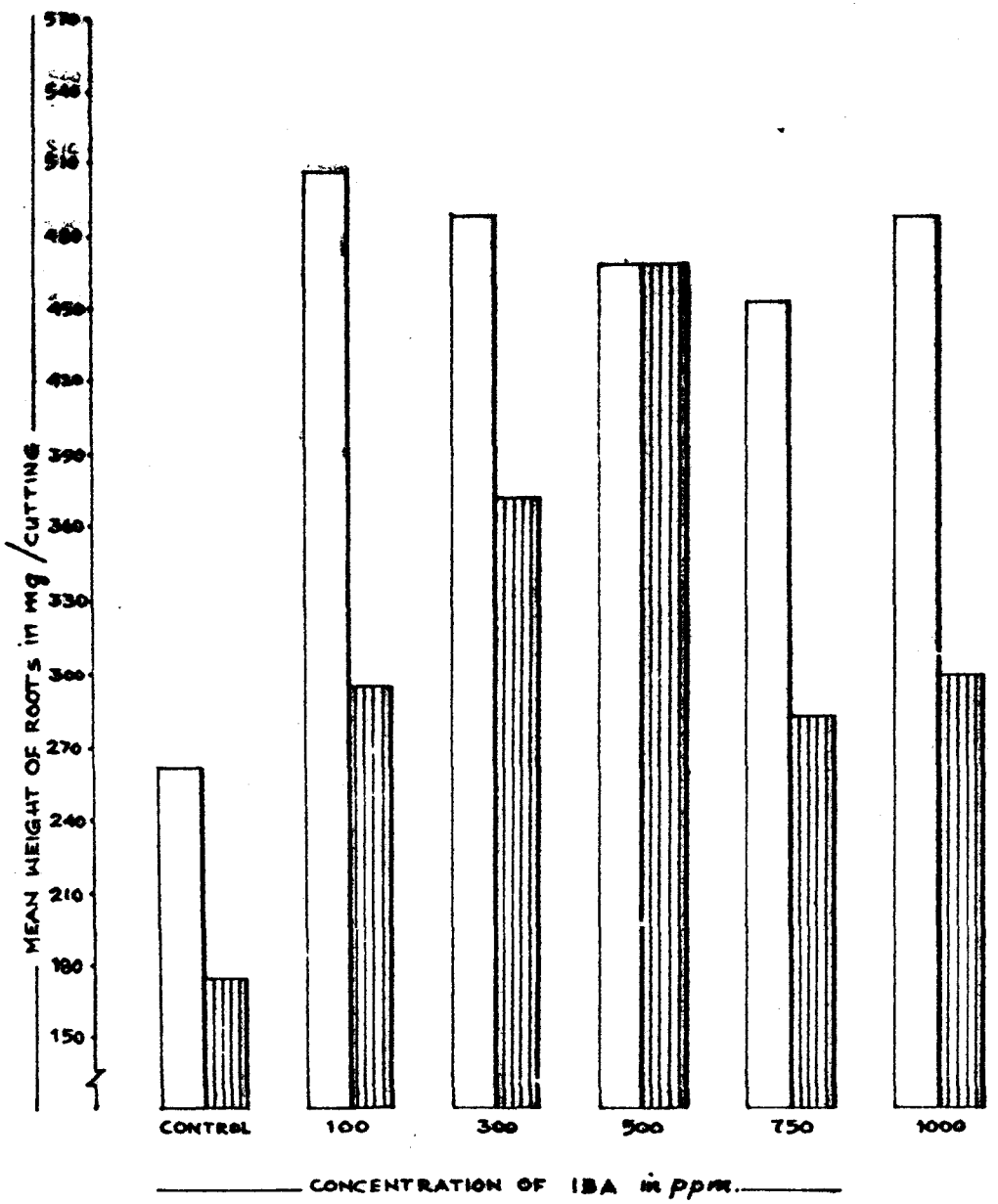


Fig-12. EFFECT OF NAA ON WEIGHT OF ROOTS

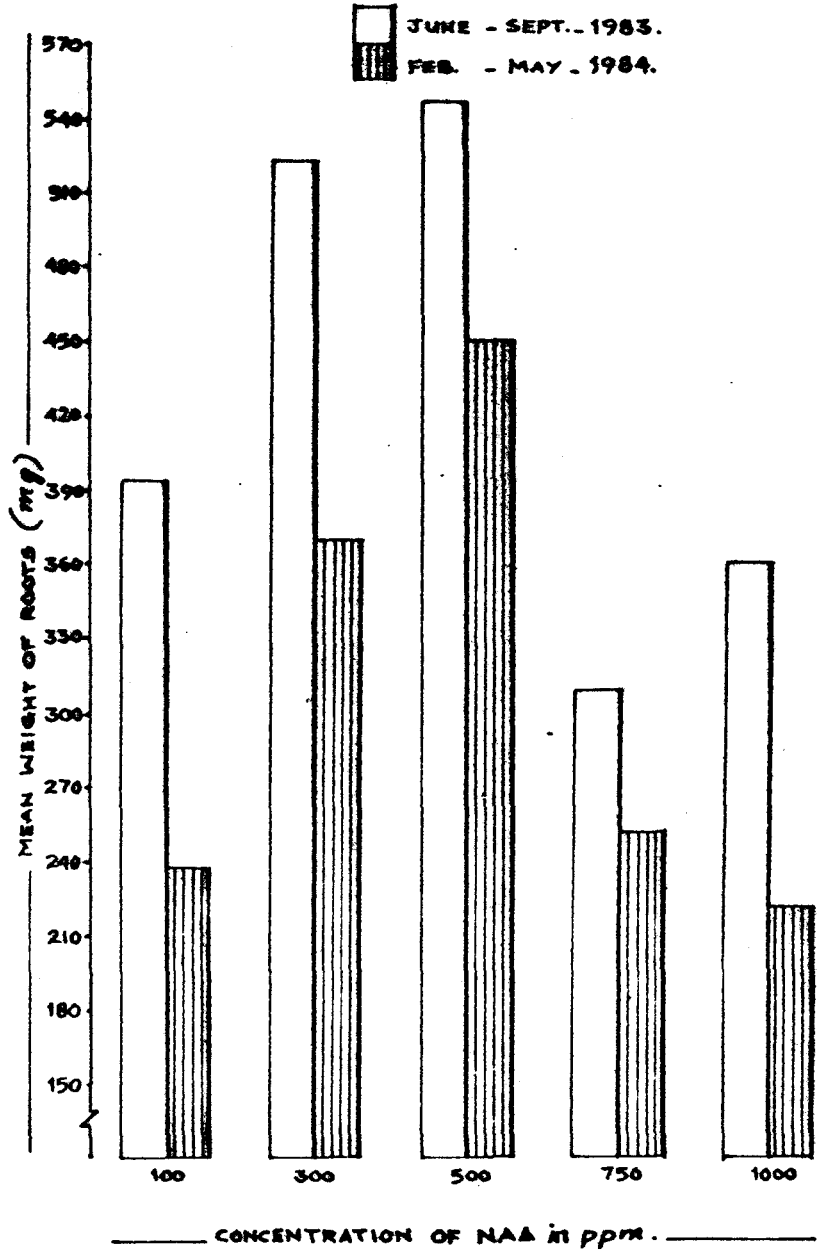
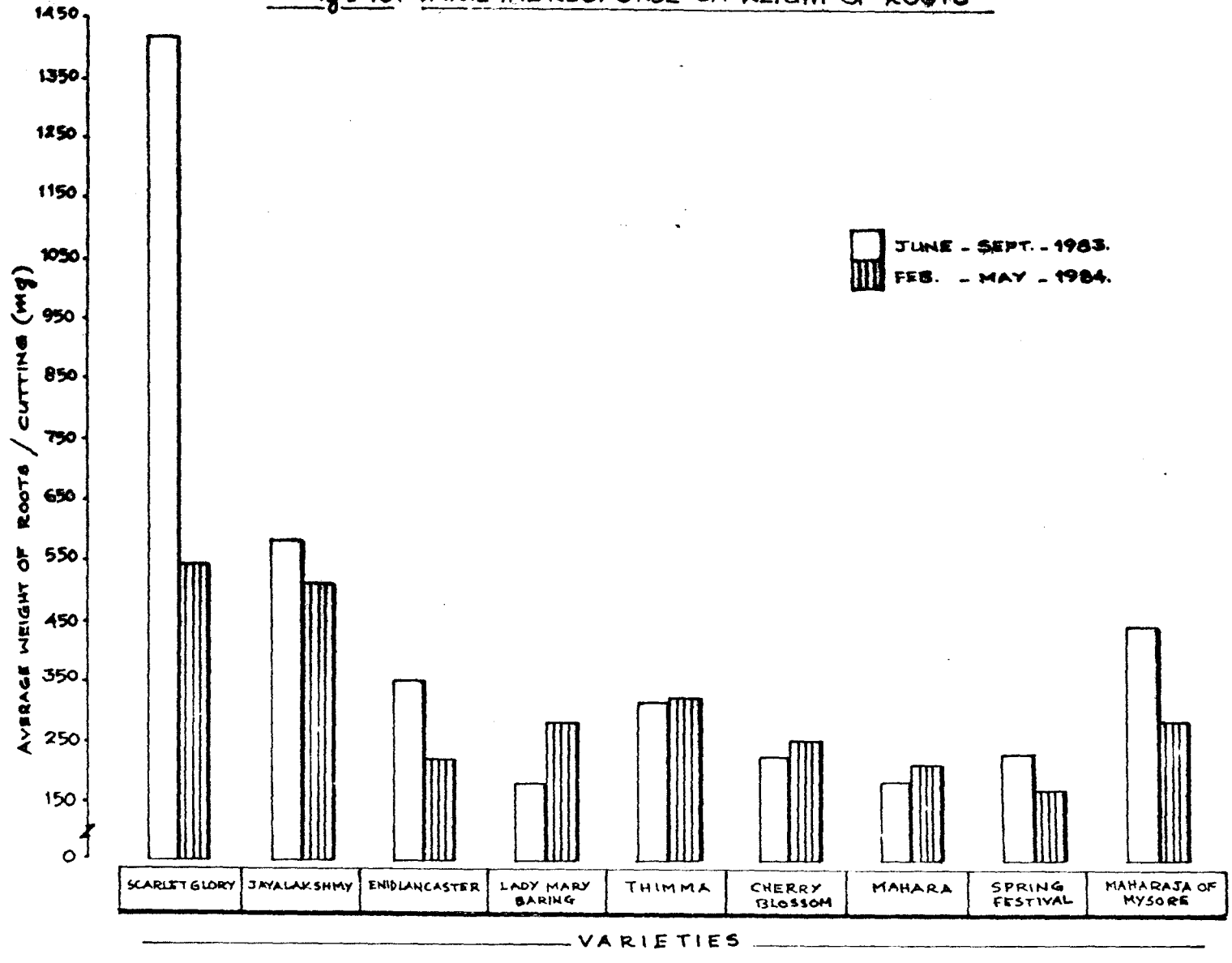


Fig - 13. VARIETAL RESPONSE ON WEIGHT OF ROOTS



all growth regulators induced a better root growth over the control (Fig.11 and 12). Between the two methods of growth regulator treatments, prolonged soak method in NAA 500 ppm, recorded the maximum fresh weight of roots (544.7 mg and 451.11 mg) irrespective of the seasons (Table 9c). The root weight was minimum during both the seasons for the cuttings which received no growth regulator treatment.

Fig. 13 depicts the variation in root weight in nine varieties of bougainvillea during rainy as well as summer seasons. The mean fresh weight of roots per cutting was maximum for 'Scarlet Glory' during both the seasons, although the root weight varied significantly between the two seasons. Cuttings of the varieties like 'Lary Mary Baring', 'Thimma', 'Cherry Blossom', 'Mahara' showed slight increase in root weight during summer, while it was more during rainy season, in other varieties viz. 'Scarlet Glory', 'Jayalakshmy', 'Enidlancaster', 'Spring Festival' and 'Maharaja of Mysore'. A positive relationship was noticed between shoot weight and root weight within a particular season (Table 8c and 9c).

4.7. Shoot/root ratio

The data on the effect of IBA and NAA treatments on shoot/root ratio at monthly intervals are furnished in

Tables 10a, 10b and 10c and the details of statistical analysis in Appendix XI. The results revealed that the growth regulators significantly influenced the shoot/root ratio, especially in the early periods. The influence was more pronounced in summer than in rainy season. It was interesting to note that the ratio was highest, one month after planting, which gradually decreased towards the third month. Among the various treatments NAA 750 ppm produced the highest ratio (15.6) during rainy season, while IBA 1000 ppm recorded the highest ratio (31.5) during summer (Table 10b). In general, the shoot/root ratio was more during summer season than in rainy season. Between IBA and NAA treatments, IBA produced comparatively higher ratio than the corresponding levels of NAA.

Among the different varieties, the shoot/root ratio varied significantly. In all the varieties, the ratio was more during summer than in rainy season. In summer, the ratio was highest (46.0) in the variety 'Cherry Blossom' and the lowest (13.17) in 'Jayalakshmy' (Table 10b). But in rainy season, the variety 'Lady Mary Baring' and 'Scarlet Glory' produced the maximum (18.2) and minimum (8.4) ratio respectively.

