Influence of growth regulators on the rooting of different types of cuttings in *Bursera penicillata* (DC) Engl.

CHANDRAMOULI H.

Division of Horticulture
University of Agricultural Sciences
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Influence of growth regulators on the rooting of different types of cuttings in *Bursera penicillata* (DC) Engl.

CHANDRAMOULI H.

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CERTIFICATE

This is to certify that the thesis entitled "Influence of growth regulators on the rooting of different types of cuttings in Bursera penicillata (DC) Engl." in partial fulfilment of the requirements for the degree of Master of Science (Agriculture) in Horticulture to the University of Agricultural Sciences, Bangalore is a bonafide record of research work carried out by Mr. Chandra Mouli H. under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma associateship, fellowship or other similar titles.

Bangalore
October, 2001

(. M. CHANDRE GOWDA)
Associate Professor
Division of Horticulture
(Major advisor)

Approved by :

Chairperson : .....
(. M. Chandre Gowda)

Members :

1. M. Vasundhara
((Mrs.) M.Vasundhara)

2. .....
(A. A. Farooqi)

3. .....
(K. Seenappa)

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INTRODUCTION

I INTRODUCTION

Richness of plants is a unique feature of the biosphere. From the very early times, mankind has been a genuine lover of perfumes of any sort which may make an agreeable appeal to his sense of smell. Many of the aromatic herbs and essential oil bearing plants have long been the basic ingredients to ancient perfumery and modern cosmetics. There is nothing in the world which can equal them in enlivening the depressed mind and in permeating it with a new spirit of buoyancy. Many of them are, over and above credited with actual disinfecting properties and are of immense value from the hygienic point of view.

Indian lavender oil is the essential oil of paramount commercial importance and is obtained from Bursera (*Bursera penicillata* (DC) Engl.) . It was introduced to India from Mexico in 1912 by two enterprising Scots men, P.J.Anderson and G.N.Hamphries. It is called as 'Linaloe or 'Ligna loe' or 'Mexican lavender' tree, whereas in India it is called as 'Indian linaloe' or 'Indian lavender' tree and its oil is known as 'Linaloe oil' in commerce. In Mexico, the heart wood of about 50-60 years old trees is used to extract essential oil, whereas in India, both fully matured and fallen husks from the fruits as well as heart wood are subjected to steam distillation for extraction of essential oil (Sastry, 1952). The Indian lavender oil has superior staying power and excellent odour. The oil is rich source of linalool and linalyl acetate.

Linaloe tree belongs to the tropical American genus 'Bursera' and family 'Burseraceae' under the natural order 'Geraniales' to which citrus also belongs. According to Hussain (1958), the genus Bursera has 45 species. Tanka (1976), in his Encyclopedia of edible plants of the world,

has given eight important species and of them, *Bursera serrata* is found growing wild in India and *Bursera penicillata* is the only species which is being commercially exploited for its oil.

Bursera penicillata is a small tree which is bushy and branchy in nature, under the climatic conditions of Bangalore, the tree starts shedding its leaves by December-January and by February. It is completely leafless and remains in the same condition till April or early monsoon season. The new flush of leaves and flowers appear more or less simultaneously during early April and profuse flowering is noticed by the third week of April (Gowda, 1983). The flowers are both unisexual and hermaphrodite. Fruit ripens during August-September. It is oval with fleshy pericarp with oil glands, green in initial stages and turns reddish pink or purple when ripe. The pericarp contains a considerable percentage of oil and thus, is the economically important part. Normally trees fruit every year. Seed is single, thick, has a hard seed coat and black in colour. It is devoid of oil glands. Except seeds, every part of the tree contains aromatic essential oil and is maximum in the pericarp of the fruits. The percentage of oil in the pericarp varies with different stages of fruit development and it is maximum when mature.