Inter correlation coefficient was worked out among the different root parameters like root number, length,

Table 10a. Effect of growth regulators on shoot : root ratio/cutting one month after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	4.49	8.83	4.92	6.13	14.41	12.21	8.46	3.75	4.70	5.59	7.28	7.34	
	Summer	2.37	8.65	14.58	16.63	22.65	23.50	13.59	11.78	7.88	8.51	5.74	12.35	
Jayalakshmy	Rainy	11.95	15.12	11.04	3.29	10.67	5.63	12.49	9.40	16.41	4.62	2.78	8.85	CD=
	Summer	28.44	8.07	5.06	4.18	12.31	18.48	5.21	10.18	8.59	24.84	17.60	12.99	2.6
Enidlancaster	Rainy	11.04	9.12	3.71	5.32	10.41	6.81	3.18	4.12	1.46	3.08	4.79	5.73	5.95
	Summer	23.72	19.64	25.04	13.92	7.66	11.57	11.63	10.29	4.63	29.12	20.40	16.11	
Lady Mary Baring	Rainy	13.36	18.92	16.78	12.53	7.08	10.65	19.49	9.97	4.40	22.47	3.86	12.64	SEM
	Summer	39.60	10.48	31.28	24.93	31.47	49.07	25.93	29.51	25.82	42.66	25.84	30.60	0.93
Thimma	Rainy	7.16	16.62	4.70	13.09	19.70	3.76	4.72	3.18	3.59	11.66	4.20	8.40	2.16
	Summer	17.80	27.45	33.00	18.17	50.66	83.00	54.21	45.01	19.50	27.32	29.85	36.95	
Cherry Blossom	Rainy	6.75	14.37	19.12	18.70	4.63	8.95	7.46	9.16	5.40	8.77	6.61	9.99	
	Summer	19.17	21.07	49.66	28.43	43.28	75.15	16.21	32.76	26.35	53.94	57.35	38.49	
Mahara	Rainy	8.76	5.71	14.61	6.84	4.55	10.50	11.14	4.00	13.72	8.56	8.12	8.77	
	Summer	17.36	35.25	65.85	35.77	49.19	38.26	21.33	37.31	31.23	33.97	47.89	37.56	
Spring Festival	Rainy	17.95	22.94	16.30	17.20	14.23	14.41	19.05	18.33	13.47	11.75	11.57	11.13	
	Summer	6.75	18.14	18.62	10.68	19.99	6.82	13.52	5.83	4.78	13.00	8.79	16.54	
Maharaja of Mysore	Rainy	8.84	17.82	20.08	11.68	2.21	1.87	12.94	12.72	14.92	3.63	21.69	11.67	
	Summer	7.36	16.00	32.03	14.39	40.67	34.87	41.26	33.99	21.76	63.28	15.42	29.19	
		10.03	14.38	12.36	10.48	9.76	8.31	10.99	8.29	8.03	8.90	7.882		
		18.06	18.30	30.57	18.56	30.87	37.81	22.54	24.06	16.73	32.96	25.39		

CD = 2.88
6.58

SEM = 1.02
2.35

Table 10b. Effect of growth regulators on shoot : root ratio/cutting two months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean
Scarlet Glory	Rainy	21.19	7.72	3.16	13.01	9.51	6.42	2.59	3.76	2.36	9.22	13.62	8.41
	Summer	13.97	17.89	21.10	18.79	19.98	43.22	12.31	19.91	16.86	25.10	19.41	20.78
Jayalakshmy	Rainy	26.48	12.17	26.73	15.20	15.80	29.70	13.98	11.08	8.64	13.60	19.66	17.50
	Summer	25.45	16.51	4.13	10.34	19.87	23.56	11.62	6.27	4.78	11.64	13.89	13.19
Enidlancaster	Rainy	7.00	4.26	8.05	7.89	10.07	16.22	7.30	4.29	10.78	16.24	7.03	9.01
	Summer	22.17	28.81	23.60	19.67	24.09	42.44	24.73	11.90	22.00	8.55	25.72	23.02
Lady Mary Baring	Rainy	16.76	19.29	22.56	16.24	23.71	21.15	7.55	18.88	16.78	20.65	17.55	18.28
	Summer	21.03	23.12	10.13	22.83	30.10	10.87	30.73	9.42	41.41	17.95	41.38	23.47
Thimma	Rainy	23.19	16.91	12.50	9.63	10.35	14.53	10.03	14.23	7.06	15.52	17.45	13.76
	Summer	11.63	18.96	8.88	6.23	49.28	16.09	21.09	27.59	9.66	14.86	22.99	18.84
Cherry Blossom	Rainy	10.21	19.23	15.56	23.87	8.28	13.46	7.46	13.58	13.78	11.50	6.57	14.07
	Summer	53.51	33.33	82.41	61.83	73.87	31.17	21.17	47.82	24.18	25.66	51.40	46.03
Mahara	Rainy	3.56	15.69	24.36	9.69	19.91	17.45	15.71	12.14	13.94	23.73	30.53	16.97
	Summer	19.78	10.68	27.32	61.09	36.75	27.67	25.98	28.52	18.05	15.47	47.40	27.78
Spring Festival	Rainy	9.58	11.10	6.55	7.49	10.97	7.65	9.65	11.44	7.81	8.32	8.56	9.01
	Summer	8.17	20.85	8.72	27.36	16.84	15.27	32.23	32.23	6.54	22.06	20.35	17.33
Maharaja of Mysore	Rainy	17.23	8.46	9.55	15.67	20.79	8.22	9.83	9.17	6.80	21.94	9.86	12.50
	Summer	15.68	41.66	61.00	46.17	15.54	47.31	26.84	16.76	28.91	55.94	50.39	35.76
		12.02	12.76	14.33	13.19	14.37	14.97	10.95	10.54	9.77	15.63	14.53	
		20.94	23.53	27.48	30.48	26.81	31.48	19.39	21.47	19.78	21.54	31.41	

CD = 4.16 / 5.64

SEM = 1.48 / 2.01

CD = 3.76 / 5.10

SEM 1.34 / 1.82

Table 10c. Effect of growth regulators on shoot : root ratio/cutting three months after planting

Variety	Season	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000	Mean	
Scarlet Glory	Rainy	4.37	3.44	4.85	5.28	5.57	3.25	5.42	4.21	1.89	4.13	12.96	5.034	
	Summer	21.38	15.59	17.65	12.02	20.67	17.22	22.68	11.45	8.35	15.08	16.77	16.27	
Jayalakshmy	Rainy	13.71	13.59	17.05	8.84	10.01	19.35	9.81	6.23	3.65	8.37	13.34	11.27	CD = 2.98 5.34
	Summer	8.22	9.25	8.58	7.02	11.08	12.73	10.92	8.34	9.02	9.91	10.34	9.58	
Enidlancaster	Rainy	9.65	9.17	11.37	6.92	13.37	12.53	9.98	11.28	10.85	13.68	8.28	10.64	SEM 1.06 1.90
	Summer	20.86	29.35	8.13	28.46	32.00	46.61	14.84	20.42	24.92	18.39	27.51	24.73	
Lady Mary Baring	Rainy	18.20	11.48	24.76	18.08	21.53	16.93	27.32	13.04	13.18	14.35	10.15	17.19	
	Summer	16.88	25.69	19.69	13.57	29.06	12.83	26.15	13.42	14.14	18.81	19.84	19.10	
Thimma	Rainy	12.88	10.17	10.48	8.81	9.22	9.40	8.45	7.94	9.87	17.83	14.78	10.95	
	Summer	14.17	13.46	16.59	8.74	34.94	12.66	18.36	12.88	11.91	9.94	16.34	15.45	
Cherry Blossom	Rainy	12.57	13.85	10.11	19.61	20.07	11.83	16.76	15.85	13.33	15.76	16.15	15.08	
	Summer	28.20	17.96	25.37	7.92	16.11	34.45	23.57	18.25	18.58	17.48	21.84	20.88	
Mahara	Rainy	5.75	16.01	11.02	11.68	20.14	13.15	19.32	15.50	16.30	11.04	27.05	15.18	
	Summer	24.45	32.49	39.48	18.58	23.73	46.54	12.12	17.56	22.45	30.34	27.13	26.68	
Spring Festival	Rainy	6.72	10.88	8.98	5.37	9.92	9.52	11.91	8.09	8.89	10.76	11.29	9.30	
	Summer	14.30	13.44	16.15	15.01	20.51	11.88	24.38	7.90	6.36	16.29	28.75	15.90	
Maharaja of Mysore	Rainy	15.95	9.43	20.04	15.31	10.11	8.34	17.76	9.91	12.62	6.53	10.30	12.39	
	Summer	24.17	37.96	36.94	19.21	46.02	27.82	20.44	11.24	14.54	23.68	23.89	25.97	
		11.40	10.95	13.18	11.10	13.33	11.59	14.08	10.23	10.06	11.39	13.81		
		19.18	21.75	20.93	14.50	26.01	24.74	19.27	13.50	14.49	17.77	21.38		

CD = 3.30
5.91

SEM = 1.17
2.11

shoot number, root weight, shoot weight and percentage of rooting and the details are presented in Appendices XII and XIII. The number of roots was positively and significantly correlated with the percentage of rooting both in rainy and summer seasons. Like wise a significant positive correlation was also observed between root length and shoot weight. Root length was positively and significantly correlated with rooting percentage in rainy season but it was not significant during summer. Significant correlation was also noticed between shoot weight and rooting percentage, number of shoots and rooting percentage, root weight and shoot weight (Appendix XII).

4.8. Changes in carbon and nitrogen during the course of rooting of cutting

The data on chemical analysis of the cuttings carried out for organic carbon (C) and total nitrogen (N) are summarised in Tables 11 and 12. From Table 11 it is evident that organic carbon content in the mother plant was significantly higher during rainy season compared to that in summer and the contents decreased as the rooting progressed. However, there was no significant change in its content between different varieties at any time of a particular season. A significant negative correlation was noticed between organic matter content and the percentage of rooting (Appendix XIV and XV) during rainy as well as summer season.

Table 11. Organic carbon content (%) in the mother plant and cuttings at fortnightly intervals

Variety	Season	Content in the mother plant (%)	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	Mean
Scarlet Glory	Rainy	42.6	34.6	23.98	22.03	21.38	19.76	19.44	26.25
	Summer	33.37	27.84	27.26	25.2	24.90	24.62	20.11	26.18
Jayalakshmy	Rainy	47.4	39.20	24.92	23.00	22.09	18.82	17.82	27.60
	Summer	32.75	32.12	32.12	30.75	30.78	28.78	21.84	29.84
Knidlancaster	Rainy	47.6	39.2	23.65	20.73	17.17	13.94	10.04	24.61
	Summer	35.96	35.3	35.3	37.5	34.96	32.4	24.14	33.65
Lady Mary Baring	Rainy	46.11	37.58	23.98	22.34	22.03	20.73	19.44	27.50
	Summer	36.9	34.23	30.11	26.24	25.19	24.11	23.51	28.56
Thimma	Rainy	49.11	37.90	24.3	23.98	23.32	21.70	19.76	28.68
	Summer	36.61	34.99	34.2	28.40	26.01	26.11	24.49	30.11
Cherry Blossom	Rainy	47.18	36.34	21.70	21.70	21.70	23.73	20.73	27.15
	Summer	36.93	36.11	34.95	34.32	29.84	29.12	27.11	32.88
Mahara	Rainy	44.51	34.61	23.54	20.88	19.44	17.82	17.49	25.20
	Summer	37.90	37.40	36.89	36.89	34.64	32.44	30.91	29.88
Spring Festival	Rainy	43.19	35.96	23.65	23.0	20.08	19.11	17.49	26.06
	Summer	37.50	36.20	36.19	33.58	32.10	30.90	30.90	33.91
Maharaja of Mysore	Rainy	46.41	40.10	23.65	20.41	20.08	20.08	18.46	27.02
	Summer	36.89	36.27	33.84	35.21	30.14	29.84	29.11	32.75

DAP = Days after planting.

The total nitrogen content of cuttings of different varieties is presented in Table 12. It could be seen that the nitrogen content varied with season and it was negatively correlated with percentage of rooting (Appendix XIV and XV). N content of the mother plants, as indicated from the cuttings taken from them, was more during summer than during rainy season in varieties like 'Scarlet Glory', 'Jayalakshmy' 'Enidlancaster' and 'Maharaja of Mysore', which recorded lower percentages of rooting in summer. Likewise, comparatively higher nitrogen content of the cuttings in varieties like 'Lady Mary Baring', 'Thimma', 'Cherry Blossom', 'Mahara' and 'Spring Festival' during rainy season was found to be associated with their lower rooting response during that season. The C/N ratio of the cuttings as seen from Table 13, showed a significant positive correlation with percentage of rooting (Appendix XIV). The ratio was higher in varieties like 'Scarlet Glory', 'Jayalakshmy', 'Enidlancaster' and 'Maharaja of Mysore', during rainy season and these were the varieties which recorded higher percentage of rooting during that season. Similarly a higher C/N ratio of varieties like 'Lady Mary Baring', 'Thimma', 'Cherry Blossom', 'Mahara' and 'Spring Festival' during summer was found to be associated with their better rooting during that period.

Table 12. Total nitrogen content (%) of the mother plant and cuttings at fortnightly intervals

Variety	Season	Mother plant	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	Mean
Scarley Glory	Rainy	1.97	1.67	0.73	0.70	0.42	0.38	0.38	0.89
	Summer	2.06	1.19	0.74	0.63	0.56	0.53	0.49	0.89
Jayalakshmy	Rainy	1.65	1.21	0.61	0.54	0.49	0.40	0.18	0.73
	Summer	1.68	1.01	0.88	0.70	0.56	0.53	0.45	0.83
Enidlancaster	Rainy	1.12	0.94	0.35	0.28	0.28	0.14	0.13	0.46
	Summer	1.58	0.95	0.87	0.80	0.80	0.77	0.68	0.92
Lady Mary Baring	Rainy	1.03	0.88	0.84	0.71	0.69	0.60	0.58	0.76
	Summer	0.63	0.44	0.46	0.39	0.35	0.30	0.24	0.41
Thimma	Rainy	1.91	1.64	0.92	0.87	0.87	0.77	0.46	1.06
	Summer	1.01	0.84	0.74	0.60	0.53	0.49	0.46	0.68
Cherry Blossom	Rainy	1.78	1.58	1.10	0.97	0.84	0.81	0.77	1.12
	Summer	0.67	0.56	0.42	0.39	0.39	0.35	0.28	0.44
Mahara	Rainy	1.53	1.08	0.98	0.91	0.83	0.77	0.74	0.98
	Summer	0.61	0.49	0.45	0.45	0.42	0.35	0.28	0.44
Spring Festival	Rainy	1.34	1.05	0.86	0.73	0.73	0.64	0.53	0.84
	Summer	0.88	0.80	0.49	0.35	0.35	0.28	0.24	0.48
Maharaja of Mysore	Rainy	1.15	0.89	0.79	0.74	0.66	0.54	0.43	0.74
	Summer	1.63	1.27	1.14	0.97	0.94	0.84	0.62	1.05

DAP = Days after planting

Table 13. C/N ratio of the mother plant and cuttings at fortnightly intervals

Variety	Season	Mother plant	15 DAP	30 DAP	45 DAP	60 DAP	75 DAP	90 DAP	Mean
Scarlet Glory	Rainy	21.62	20.72	32.84	31.74	50.90	47.04	51.15	36.55
	Summer	16.19	23.39	36.83	40.0	44.46	46.45	41.04	35.47
Jayalakshmy	Rainy	39.17	23.75	40.85	42.59	45.08	47.05	99.00	48.21
	Summer	19.49	31.80	36.50	43.92	54.96	54.30	48.00	41.20
Enidlancaster	Rainy	42.5	41.70	67.54	74.03	61.32	99.57	77.23	66.27
	Summer	22.75	37.15	40.5	46.8	43.7	42.07	35.50	38.35
Lady Mary Baring	Rainy	44.67	42.70	28.54	31.96	31.92	34.55	33.57	35.40
	Summer	58.57	69.85	65.45	67.28	71.97	80.36	97.95	63.71
Thimma	Rainy	25.7	23.10	26.41	27.56	26.80	28.18	42.95	28.67
	Summer	36.2	41.65	46.21	47.33	49.07	53.28	53.23	46.71
Cherry Blossom	Rainy	26.50	23.0	19.72	22.37	25.83	25.59	26.92	24.27
	Summer	55.1	64.48	83.19	88.0	76.57	83.20	96.82	78.18
Mahara	Rainy	29.09	32.04	28.10	22.06	23.48	23.14	23.63	25.93
	Summer	62.1	37.44	81.87	81.97	82.47	92.68	110.39	78.43
Spring Festival	Rainy	31.86	34.24	27.5	31.5	27.5	29.85	33.0	30.70
	Summer	42.60	44.69	73.85	95.94	91.71	110.35	128.75	83.98
Maharaja of Mysore	Rainy	28.4	45.05	29.93	27.58	27.13	37.18	42.93	34.02
	Summer	22.63	26.98	29.68	34.23	32.06	35.52	46.95	32.57

DAP = Days after planting

Rooting efficiency of varieties

On the basis of the parameters like percentage of rooting, root number, root length, shoot weight and root weight, it was found that rooting efficiency varied between varieties and an arbitrary classification could be made. The varieties 'Scarlet Glory' and 'Jayalakshmy' were found easy rooting compared to 'Cherry Blossom', 'Spring Festival' and 'Thimma' which exhibited very poor rooting, while the varieties 'Lady Mary Baring', 'Enidlancaster' and 'Mahara' and 'Maharaja of Mysore' were medium rooting types.

Expt.II. Micropropagation studies

Results obtained from the series of experiments conducted on standardisation of micropropagation technique in bougainvillea are presented below.

4.1. Callus formation

4.1.1. Effect of explant sources

The different explants tried and their influence on callus formation are given in Table 14. All the explants tried viz. immature axillary stem segment, shoot apex, mature leaf tissue and mature dormant buds were capable of callus formation and the callus formed was creamy white initially which later turned pale yellow and was spongy in texture. Those developed from leaf discs were comparatively compact. The callus development from different explant sources may be seen in Plates V, VI and VII.

Table 14. **Effect of different explant sources on callus formation**

Explant sources	Number of days taken for callusing	Number of cultures callused	Percentage of cultures forming callus	Callus growth after 5 weeks
Shoot apices	6-7	6/7	85.7	++++
Immature axillary stem pieces	6-7	6/8	75.0	++++
Mature dormant buds	25-30	4/9	44.4	+
Embryonic leaves	6-8	4/6	66.6	+++
Mature leaf disc	22-26	3/10	33.3	+

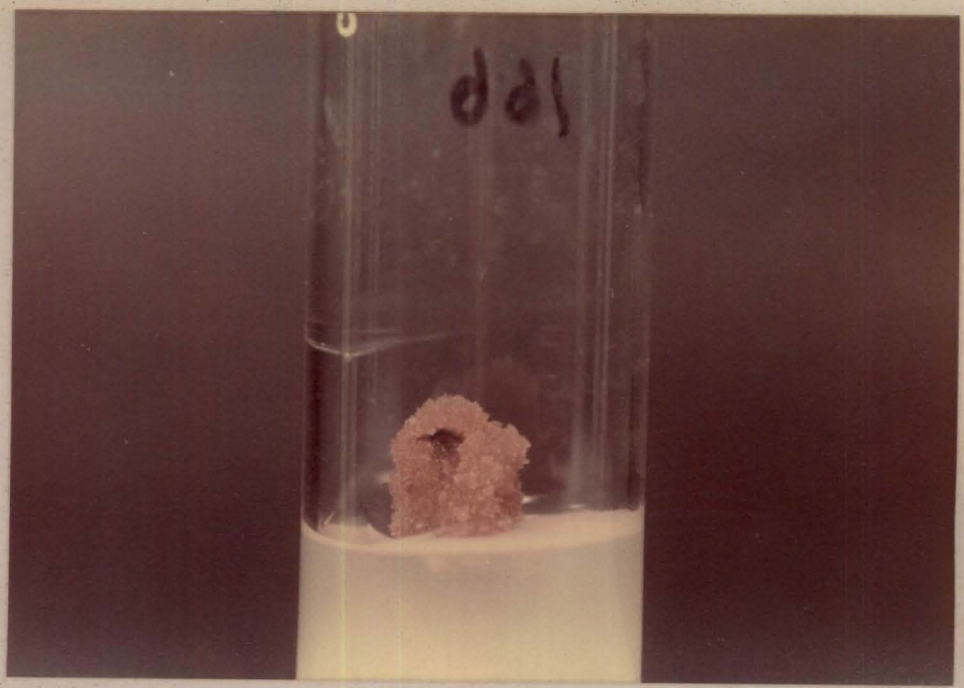
Chi-square values - 32.26**

** Significant at 1 per cent level of probability.

Rating - + Size of the callus upto about 0.5 cm diametre.
+++ Size of the callus upto about 1.5 cm diametre.
++++ Size of the callus upto about 2.0 cm diametre.

Plate V. Development of callus from immature axillary stem segments

Plate VI. Development of callus from shoot apex


25mm

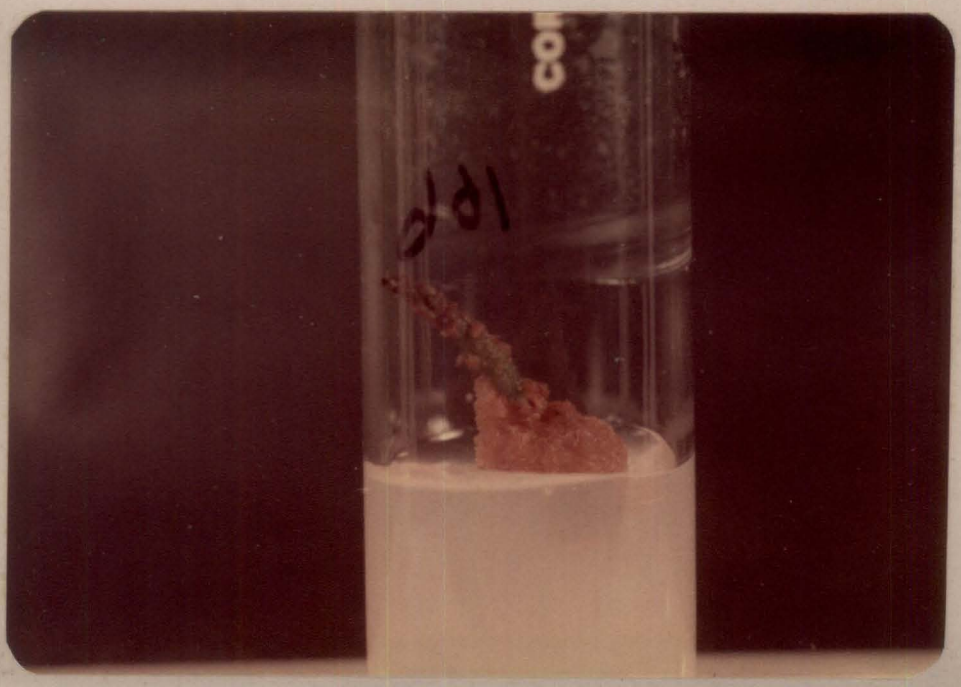

25mm

Plate VII. Development of callus from mature leaf tissue

20mm

The percentage of callus formation from shoot apices and immature axillary stem segments were significantly higher than that from mature dormant buds and leaf discs (Appendix XVI). Shoot apices and immature stem segments recorded 85.7 and 75.0 per cent callus formation respectively (Table 14) as against 44.4 and 33.3 per cent respectively from mature dormant buds and mature leaf discs. It was further observed that the shoot apices and axillary stem segment initiated callus within one week of culturing. The size of the callus formed, measured after five weeks in culture, also was maximum for these explants, besides having a very fast growth rate (Table 14a).

The shoot apices showed slight swelling at the base four days after culturing, while the immature axillary stem segment exhibited swelling all over the tissue. Subsequently callus formation took place in both cases after six to seven days. The whole explant callused in the latter, while in the shoot apices it was confined to the basal portion (Plate V and VI). When embryonic leaves were cultured, the leaves crinkled and swelled within four to five days. The callus initiation was observed in about ten days of culturing and the callus showed rapid growth rate upto five to six weeks. The growth slowed down after this period.

Table 14a. Influence of explant sources on growth rate of callus cv. 'Mahara'

Explant Source	2nd week	3rd week	4th week	5th week	6th week
Shoot apices	+	++	+++	++++	++++
Immature axillary stem pieces	+	++	+++	++++	++++
Mature dormant buds	-	-	+	+	+
Embryonic leaves	+	++	++	+++	+++
Mature leaf discs	-	+	+	+	++

Rating - + Size of the callus upto about 0.5 cm
++ Size of the callus upto about 1.0 cm
+++ Size of the callus upto about 1.5 cm
++++ Size of the callus upto about 2.0 cm
- No callus growth

Callus formation from mature leaf discs and mature dormant buds started 22 to 26 and 25 to 30 days respectively, after culturing. It had only very slow rate of growth and soon after the initiation, further growth was stopped.

4.1.2. Effect of phytohormones

4.1.2a. Effect of NAA and BA

The influence of phytohormones like NAA, and BA on callus formation may be seen from the Table 15. Callusing occurred in all the 16 combinations of NAA and BA tried as well as in the control. However maximum number of explants callused when the explants were grown on medium containing NAA at 1.0 mg/l. All leaves of BA tried were equally effective. Pair wise comparison of different levels of NAA confirmed the significant superiority of NAA 1.0 mg/l over other levels (Appendix XVII). Callus initiated after one week had a very fast growth rate and the size of the callus doubled within five weeks after culturing.

Incorporation of 15 mg/l of adenine was found to have a beneficial effect on the duration for initiation of the callus as well as on its further growth (Table 15a). Adenine in the presence of NAA and BA resulted in earlier callus initiation and rapid rate of growth. Initial size of the callus almost doubled within three to four weeks of

Table 15. Effect of NAA/BA on callus formation in the shoot apex culture of bougainvillea cv. 'Mahara'

Treatment (mg/l)	Number of days taken for callus initiation	Number of cultures callused	Percentage of cultures callused	Size of the callus
Control	26-29	2/9	22.2	+
NAA 0.5 + BA 0.2	14-17	4/4	100.0	++
NAA 0.5 + BA 0.5	14-17	4/4	100.0	+++
NAA 0.5 + BA 0.8	14-17	4/5	80.0	++
NAA 0.5 + BA 1.0	13-15	3/6	50.0	++
NAA 1.0 + BA 0.2	11-13	5/8	62.5	++
NAA 1.0 + BA 0.5	11-14	5/9	66.6	++
NAA 1.0 + BA 0.8	11-13	7/7	100.0	++
NAA 1.0 + BA 1.0	11-13	7/7	100.0	+++
NAA 1.5 + BA 0.2	12-16	8/8	100.0	+++
NAA 1.5 + BA 0.5	12-15	6/7	85.7	++
NAA 1.5 + BA 0.8	12-15	4/5	80.0	++
NAA 1.5 + BA 1.0	12-14	2/5	40.0	+++
NAA 2.0 + BA 0.2	14-16	2/4	50.0	+++
NAA 2.0 + BA 0.5	11-15	3/5	60.0	+++
NAA 2.0 + BA 0.8	11-14	4/4	100.0	+++
NAA 2.0 + BA 1.0	11-14	4/7	57.14	+++

Chi-square values - 2.01

Rating - + Size of callus upto about 0.5 cm diametre
++ Size of callus upto about 1.0 cm diametre
+++ Size of callus upto about 1.5 cm diametre

15a. Effect of adenine 15 mg/l, NAA & BA on callus formation in the shoot apex culture of bougainvillea var.'Mahara'

Treatment (mg/l)	Number of days taken for callus initiation	Number of cultures callused	Percentage of cultures callused	Size of callus
Control	20-28	1/8	12.5	+
NAA 0.5 + BA 0.2	10-12	3/5	60.0	++
NAA 0.5 + BA 0.5	10-12	3/3	100.0	++
NAA 0.5 + BA 0.8	10-12	3/5	60.0	++
NAA 0.5 + BA 1.0	10-12	3/4	75.0	++
NAA 1.0 + BA 0.2	8-9	8/9	88.8	++
NAA 1.0 + BA 0.5	8-9	12/12	100.0	+++
NAA 1.0 + BA 0.8	8-9	11/11	100.0	++
NAA 1.0 + BA 1.0	8-10	10/11	90.9	+++
NAA 1.5 + BA 0.2	10-11	2/3	66.6	+++
NAA 1.5 + BA 0.5	10-11	4/4	100.0	+++
NAA 1.5 + BA 0.8	9-11	8/9	88.8	++
NAA 1.5 + BA 1.0	10-11	5/8	62.5	+++
NAA 2.0 + BA 0.2	7-9	4/6	66.6	+++
NAA 2.0 + BA 0.5	7-9	5/6	83.3	++++
NAA 2.0 + BA 0.8	7-10	3/4	75.0	++++
NAA 2.0 + BA 1.0	7-9	3/4	75.0	++++

Chi-square value - 90.93**

** Significance at 1 per cent level of probability.

Rating - + Size of the callus upto about 0.5 cm diametre
++ Size of the callus upto about 1.0 cm diametre
+++ Size of the callus upto about 1.5 cm diametre
++++ Size of the callus upto about 2.0 cm diametre

culturing. The size of the callus mass formed was more when adenine was incorporated to the medium.

4.1.2b. Effect of NAA and KIN

The effect of kinetin on callus growth could be seen from the data presented in Table 16. All the four levels of kinetin tried (0.2, 0.5, 0.8 and 1.0 mg/l) in combination with NAA at 1.0 and 2.0 mg/l, were found less effective in terms of percentage of the cultures callused, when compared to the corresponding levels of BA. Statistical comparison of different levels of the two cytokinins also confirmed the significant superiority of BA over kinetin (Appendix XVIII). Moreover, the time taken for callus initiation was also more, when NAA and kinetin were used. However, the size of the callus mass formed after five weeks, did not show any significant difference.

4.1.2c. Effect of IAA and BA

Table 17 presents the data on the effect of the natural auxin-IAA and the cytokinin-BA on callus formation from shoot apices. It was observed that all levels of IAA (0.5, 1.0, 2.0, 3.0 and 5.0 mg/l) in combination with BA at 0.5 mg/l were almost equally effective. Eventhough there was no difference in the number of cultures callused, the size of the callus mass increased with the concentration of IAA. At lower concentration the explants showed a

Table 16. Effect of NAA/KIN on callus formation in the shoot apex culture of bougainvillea cv. 'Mahara'

Treatments (mg/l)	Number of days taken for callus initiation	Number of cultures callused	Percentage of cultures callused	Size of callus after 5 weeks
Control	--	--	--	--
NAA 1.0 + KIN 0.2	15-18	2/4	50.0	+++
NAA 1.0 + KIN 0.5	14-16	2/5	40.0	+++
NAA 1.0 + KIN 0.8	14-18	2/6	33.3	++
NAA 1.0 + KIN 1.0	16-19	3/4	75.0	++
NAA 2.0 + KIN 0.2	15-17	2/4	50.0	+++
NAA 2.0 + KIN 0.5	15-17	4/5	80.0	+++
NAA 2.0 + KIN 0.8	15-18	1/4	25.0	++
NAA 2.0 + KIN 1.0	15-17	1/4	25.0	++

Chi-square values - 2.61

Rating - + Size of the callus upto about 0.5 cm diametre
++ Size of the callus upto about 1.0 cm diametre
+++ Size of the callus upto about 1.5 cm diametre

17. Effect of IAA/BA on callus formation in the shoot apex culture of bougainvillea cv. 'Mahara'.

Treatments	Number of days taken for callus initiation	Number of cultures callused	Percentage of cultures callused	Size of callus
IAA 0.5 + BA 0.5	18-20	4/10	40.0	+
IAA 1.0 + BA 0.5	18-20	5/12	41.6	+
IAA 2.0 + BA 0.5	15-16	6/11	54.5	++
IAA 3.0 + BA 0.5	15-16	3/9	33.3	++
IAA 4.0 + BA 0.5	14-17	4/10	40.0	+++

Chi-square value - 0.480

Table 18. Effect of 2,4-D, BA and KIN on callus formation in the shoot apex culture of bougainvillea cv. 'Mahara'

Treatments (mg/l)	Number of days taken for callus initiation	Number of cultures callused	Percentage of cultures callused	Size of callus
Control	22-24	2/8	25.0	+
2,4-D 0.1 + BA 0.5	22-24	3/6	50.0	++
2,4-D 0.5 + BA 0.5	22-24	4/6	66.6	++
2,4-D 1.0 + BA 0.5	23-24	5/6	83.3	++
2,4-D 2.0 + BA 0.5	23-24	2/6	33.3	++
2,4-D 0.1 + KIN 0.5	26-27	2/6	33.3	++
2,4-D 0.5 + KIN 0.5	25-26	3/6	50.0	+
2,4-D 1.0 + KIN 0.5	26-27	2/6	33.3	+
2,4-D 2.0 + KIN 0.5	27-28	2/6	33.3	+

Chi-square value - 4.45
Rating - + Size of callus upto about 0.5 cm diametre
++ Size of callus upto about 1.0 cm diametre

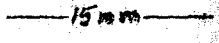
tendency to elongate rather than to form callus at their basal portion. Explants grown on medium containing lower concentration of IAA elongated 2 to 3 times than those grown on medium with a higher concentration of IAA.

4.1.2d. Effect of 2,4-D, BA and KIN

When shoot apices were grown on MS solid medium supplemented with 2,4-D at 0.1, 0.5, 1.0 and 2.0 mg/l, in combination with BA or KIN each at 0.5 mg/l, the callus initiation was very much delayed by 22to 28 days (Table 18). None of treatments showed any significant superiority over the other but a combination of 2,4-D 1.0 mg/l + BA 0.5 mg/l was found better than the other treatments, in which case a maximum of 83.3 per cent explants callused. 2,4-D/BA combination was found to be more effective than 2,4-D/KIN combination, in which case of the latter the maximum percentage of cultures callused was only 50. After about 1 to 1½ weeks after initiation of the callus snow white globular mass of size about 3 to 5 mm diameter appeared over the callus tissue, which subsequently covered almost the entire periphery of the callus mass.

Leaf discs as well as embryonic leaves when cultured on a medium containing NAA and BA, also developed such white globular solid mass from the cut ends (Plate VIII) 40 to 45 days after culturing. But leaf discs when grown on 2,4-D

Plate VIII. Development of snow white globular mass from leaf tissue in the presence of 2,4-D and BA.


15 mm

15 mm

and BA containing medium, development of such white globular mass occurred as early as in two weeks after culturing. They were separated from the mother tissue and subcultured to MS solid medium devoid of auxins and also in presence of different concentration and combination of growth regulators but failed to redifferentiate. Formation of such white patches was also noticed after subculturing the callus derived from stem explants, to a medium containing NAA and BA, also failed to redifferentiate.

The overall effect of the three auxins viz. NAA, IAA and 2,4-D was compared statistically and it was found that NAA was significantly superior to other two auxins (Appendix XIX) with maximum number of cultures producing callus, in addition to causing an earlier initiation of callus.

4.1.3. Varietal response

Callus formation from the shoot apices of three varieties of bougainvillea viz. 'Mahara', 'Cherry Blossom' and 'Spring Festival' was studied and the results are presented in Table 19. Significant variation existed between the three varieties studied with respect to the percentage of cultures callused (Appendix XX). The number of explants callused was maximum with the var. 'Mahara' followed by 'Cherry Blossom'. In these varieties the

Table 19. Varietal response of bougainvillea shoot apices on callus formation

Treatments	Var. Mahara				Var. Cherry Blossom				Var. Spring Festival			
	No.of days taken for callus initiation	Callus formed Number	Per-centage	Size[1]	No.of days taken for callus initiation	Callus formed Number	Per-centage	Size[1]	No.of days taken for callus initiation	Callus formed Number	Per-centage	Size[1]
NAA 1.0 + BA 0.5	11-14	12/12	100	+++	15-16	4/8	50.0	++	15-17	3/7	42.8	++
NAA 1.5 + BA 0.5	12-15	6/7	85.7	++	15-16	7/7	100.0	++	14-16	4/8	50.0	++
NAA 2.0 + BA 0.5	11-15	3/5	60.7	+++	14-17	5/8	62.5	++	15-17	2/6	33.3	++
NAA 1.0 + BA 1.0	8-9	10/11	90.0	+++	12-16	7/9	77.7	++	13-17	4/8	50.0	++
NAA 1.5 + BA 1.0	8-10	3/4	75.0	+++	12-16	6/10	60.0	++	12-17	2/4	50.0	++
NAA 2.0 + BA 1.0	8-10	3/4	75.0	+++	12-16	6/8	75.0	++	13-16	3/7	42.8	++

Chi-square value - 35.9** ** Significant at 1 per cent level of probability

Rating - ++ Size of callus upto about 1.0 cm diametre
+++ Size of callus upto about 1.5 cm diametre

1 - Callus size after 4 weeks of growth.

initiation of callus was also earlier. In 'Spring Festival' only 50 per cent of the shoot apices cultured, produced callus.

4.2. Callus proliferation

Callus mass was cut into small pieces and subcultured for proliferation on MS solid medium containing NAA 1.0 mg/l+ BA 0.5 mg/l. It started proliferation and within two weeks filled almost the entire surface of the medium (Plate IX). But the active growth period was much shorter than that on the primary medium. The tissue developed brownish colour sooner and ultimately became black arresting further proliferation.

4.3. Regeneration from callus

Actively proliferating callus pieces were subcultured to MS medium containing IAA at 0.01, 0.1, 0.2 and 0.4 mg/l, together with 0.01 mg/l GA_3 and 0.05 mg/l each of biotin and calcium pantothenate and incubated for a period of 2 to 3 months with periodical subculturing. The callus in all cases turned black without any regeneration.

4.4. Multiple shoot formation from shoot apex culture

4.4.1. Effect of IAA and BA

Table 20 presents the results of the effect of IAA and BA on inducing multiple shoot formation from the shoot apices of bougainvillea var. 'Mahara'. Results pointed out

Table 20. Effect of IAA and BA in inducing multiple shoot formation in the shoot apex culture of bougainvillea cv. 'Mahara'

Treatment (mg/l)	Number of cultures produced shoot	Percentage	Number of shoots per culture	Mean length of shoot [1]	Number of leaves
Control	--	--	--	--	--
IAA 1.0 + BA 0.5	4/8	50.0	1	5.22	4.8
IAA 2.0 + BA 0.5	4/9	44.4	1	7.46	5.4
IAA 3.0 + BA 0.5	3/7	42.8	1	4.90	4.4
IAA 4.0 + BA 0.5	4/8	50.0	1	2.10	3.8
IAA 1.0 + BA 1.0	5/7	71.42	1.3	3.50	8.34
IAA 1.0 + BA 3.0	5/7	71.42	1.33	3.83	9.21
IAA 1.0 + BA 9.0	4/5	80.0	2.66	1.66	4.24
IAA 1.0 + BA 16.0	6/8	60.0	2.66	1.66	3.98

Chi-square value - 5.69

1 - 8 weeks after inoculation.

Plate IX. Callus proliferation on MS solid medium in the presence of NAA 1.0 mg/l + BA 0.5 mg/l.


25 mm

that the concentration of BA influenced the number of additional shoots produced, a higher concentration favouring multiple shoot development. However, single shoots developed at lower concentrations of BA also. Mean number of shoots produced per culture was maximum (2.66) in the presence of BA at 9.0 and 16.0 mg/l + IAA at 1.0 mg/l. It was further observed that lower concentration of IAA had a pronounced effect on shoot elongation (Plate X). Mean elongation of shoot when recorded 8 to 9 weeks after culturing was maximum (7.46 cm) on a medium containing IAA 2.0 mg/l + BA 0.5 mg/l. At higher concentrations of IAA the shoot apices callused at their bases. It was further observed that higher concentration of BA adversely affected the elongation of shoot. Shoots grown on such medium also produced leaves which were pale yellow in colour. In certain cases only microsized leaves were found which failed to develop further and leaves easily shed.

4.4.2. Effect of BA and KIN

Cytokinin such as BA and kinetin were used either alone or in combination to study their effect on inducing multiple shoots. It could be seen from the Table 21a that only lesser number of explants developed shoots in the presence of lower concentrations of these two cytokinins, while at concentrations of 1.0 and 2.0 mg/l of BA and kinetin, more number of explants developed shoots.

Plate X. *In vitro* shoot development from shoot apex of the var. 'Mahara' in the presence of IAA 2.0 mg/l + BA 0.5 mg/l.

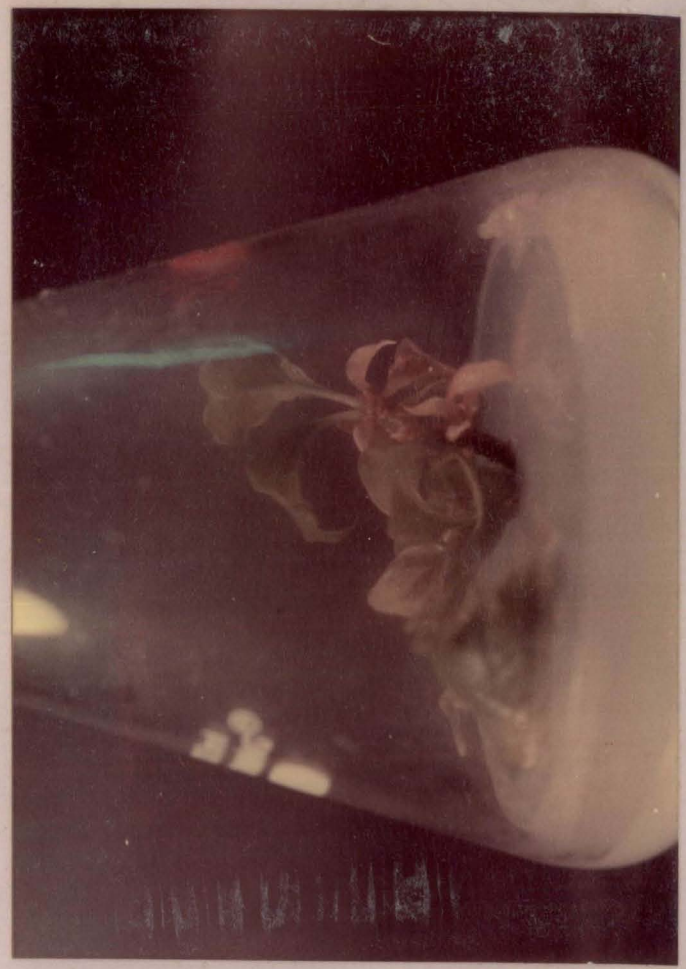


Table 21a Effect of BA and KIN in inducing multiple shoot formation in the shoot apex culture of bougainvillea cv. 'Mahara'.

Treatments (mg/l)	Number of cultures produced shoot	Percentage	Mean number of shoots/ culture	Mean length of shoots/ culture	Mean number of leaves
BA 0 + KIN 0.5	2/6	33.3	1	1.20	2.66
BA 0 + KIN 1.0	1/5	20.0	1	1.16	3.30
BA 0 + KIN 2.0	2/6	33.3	1	1.13	2.33
BA 0.5 + KIN 0	2/4	50.0	1	1.16	6.33
BA 0.5 + KIN 0.5	3/6	50.0	1	1.16	5.21
BA 0.5 + KIN 1.0	3/7	42.8	1	1.33	5.01
BA 0.5 + KIN 2.0	4/8	50.0	1	1.93	9.66
BA 1.0 + KIN 0	5/8	62.5	1	2.03	7.33
BA 1.0 + KIN 0.5	4/7	57.0	1	2.53	9.33
BA 1.0 + KIN 1.0	5/6	83.3	1	2.63	7.33
BA 1.0 + KIN 2.0	4/7	57.1	2.16	2.63	9.23
BA 2.0 + KIN 0	6/7	85.7	3.0	1.41	15.00
BA 2.0 + KIN 0.5	5/7	71.4	3.33	1.20	13.80
BA 2.0 + KIN 1.0	6/6	100.0	2.0	1.43	12.33
BA 2.0 + KIN 2.0	4/6	66.6	2.33	1.43	12.00

Chi-square value - 14.64

Only single shoots developed in the presence of lower concentrations of BA and Kinetin. Mean number of shoots per culture were maximum (3.33) with BA 2.0 mg/l + KIN 0.5 mg/l, closely followed by BA 2.0 mg/l, in which an average of 3.0 shoots/culture were produced. Multiple shoots developed on this medium had normal healthy green leaves (Plate X).

4.4.3. Effect of adenine sulphate

Addition of adenine sulphate at 50.0 mg/l to solid MS medium in the presence of IAA and cytokinins BA or KIN resulted in the induction of more number of shoots per culture (Table 21b). The number of shoots was slightly enhanced in all the treatments. Maximum number of shoots per culture (4.25) were produced in treatments with a combination of BA 9.0 mg/l + IAA 1.0 mg/l and BA 16.0 mg/l + IAA 1.0 mg/l. All the shoots produced from the explants, developed in clusters with reduced internodal length (Plate XI). When the concentration of BA in the medium was increased to 9.0 mg/l and above, shoots developed on that medium had pale yellow leaves. Elongation of the shoots was more in the presence of lower concentration of BA.

4.5. In vitro root formation from shoot apex culture

The effect of various auxins such as NAA, IAA and BA on *in vitro* root formation from shoot apex cultures was

Table 21b. Effect of adenine sulphate 50 ppm in inducing multiple shoot formation

Treatments (mg/l)	Number of cultures produced shoot	Percentage	Mean number of shoots/ culture	Mean length of shoots	Mean number of leaves
BA 2.0	4/6	66.6	2.0	2.73	6.5
BA 2.0 + KIN 0.5	5/7	71.44	3.5	2.04	6.2
BA 2.0 + KIN 1.0	4/6	66.6	3.0	2.55	7.66
BA 2.0 + KIN 2.0	3/8	37.5	3.0	2.0	6.75
BA 3.0 + IAA 1.0	4/5	80.0	3.0	2.25	5.75
BA 9.0 + IAA 1.0	5/5	100.0	4.25	1.40	12.6
BA 16.0 + IAA 1.0	5/5	100.0	4.25	1.18	13.0

Plate XI. In vitro shoot proliferation in the presence of higher concentration of cytokinins and adenine sulphate.

studied both on full strength and half strength MS solid medium under light as well as dark conditions. Data presented in Table 22 showed that among the various treatments, half strength MS medium (½ MS) supplemented with IBA 1.6 mg/l produced maximum number of rooted plantlets (85.7 per cent), followed by IBA at 0.8 mg/l (80.0 per cent). No significant difference was noticed in the percentage of rooted plantlets produced, on full strength and half strength MS medium. However, the percentage of rooting were slightly increased in the latter. All the treatments was almost equally effective over control in which case, none of the explant rooted. From the present study it was also noticed that well developed and profuse root development was noticed when full MS and ½ MS media were used (Plate XII and XIII). Prominent tap root like growth was noticed in almost all of the rooted plantlets. The development of root hairs were clearly visible on the root portions above the agar medium (Plate XIII). Rooting did not occur in explants kept under light except for a few cultures.

The concentration of the major and minor nutrients seemed to have influenced the number of days taken for rooting. In all the treatments that received half strength of MS salt, root initiation occurred much earlier (Table 22).

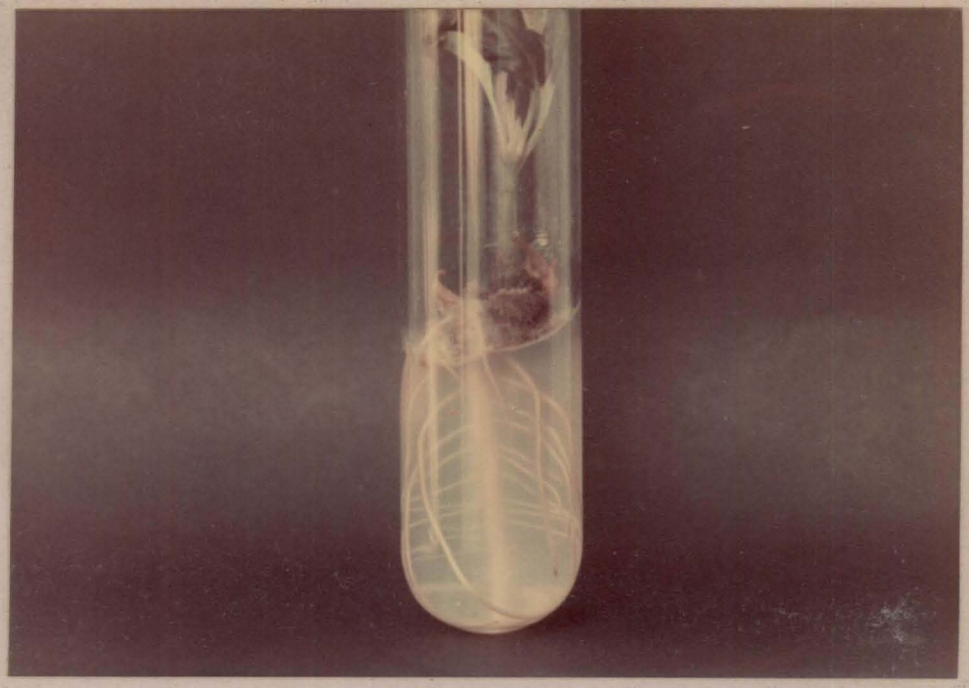
Table 22. Effect of auxins and salt concentration of the medium on *in vitro* rooting of shoot apex cultures of bougainvillea cv. 'Mahara'

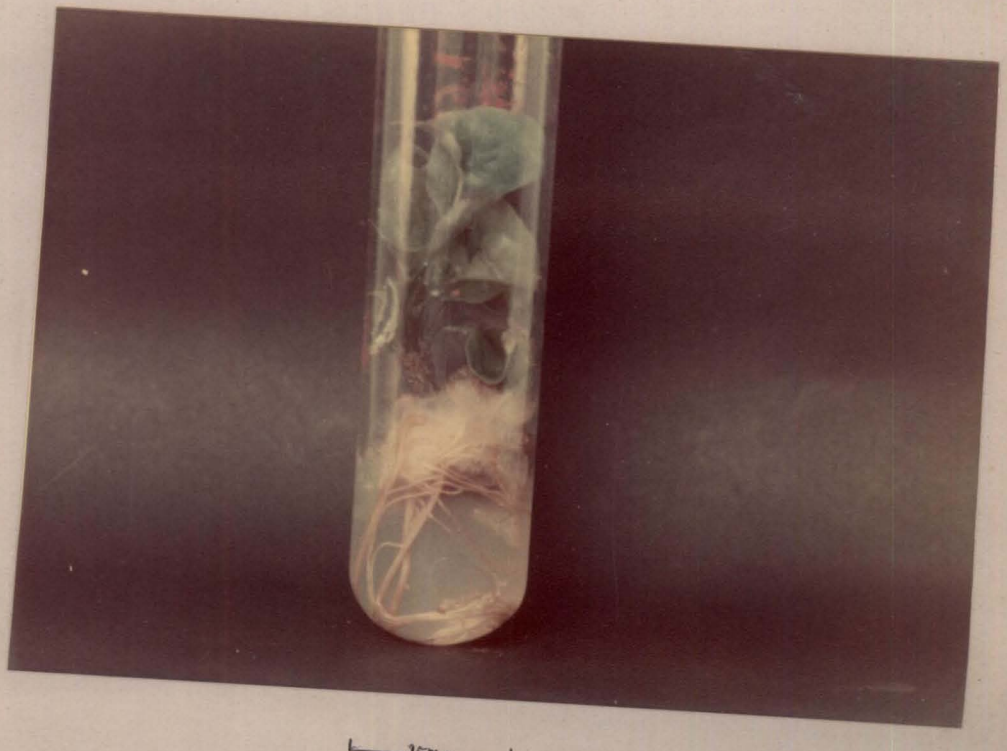
Treatments (mg/l)	Number of days taken for rooting		Number of cultures rooted	Percentage of rooting	Mean Number of roots	Mean length of roots (cm)
	Fresh explant	Precallused shoot				
Control	—		—	—	—	—
MS + NAA 2.0	66-70	53-58	3/6	50.0	2.66	11.40
½ MS + NAA 2.0	45-50	32-35	4/6	66.6	2.50	6.8
MS + NAA 3.0	65-68	50-52	3/7	42.8	2.43	14.61
½ MS + NAA 3.0	48-56	34-44	4/7	57.1	3.25	8.35
MS + IAA 3.0	75-83	59-66	4/7	57.1	2.33	9.66
½ MS + IAA 3.0	59-68	32-38	4/6	66.6	3.50	6.83
MS + IAA 4.0	68-73	30-35	6/10	60.0	2.00	9.76
½ MS + IAA 4.0	43-47	23-27	7/11	63.6	3.00	6.20
MS + IAA 6.0	60-68	27-38	3/7	42.8	2.33	8.64
½ MS + IAA 6.0	44-49	22-25	5/8	62.5	3.66	7.56
MS + IBA 0.1 + IAA 0.1	—	—	—	—	—	—
½ MS + IBA 0.1 + IAA 0.1	—	—	—	—	—	—
MS + IBA 0.2 + IAA 0.2	50-55	40-44	1/6	16.6	3.00	14.40
½MS + IBA 0.2 + IAA 0.2	44-50	24-32	5/10	50.0	3.00	6.44
MS + IBA 0.4 + IAA 0.4	50-54	32-40	3/9	33.3	3.33	9.60
½ MS + IBA 0.4 + IAA 0.4	30-38	22-28	4/6	66.6	6.25	5.10
MS + IBA 0.8	45-48	28-33	6/10	60.0	4.0	9.15
½ MS + IBA 0.8	24-27	16-19	4/5	80.0	4.91	4.11
MS + IBA 1.6	46-52	23-30	5/10	50.0	4.11	6.86
½ MS + IBA 1.6	26-29	16-18	6/7	85.7	4.21	4.01

Chi-square value - 11.527

Plate XII. *In vitro* rooting of shoot apex on MS medium.

Plate XIII. *In vitro* rooting of shoot apex on ½ MS medium.

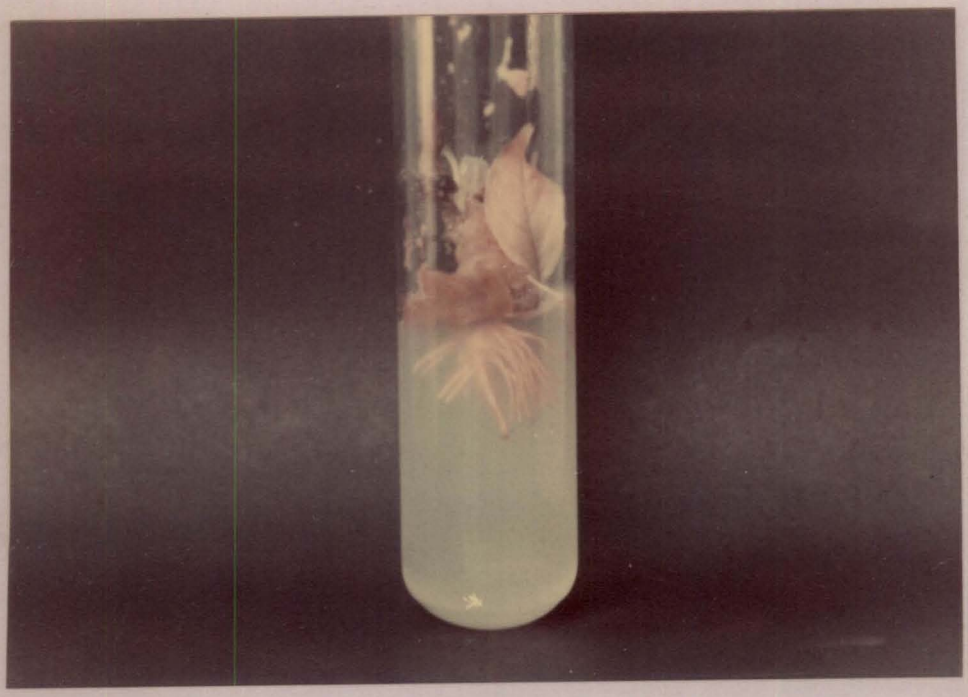

25mm


25mm

This period was further shortened when precallused shoots were employed for rooting rather than fresh shoot apices. The earliest rooting was obtained with precallused shoots on ½ MS medium containing 1.6 mg/l IBA, within 16 to 18 days of culturing. Soon, the leaves turned yellow and gradually shed, but those grown on full strength MS medium remained healthy.

Data on the number of roots and the length of roots are given in Table 22. It was observed that the number of roots produced per culture was more in IBA containing medium, than NAA or IAA containing one. Maximum number of roots (6.25) per culture was produced in the presence of IBA 0.4 mg/l + IAA 0.4 mg/l on half strength MS salt. Length of the roots also showed variation with the composition of the medium. Longer roots were produced from explants grown on full MS medium. The length of roots produced on IBA containing medium was comparatively shorter (Plate XV). Elongation of the root was slower on half strength MS medium compared to full strength MS medium.

The rooted plantlets were transferred to pots filled with sterile sand and soil. They remained healthy for about two weeks but later the leaves turned yellow and ultimately shed completely.



25 mm

Plate XIV. *In vitro* rooting of shoot apex on ½ MS medium in the presence of IBA.

DISCUSSION

DISCUSSION

Bougainvilleaswith their wide adaptability to varied climatic conditions, are grown in the gardens throughout tropics and subtropics for their brilliantly coloured sprays of bracts. Cuttage forms the most popular method of propagation in this ornamental shrub, although methods like layering or budding are attempted when cuttings fail to strike roots. Application of growth regulators have been found to increase the rooting percentage in bougainvillea (Mukhopadhaya and Bose, 1966; Kale and Bhujbal, 1972; Seetharama and Mohanram, 1972; Gandothra *et al*. 1975; Yadav *et al*. 1977; Philip and Gopalakrishnan, 1982). The response of different varieties to growth regulators, however, vary from place to place depending upon several environmental factors. In view of this fact it becomes necessary to standardise the concentration of the growth regulator and the duration of their treatment by detailed experimentation in any particular agroclimatic situation.

Even with the application of growth regulators, cuttings of many varieties very often fail to root to any appreciable extent probably due to peculiar genetic make up of those varieties. In such case *in vitro* culture might probably provide a satisfactory method of commercial production of propagules. Tissue culture appears to offer

excellent scope for large scale production of plants comparatively with ease in bougainvillea. The present studies on macro and micropropagation of certain varieties of bougainvillea were undertaken in order to standardise methods that would ultimately help the multiplication of this plant on a commercial basis. The results of a series of work done on rooting of cuttings with the aid of growth regulators and on tissue culture techniques in the Department of Pomology and Floriculture during the year 1983-'84 are discussed in this chapter.

Effect of growth regulators on rooting of cuttings

The results of the studies on rooting of cuttings have amply illustrated the effectiveness of growth regulators in enhancing rooting in bougainvillea cuttings. Out of the two growth regulators tried viz. IBA and NAA, the former appeared to be more effective in terms of percentage of rooting of cuttings. A concentration of 500 ppm IBA gave the best rooting percentage ie. over 63 per cent both during rainy and summer seasons. The effectiveness of auxin in general and IBA in particular in induction of rooting of cuttings has been well amplified by several workers in a variety of plant species (Linder, 1939; Williams, 1943; Audus, 1959; Mukhopadhaya and Bose, 1966; Kale and Bhujbal, 1972; Seetharama and Mohanram, 1972;

Bose et al. 1975; Gandothra et al. 1975; Yadav et al. 1977; Singh and Motilal, 1979; Singh, 1980; Beel and Schelstraete, 1981; Shailendrarajan and Santaram, 1982; Philip and Gopalakrishnan, 1982).

Mukhopadhaya and Bose (1966) and Kale and Bhujbal (1972) found that in bougainvillea IBA was more effective than other types of growth regulators. The inhibitory effect of auxin above 500 ppm is noteworthy. Such effect was observed by Gandothra et al. (1975) in bougainvillea cv. 'Mary Palmer'. The detrimental effects of higher concentration of growth regulators call for their use at optimum concentrations.

The rooting efficiency of applied auxins are generally assessed by the number of roots produced per cutting, their length, and weight since these factors ultimately decide the final percentage of establishment of the cuttings planted (Hartmann and Kester, 1975). Cuttings treated with IBA 300 and 500 ppm produced the largest number of roots which is indicative of the fact that the number of roots produced had a definite bearing on the percentage of success in rooting. It may be mentioned here that maximum percentage of rooting was also noticed in the treatment IBA 500 ppm followed by IBA 300 ppm. That the applied auxins activated the cambium and enhanced root differentiation had been demonstrated by earlier workers (Pontikis et al. 1979).

The production of more number of roots in auxin treated cuttings is often attributed to the mobilisation of reserve food material from the terminal to the basal regions of the cuttings (Strydom and Hartmann, 1960).

The present study also showed that the root production was maximum during the early stages. The root number increased upto two months after planting, then decreased slightly which showed that all the roots produced initially did not remain intact till the end. This could take place due to the deterioration of certain roots in the initial stages or due to the nondevelopment of certain roots while certain others make rapid growth. In the case of cuttings it has been observed that the ultimate survival is decided by those roots which make a rapid growth and establish.

Although the length of roots was not significantly influenced by growth regulator treatments, a tendency for higher root length in auxin treated cuttings was always apparent. Effect of auxins in inducing longer roots in the treated cuttings of bougainvillea was reported by Seetharama and Mohanram (1972) and Yadav et al. (1977).

The present study also showed that auxin treatment had a pronounced effect on weight of roots per cutting.

IBA and NAA treatment which recorded a higher shoot weight had shown correspondingly increase in their root weight also. This clearly indicated that developing shoots might have contributed auxins or other integral factors and photosynthate for the vigorous growth of roots. The results are in conformity with the findings of Cripps (1971).

Sprouting of shoots is an indication of root initiation in cuttings of most of the plants. In bougainvillea it was observed that the initial sprouting of shoots is not indicative of the root 'strike'. Some of the earlier produced shoots withered away in the course of time. The auxins tried in the present study did not influence significantly the number of shoots produced in both the seasons, but during rainy season the cuttings produced more number of shoots. Thus the results indicated that the basal application of auxins for rooting need not necessarily affect the shoot production. On the contrary, the auxin treatment of the cuttings significantly increased the fresh weight of shoots in both the seasons particularly in summer. This could be attributed to the redistribution and mobilisation of nitrogen in the treated cuttings resulting in a higher shoot weight. The relatively higher content of N during summer in some of the varieties studied, explains the higher shoot weight during that period.

The mean shoot/root ratio recorded indicated that the ratio increased upto two months after planting and then decreased by third month. This could be attributed to the differential growth of shoot and root. Initially shoot weight was very high with poor root growth. Later the growth of roots was faster compared to shoot, thus narrowing down the ratio in the subsequent periods. In general, between IBA and NAA treatment, the ratio was higher for the former which might be due to the favourable effect of IBA on shoot production.

Influence of season on rooting

From the present investigations, it became clear that weather parameters especially rainfall influenced to some extent the rooting of cuttings. Highest rooting was obtained when the rainfall was maximum (Appendix V). However, it could be seen that rooting was more influenced by varieties than the season. While summer was favourable to varieties like 'Lady Mary Baring', 'Thimma', 'Cherry Blossom', 'Mahara' and 'Spring Festival', rainy season was the best for other varieties like 'Scarlet Glory', 'Jayalakshmy', 'Enidlancaster' and 'Maharaja of Mysore'. Contrary to the general belief that rainy season is the best for bougainvilleas, the present studies have clearly brought out that season is not a limiting factor in rooting

of cuttings of bougainvillea. Even when varieties were in bloom rooting could be successfully achieved. This would indicate that by proper manipulation of environmental conditions, in Kerala rooting of cuttings could be successful on a commercial basis all the year round in bougainvilleas. As mentioned earlier the varieties react differently, probably certain internal factors being responsible for the differential behaviour (Mukhopadhaya and Bose, 1966; Seetharama and Mohanram, 1972; Hartmann and Kester, 1975; Beel and Schelstraete, 1981).

Mode of treatment of growth regulators

The method of treatment of cuttings with growth regulators has been found to be a deciding factor in rooting of cuttings in many species (Williams, 1943; Woszszynska and Borys, 1976; Gorecka, 1979; Kwack and Chung, 1980). In the present study it was found that prolonged dip was the most congenial method of treatment of cuttings. As stated by Gorecka (1979), the ultimate effect of a growth regulator treatment was decided by the amount of substance absorbed by the cuttings and not by the concentration alone. In a slow dip (soaking) treatment the higher amount of growth regulators absorbed perhaps directly effected a better rooting than in quick dip treatment. The quick dip method with higher concentration

often might lead to tissue deterioration resulting in reduced success (Lingaraj, 1960; Gandothra et al. 1975). Bougainvillea, although is a hardy species perhaps is delicate to the growth regulator treatments.

Changes in carbon and nitrogen content

The reduction in the content of carbon in cuttings as the rooting progressed suggests that the carbohydrate was utilised for root production. However, its content in different varieties, which presumbly be genitically linked, was not directly correlated with the rooting propensity. The N content of the cuttings was significantly but negatively correlated with rooting. The lesser N content of varieties like 'Scarlet Glory', 'Jayalakshmy', 'Enidlancaster', 'Maharaja of Mysore' during rainy season and that of 'Lady Mary Baring', 'Thimma', 'Cherry Blossom', 'Mahara' and 'Spring Festival' during summer was found correlated with better rooting of these varieties in the respective seasons. C/N ratio of the cuttings showed significant positive correlation with rooting which is also in agreement with the results obtained by Basu and Ghosh (1974), who concluded that higher C/N ratio generally induced better rooting co-factor activity.

Micropropagation studies

The results of the experiments on tissue culture was aimed at the standardisation of a suitable technique as well

as to make a preliminary analysis of the response of certain varieties to *in vitro* culture.

In the present studies it was observed that in bougainvillea, callus could be easily obtained from almost all the explant sources, viz. immature axillary stem, segments, mature dormant buds, shoot apices, embryonic and mature leaf tissue. But more rapid and prolific growth of callus was observed from the young tissues like shoot apices and immature axillary stem segments, than from mature dormant buds and leaf tissues. The differential response of various explant sources to morphogenesis *in vitro* was found to be directly related to maturity of the tissue by Marashige (1974). Young explant consisting of meristematic and mitotically active cells, is highly favourable for callus initiation and subsequent regeneration and the morphogenic competence of any tissue decrease with maturity (Pierik *et al*. 1974).

The studies conducted on the influence of auxins and cytokinins on callus formation revealed that, callus formation could take place with all the three auxins tried viz. IAA, NAA and 2,4-D, in combination with cytokinins BA or KIN. Among these, NAA/BA combination proved best and moreover, KIN was less effective compared to BA. The favourable effect of NAA and BA on callus formation has

been well amplified by Scorza and Janick (1976) and Awad and Bank (1981). Contrary to the general belief that 2,4-D is a potent auxin capable of inducing callus growth, in bougainvillea explants callus initiation was delayed very much with a poor rate of growth by this auxin. Occurrence of callus initiation from explants grown on MS medium devoid of any growth regulator in the present study is noteworthy. While callusing takes place in majority of the plant species in the presence of an auxin and cytokinin combination, in bougainvillea, the initiation and growth of callus atleast in a few cultures on hormone free medium perhaps indicate the possibility of higher levels of endogenous hormones present in the tissues. A similar case was observed by Hussey (1978) in lilium and hyacinth.

Though literature concerning the stimulatory effect of optional organic compounds on callus growth is quite scanty, it could be seen in the present study that adenine exerted a favourable effect on callus initiation and its growth. In tobacco callus culture, adenine was reported to have a pronounced effect on callus growth by Skooj and Tsui (1968).

Among the three varieties of bougainvillea viz. 'Mahara', 'Cherry Blossom', and 'Spring Festival', shoot apices of the first one performed best on MS medium supplemented with NAA 1.0 mg/l + BA 0.5 mg/l with regard to the percentage of cultures callused. A distinct varietal variation could be observed in the morphogenic response of these varieties which could be attributable to the genotype (Welander, 1977).

It was further observed that concentration of cytokinins-BA/KIN was an important factor for shoot proliferation. Shoot apices of the cv. 'Mahara' produced multiple shoots in the presence of higher concentration of cytokinins, but only single shoots were produced in the presence of lower concentration. Cytokinins alone could not produce a satisfactory growth of the shoots. At concentrations above 9.0 mg/l, the leaves produced appeared pale yellow and abnormal, which showed a tendency to abscise easily. Such effects have been noticed by Welander (1977) and Hosoki and Asahira (1980). But in combination with IAA, cytokinins produced rapid elongation and normal growth of shoots. In Bougainvillea glabra cv. 'Magnifica' Sharma et al. (1981) also noticed that higher concentration of cytokinins like BA produced an inhibitory effect on shoot elongation but auxins tended to elongate the shoots.

Incorporation of optional additives like adenine sulphate was found to have a synergistic effect on shoot proliferation. When adnine was added, mean number of shoot produced per culture was maximum (4.25). The promotary effect of these organic constituents on shoot growth has been reported by Skoog and Tsui (1968) and Silberstein et al. (1983) who found that these substances in combination with an auxin and cytokinin promoted shoot growth consequent to the cell enlargement and proliferation.

The rooting response of shoot apices of poor rooting types of bougainvillea cv. 'Mahara' was studied on MS as well as on ½ MS medium. Rooting could be obtained on these media in the presence of all the three auxins tried viz. IBA, NAA and IAA, but maximum explants rooted (85.7 per cent) on ½ MS medium containing IBA 1.6 mg/l. The fact that none of the explants rooted on MS medium devoid of any auxin indicated the essentiality of exogenous supply of these substances for satisfactory rooting. Though root initiation was much early and the rooting percentage was slightly more on ½ MS medium, a better growth and elongation of the roots were observed on MS medium. Perhaps this might be due to the inadequacy of nutrients for root growth on ½ MS salt medium, compared to MS medium. The influence of salt concentration of the medium and the promotary effect of

IBA on *in vitro* rooting has been demonstrated by Rugini and Verma (1983), Borowska (1983) and Hutchinson (1984), who found that ½ MS medium was superior to MS medium for better rooting of explants in many of the woody species like cherry and apple. Further, it was revealed from the present study that the concentration of major and minor nutrients had a direct influence on the number of days taken for root initiation as well as for its elongation and growth. Root initiation was delayed on MS medium in contrast to ½ MS medium.

Though significant progress has been made in the culture of plant tissues and cells *in vitro*, many variables influence the behaviour of cells in culture. As suggested by Skoog and Miller (1957) though the relative concentration of auxins and cytokinins forms the key factor for successful regeneration of plants *in vitro*, other environmental factors such as light, pH, season and 'cultivar' play their own roles.

Adventitious root formation in cuttings is also influenced by several factors such as variety, environment, physiological stage of growth and pretreatment, besides applied auxins. When ordinary methods of rooting of cuttings fail, *in vitro* culture come to the rescue of

researchers. In the present study, the finding that the poor rooting cv. 'Mahara' could be successfully rooted upto 85 per cent via tissue culture technique points out to the wider utility of *in vitro* culturing. A combination of macro and micropropagation methods might ultimately prove a potentially viable tool for rapid multiplication of choice plant species in the coming future.

SUMMARY

SUMMARY

The present investigations were carried out in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during the period 1983-'84. The experiments consisted of studies on the effect of growth regulators on rooting of cuttings and standardisation of tissue culture technique for bougainvillea.

1. In the experiments on rooting of cuttings all the levels of IBA and NAA tried were found to be significantly superior to control. IBA 300 and 500 ppm were equally effective. Among the nine varieties tried, the highest rooting was achieved in the variety 'Jayalakshmy' and the least in 'Cherry Blossom'. Prolonged soaking for a period of six hours in dilute solutions of IBA and NAA was found to be more effective than quick dip in concentrated solutions.

2. Influence of weather parameters like rainfall and temperature on rooting was evident. Maximum rooting was obtained when the rainfall was maximum and when the variation in mean daily temperature was less.

3. The mean number of roots per cutting was significantly higher when cuttings were treated with growth regulators.

Maximum number of roots was produced after two months in IBA 300 ppm treatment during both the seasons.

4. Among the different treatments, the longest roots were obtained with NAA 300 ppm irrespective of the seasons. The varietal influence was however, significant.

5. The number of shoots per cutting was maximum in the var. 'Mahara' during rainy season, but in 'Spring Festival' during summer.

6. Treatment of cuttings with IBA induced more shoot growth than NAA treatment. Highest shoot weight was recorded in IBA 300 ppm treatment. Among the different varieties, 'Jayalakshmy' and 'Scarlet Glory' produced maximum fresh weight of shoots per cutting. The shoot weight was more during summer in all the varieties except in 'Jayalakshmy', where it was more during rainy season.

7. The mean fresh weight of roots was significantly higher in all the dilute solution treatments of IBA and NAA and it was maximum in NAA 500 ppm treatment. In all the treatments and varieties, root weight was more during rainy season than in summer.

8. Shoot/root ratio was more during summer in all the treatments and varieties. The ratio was maximum in the

early periods of rooting in IBA 1000 ppm treatment during both the seasons and the variety 'Cherry Blossom' produced highest ratio during summer season.

9. Organic carbon and nitrogen content was negatively correlated with rooting percentage, while C/N ratio was positively correlated.

10. Aseptic culture of various explant sources on MS solid medium showed that almost all plant parts were capable of callus formation but it was more pronounced in young tissues than in mature ones. Callus formation was maximum and rapid when shoot apices were used as the explant. On the contrary, callus initiation was very much delayed upto 25 to 30 days when mature dormant buds and leaves were used.

11. Callus formation occurred with the three auxins namely NAA, IAA and 2,4-D containing medium. Maximum number of cultures callused and the earliest callus formation was obtained in the presence of NAA 1.0 and BA 0.5 mg/l. All the levels of BA were equally effective. Kinetin was less effective compared to BA. Callus initiation was very much delayed in the presence of 2,4-D.

12. Among the three varieties namely 'Mahara', 'Cherry Blossom' and 'Spring Festival' shoot apices from 'Mahara' produced maximum percentage of cultures callused while, it was minimum in 'Spring Festival'.

13. Shoot proliferation from shoot apices could be achieved on MS solid medium in the presence of higher concentrations of BA but the shoots produced were abnormal, with pale yellow leaves which showed a tendency to shed easily. Only single shoots were produced in the presence of lower concentrations of BA. Incorporation of IAA had pronounced effect on shoot elongation and the longest shoots were obtained in the presence of 2.0 mg/l IAA. Maximum number of cultures produced multiple shoots on MS medium containing BA 2.0 mg/l + KIN 1.0 mg/l. Adenine sulphate when added to the medium together with BA at 9.0 mg/l and 16.0 mg/l produced synergistic effect on shoot production.

14. <u>In vitro</u> rooting of fresh apical as well as precallused shoots were readily achieved on full and half strength MS medium in the presence of the three auxins namely IAA, NAA and IBA. The duration of root initiation was reduced on half strength MS medium and was reduced further when precallused shoots were used for rooting.

Highest percentage of rooting was achieved on ½ MS medium supplemented with IBA 1.6 mg/l. Number of roots/culture was more in IBA treatment than in IAA and NAA containing one. But the longest roots were produced in the presence of NAA 3.0 on full MS medium.

15. The preliminary trials on transplanting the rooted plantlets in sand and soil medium did not succeed. This aspect requires further detailed investigations.

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* Originals not seen.

APPENDICES

APPENDIX I

Chi-square values for comparison of treatments on rooting percentage of bougainvillea during rainy season

Treatments	Control (w/o growth)	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000
Control		3.07	51.93**	64.03**	4.32*	4.32*	10.13**	16.9**	11.0**	2.92	3.40
IBA 100 ppm			6.26**	2.82	14.35**	14.35**	5.76*	2.15	5.13*	14.97**	13.95**
IBA 300 ppm				0.50	38.5**	38.5**	23.21**	15.16**	21.56**	39.32**	37.77**
IBA 500 ppm					29.16**	29.16**	15.68**	9.47**	15.01**	29.8**	28.4**
IBA 750 ppm						0.45	2.18	58.08**	0.44	0.04	1.82
IBA 1000 ppm							2.18	58.08**	0.44	0.04	1.82
NAA 100 ppm								1.8	1.001	0.40	1.99
NAA 300 ppm									0.538	51.78**	4.8*
NAA 500 ppm										2.38	1.99
NAA 750 ppm											0.04

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX II

Chi-square values for comparison of treatments on rooting percentage of bougainvillea during summer season

Treatments	Control	IBA 100	IBA 300	IBA 500	IBA 750	IBA 1000	NAA 100	NAA 300	NAA 500	NAA 750	NAA 1000
Control		13.4**	28.4**	112.4**	15.4**	45.5**	5.34*	37.2**	32.5**	25.5**	18.71**
IBA 100 ppm			3.02	51.28*	0.11	10.2**	1.66	6.44*	4.57*	2.17	0.45
IBA 300 ppm				30.52**	1.78*	2.35	2.35	8.76**	0.75	2.17	0.87
IBA 500 ppm					45.77**	15.53**	69.5**	21.1**	24.9**	31.9**	40.6**
IBA 750 ppm						8.61**	2.43	5.15*	3.50	1.45	0.22
IBA 1000 ppm							19.5**	0.36	0.45	2.77	5.76*
NAA 100 ppm								15.13**	12.2**	8.00**	4.39*
NAA 300 ppm									0.11	1.003	3.00
NAA 500 ppm										0.362	1.78
NAA 750 ppm											0.447

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX III

Chi-square values for comparison of different varieties of bougainvillea on rooting percentage during rainy season

Varieties	Scarlet Glory	Jaya-lakshmy	Enidlan-caster	Lady Mary Baring	Thimma	Cherry Blossom	Mahara	Spring Festival	Maharaja of Mysore
Scarlet Glory		0.009	39.3**	182.42**	218.37**	214.3**	174.74**	235.0**	117.9**
Jayalakshmy			52.62**	207.00**	245.00**	269.46**	199.62**	260.06**	142.0**
Enidlancaster				56.04**	78.0**	93.03**	51.56**	0.89	2.74
Lady Mary Baring					17.7**	4.73*	0.122	3.92*	7.64**
Thimma						0.83	3.14	0.341	17.25**
Cherry Blossom							32.8**	0.068	7.00**
Mahara								5.2*	6.02*
Spring Festival									23.00**

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX IV

Chi-square values for comparison of different varieties of bougainvillea on rooting percentage during summer season

Varieties	Scarlet Glory	Jaya-lakshmy	Enidlan-caster	Lady Mary Bering	Thimma	Cherry Blossom	Mahara	Spring Festival	Maharaja of Mysore
Scarlet Glory		1.33	140.0**	70.4**	31.3**	69.4**	41.65*	57.18**	59.91**
Jayalakshmy			165.03**	88.70**	44.5**	88.03**	56.46**	74.23**	77.31**
Enidlancaster				13.47**	42.09**	13.92**	31.54**	20.43**	18.82**
Lady Mary Bering					8.48**	0.01	4.13*	0.843	0.541
Thimma						7.45**	0.66	3.75**	4.49*
Cherry Blossom							3.89*	0.73	0.45
Mahara								1.11	1.53
Spring Festival									0.014

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX V

Monthly weather data and percentage rooting of bougainvillea

Month	Rooting percentage	Temperature (°C)		Mean Relative humidity	Rainfall	
		Mean maximum	Mean minimum		Quantity (mm)	Number of rainy days
June 1983	35.53	31.9	24.5	79.0	387.2	19
July 1983	62.51	29.7	23.7	87.0	580.6	21
August 1983	77.95	29.1	23.8	87.0	754.7	26
September 1983	71.48	29.5	23.4	84.0	494.6	24
February 1984	38.9	34.3	24.2	56.0	27.0	3
March 1984	64.6	35.2	24.3	67.0	18.9	2
April 1984	73.25	34.5	24.9	72.6	109.2	9
May 1984	81.58	24.5	25.8	71.0	140.6	6

APPENDIX VI

Analysis of variance table for number of roots/cuttings one month, two months and three months after planting

Source	df	Mean squares					
		1st month		2nd month		3rd month	
		Rainy season	Summer season	Rainy season	Summer season	Rainy season	Summer season
Total	197	-	-	-	-	-	-
Variety	8	802.23**	216.35*	1786.63**	379.53**	1208.05**	274.04**
Treatment	10	256.78**	179.60**	539.57**	103.09*	306.90**	79.08**
Interaction	80	44.36	29.21	90.27	36.18	93.45	19.51
Error	99	29.73	23.72	97.05	25.77	60.02	13.73

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX VII

Analysis of variance table for length of roots/cuttingsone month, two months and three months after planting

Source	df	Mean squares					
		1st month		2nd month		3rd month	
		Rainy season	Summer season	Rainy season	Summer season	Rainy season	Summer season
Total	197	-	-	-	-	-	-
Variety	8	0.240	0.463*	12.44**	2.35*	33.30**	15.02*
Treatment	10	0.634**	0.295**	2.59*	2.31	7.26	6.85*
Interaction	80	0.262	7.82	1.11	1.01	4.75	4.18
Error	99	0.177	6.67	0.75	1.15	3.49	2.84

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX VIII

Analysis of variance table for number of shoots/cutting one month, two months and three months after planting

Source	df	Mean squares					
		1st month		2nd month		3rd month	
		Rainy season	Summer season	Rainy season	Summer season	Rainy season	Summer season
Total	197	-	-	-	-	-	-
Variety	8	9.26	6.46**	3.30	3.09**	2.05	1.55**
Treatment	10	7.28	2.50*	1.393	1.76*	1.09	0.54
Interaction	80	5.18	1.55	1.74	1.07	0.969	0.776
Error	97	5.75	1.12	1.82	0.676	1.217	0.044

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX IX

Analysis of variance table for fresh weight of shoot/cutting one month, two months and three months after planting

Source	df	Mean squares					
		1st month		2nd month		3rd month	
		Rainy season	Summer season	Rainy season	Summer season	Rainy season	Summer season
Total	197	-	-	-	-	-	-
Variety	8	2.466**	3.694**	15.029**	17.232**	57.09**	45.94**
Treatment	10	1.351**	1.460**	2.644	10.431**	7.884	13.92**
Interaction	80	0.453	0.531	1.229	3.358	3.335	3.247
Error	97	0.336	0.259	1.776	0.224	5.067	2.064

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX X

Analysis of variance table for fresh weight of roots/cutting one month, two months and three months after planting

Source	df	Mean squares					
		1st month		2nd month		3rd month	
		Rainy season	Summer season	Rainy season	Summer season	Rainy season	Summer season
Total	197	-	-	-	-	-	-
Variety	8	77520.9**	50757.6**	816711.7**	12254.8**	340517.8**	38605.0**
Treatment	10	9168.7**	6240.5**	110344.3*	30474.4**	153103.2**	16505.0**
Interaction	80	8804.2*	1691.78**	56036.5	6338.3*	148150.8	22496.6
Error	97	2232.8	313.73	27665.8	2357.6	119538.5	23475.2

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX XI

Analysis of variance table for shoot:root ratio/cutting one month, two months and three months after planting

Source	df	Mean squares					
		1st month		2nd month		3rd month	
		Rainy season	Summer season	Rainy season	Summer season	Rainy season	Summer season
Total	197	-	-	-	-	-	-
Variety	8	214.66**	3012.4**	324.71**	2271.2**	291.33**	722.5**
Treatment	10	74.45**	907.43**	73.55	428.9**	37.39	30.54
Interaction	80	43.16	261.19*	54.38	311.47	29.91	89.22
Error	99	19.04	99.40	39.69	72.95	25.01	80.10

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX XII

Intercorrelation matrix between root number, root length, shoot number, root weight, percentage of rooting and shoot weight of bougainvillea cuttings during rainy season

	Root number	Root length	Shoot number	Root weight	Percentage rooting	Shoot weight
Root number	1.00**	0.32**	-0.498**	0.270*	0.652**	0.48**
Root length		1.00**	0.50**	0.097	0.51**	0.55**
Shoot number			1.00**	0.12	0.577**	0.550**
Root weight				1.00**	0.241*	0.254*
Percentage rooting					1.00**	0.641**

* Significance at 5 per cent level of probability

** Significance at 1 per cent level of probability

APPENDIX XIII

Intercorrelation matrix between root number, root length, shoot number, root weight, percentage of rooting and shoot weight of bougainvillea cuttings during summer season

	Root number	Root length	Shoot number	Root weight	Percentage rooting	Shoot weight
Root number	1.00*	0.25*	-0.053	0.65**	0.558**	0.35**
Root length		1.0**	0.019	0.60**	0.22	0.44**
Shoot number			1.0**	0.083	0.556**	-0.050
Root weight				1.0**	0.591**	0.467**
Percentage rooting					1.0**	0.417**

* Significant at 5 per cent level of probability

** Significant at 1 per cent level of probability

APPENDIX XIV

Correlation matrix among organic carbon content, total nitrogen, C/N ratio and percentage of rooting of bougainvillea cuttings during rainy season

	Total nitrogen	C/N	Percentage of rooting
Organic carbon	0.756**	-0.308**	-0.626**
Total nitrogen		-0.707**	-0.686**
C/N ratio			0.512**

APPENDIX XV

Correlation matric among organic carbon content, total nitrogen, C/N ratio and percentage of rooting of bougainvillea cuttings during summer season

	Total nitrogen	C/N	Percentage of rooting
Organic carbon	-0.151	0.05	-0.591**
Total nitrogen		0.016	-0.027
C/N ratio			0.164

** Significant at 1 per cent level of probability

APPENDIX XVI

Pairwise comparison of different explant sources for their effect on callus formation

	Mature dormant buds	Shoot apices	Embryonic leaves	Mature leaf disc
Immature Axillary stem pieces	2.68	0.79	0	2.40
Mature dormant buds		7.91**	3.89*	0.174
Shoot apices			0.02	3.13
Embryonic leaves				0.82

APPENDIX XVII

Chi-square values for comparison of different levels of NAA

NAA (mg/l)	1.0	1.5	2.0
0.5	20.77**	5.34*	4.10*
1.0		3.23*	6.42*
1.5			0.0017

* Significance at 5 per cent level of probability
** Significance at 1 per cent level of probability

APPENDIX XVIII

Chi-square values for comparisons of different levels of KIN and BA for the effect on callus formation

	mg/l	KIN 0.5	KIN 0.8	KIN 1.0	BA 0.2	BA 0.5	BA 0.8	BA 1.0
KIN	0.2	0.14	0.05	0.18	0.5	12.19**	11.44**	11.41**
KIN	0.5		0	0.045	10.9**	13.35**	12.53**	12.53**
KIN	0.8			0.41	9.98**	16.37**	16.38**	16.38**
KIN	1.0				11.12**	18.31**	17.31**	17.31**
BA	0.2					4.08*	7.46**	7.46**
BA	0.5						0	0
BA	0.8							0

APPENDIX XIX

Chi-square values for comparison of three auxins for the effect on callus formation

	mg/l	IAA 2.0	NAA 1.0	NAA 2.0	2,4-D 1.0	2,4-D 2.0
IAA	1.0	0.132	7.90**	7.10**	0.04	6.79**
IAA	2.0		1.98	1.37	0.60	1.37
NAA	1.0			0.603	5.9*	0.31
NAA	2.0				1.37	0.071
2,4-D	1.0					1.37

* Significance at 5 per cent level of probability
** Significance at 1 per cent level of probability

APPENDIX XX

Chi-square values for comparison of the three varieties for the effect on callus formation

	Cherry Blossom	Spring Festival
Mahara	3.79*	10.19**
Cherry Blossom		6.54**

* Significant at 5 per cent level of probability
** Significant at 1 per cent level of probability

STANDARDISATION OF MACRO AND MICRO PROPAGATION TECHNIQUES IN BOUGAINVILLEA

BY

AISHABI K. A.

ABSTRACT OF A THESIS

submitted in partial fulfilment of

the requirement for the degree

Master of Science in Horticulture

Faculty of Agriculture

Kerala Agricultural University

Department of Pomology and Floriculture

COLLEGE OF HORTICULTURE

Vellanikkara - Trichur

KERALA — INDIA

1985

ABSTRACT

The present investigations were carried out at the College of Horticulture, Kerala Agricultural University, during 1983-'84 to standardise the asexual propagation in bougainvillea through conventional as well as micropropagation techniques. For macropropagation studies, cuttings from nine varieties were treated with IBA and NAA each at 100, 300, 750 and 1000 ppm concentrations. The results revealed that all the treatments, particularly IBA were significantly superior to the control. Soaking the basal ends of the cuttings in IBA 500 ppm solution for a period of six hours, gave maximum percentage of rooting both in rainy and summer seasons. The rooting response of different varieties varied considerably. Maximum percentage of rooting was obtained in the variety 'Jayalakshmy' and minimum in 'Cherry Blossom' during both the seasons. Organic carbon and total nitrogen content showed a significant negative correlation with percentage of rooting. A positive correlation was however observed between C/N ratio and rooting percentage. Rooting was more when the rainfall was maximum and variation in mean temperature was less. Root number, root length and root weight were significantly increased in all the auxin treated cuttings than in the control. Number of roots, length and fresh weight of roots/cutting were more during

rainy season, than in summer. Fresh weight of the shoot/ cutting and shoot/root ratio were more during summer than in rainy season.

Micropropagation studies carried out using the explants from 'shy' rooting varieties of bougainvillea indicated that, of the different explant sources tried, shoot apices and immature axillary stem segments were the most potent sources for callus formation. All the three auxins tried namely, IAA, NAA and 2,4-D were capable of initiating callus, but 2,4-D delayed the period of initiation. MS medium supplemented with NAA and BA was found to be most suitable for callus formation, and maximum number of cultures callused in the presence of NAA 1.0 mg/l + BA 0.5 mg/l. KIN was found to be less effective than BA.

Maximum number of cultures callused, in the case of explants collected from the variety 'Mahara', compared to that from 'Cherry Blossom' and 'Spring Festival'. Attempts to induce proliferation of axillary buds showed that, MS medium containing BA 2.0 mg/l + IAA 1.0 mg/l was optimum. Higher concentrations of BA (9.0 mg/l and above), though induced more number of shoots, they were abnormal and unhealthy. Addition of adenine sulphate 50 mg/l produced

a synergistic effect on shoot production. Fresh shoot apices and precallused shoots rooted readily on full and half strength MS medium supplemented with different auxins like IAA, NAA and IBA. Percentage of rooting was maximum on half strength MS medium in the presence of IBA 1.6 mg/l. The time required for root initiation was reduced on half strength MS medium and further when precallused shoots were used. Field transplantation of the rooted plantlets was not successful and further studies in this aspect is necessary.