Green fruits yield about 2 per cent oil as compared to 12-16 per cent in dried husk. The pericarp of matured fruit falls down in the form of husk. This husk is collected and used for extraction of oil by steam distillation. The percentage of oil in other parts like foliage, mature heart wood and roots ranges from 0.15 to 0.3 per cent ,7 to 9 per cent, and 0.27 per cent respectively. The oil is pleasant smelling and has linalool as its major constituent (60-70%). It also contains linalol monoxide, methyl heptanone, 1-methyl heptanol. nerol, alpha terpenol and geranoil in small quantities.

The oil has a specific gravity 0.9050 at 15^{O}C, optical rotation 6^{O}57', refractive index 1.45182 at 20^{O}C, zero acid number, ester number 270.7 (which is equivalent of 94.7% of linalyl acetate) and soluble in 5 volumes or more of 70 percent alcohol. These are the chemical and the physical properties of Bursera oil of high standard (Guenther, 1951).

The Bursera oil is mainly used in cosmetic and soap industries. It is used as a fixative for costly essential oils like rose oil. jasmine oil, tuberose oil, rosemary oil, lilae oil etc., and it is a perfume by itself. It is also used in flavouring food and confectionary and is also used as germicide in medicine.

Bursera can be propagated both by seeds and cuttings. Seeds require about 6 months to germinate and their germination percentage is very poor which is caused by hard seed coat and internal dormancy. Probably this is the reason why this method is not being used (Hussain, 1958).

At present Bursera is propagated mainly by using the cuttings of 0.5 to 1.0 m length and 1 to 3 cm girth, which is a cumbersome method and consumes a lot of labour, time consuming and costly and the cuttings not only take a long time of 4 to 6 months to root but rooting in them is sparse and erratic resulting in poor field establishment. Hence, there is a need to study the influence of external application of growth regulators as well as standardizing the type of planting material to improve rooting and make available the planting material for increasing acreage under this crop. Therefore in the present investigation, an attempt was made to study the influence of planting material and growth regulators on the rooting behaviour of *Bursera penicillata* (DC) Engl with the following objectives :

1. To study the effect of growth regulators on rooting of different types of cuttings in Bursera;
2. to study their interaction effects; and
3. to study the field establishment and survival of rooted cuttings;

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

A very meggar information is available on propagation of *Bursera peniciliata* (DC) Engl. through cuttings. In the present review, the research work on propagation of other related but economically important plants species have been included.

2.1 Studies on the propagation of *Bursera peniciliata* through cuttings

Among the methods of vegetative propagation, propagation by cuttings is the easiest and widely employed which is usually followed in easy to root species. In cuttings, growth substances applied exogenously, are found to enhance early and good root formation. Now a days many of the difficult to root plants are made to root easily by applying plant growth substances.

Various classes of growth regulators such as auxins, cytokinins, gibberellins and ethylene influence root initiation in cuttings. Of these, auxins have greater effect on root formation in cuttings. In addition of these groups, various growth retardants and promoters may have a less direct role in adventitious root formation (Krul, 1968). The research work on propagation of *Bursera penicillata* (DC) Engl. through cuttings is very much limited . Hence the review of literature on root formation in *Bursera penicillata* (DC) Engl. and various tree species and other related plants is briefly reviewed in this chapter under the following headings.

2.1.1 Effect of maturity of shoot on rooting of cuttings

2.1.2 Effect of water soaking as a pre-plant treatment

2.1.3 Effect of Auxins on rooting of cuttings

2.1.4 Auxin synergism

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2.1.7.3 Role of carbohydrates

2.1.7.4 Role of phenolic compounds

2.1.7.5 Role of root promoting co-factors

2.1.1 Effect of maturity of shoot on rooting of cuttings

The maturity of shoot plays a very important role in some difficult to root plant species. It is found that any treatment which induce maturity in the cuttings, would be beneficial for promoting better rooting. Therefore evidences for a relationship between maturity of shoot and rooting potentiality were established in apples by Stoutemyer (1937) in olive, Walnut and peach by Garner (1958).

Rooting capacity of cuttings depends on the type of wood and portion of the plant from which they are taken (Scarborough, 1971). Vadivel *et al.* (1981) reported that hard wood cuttings of cinnamon rooted better than semi-hard wood cuttings. Similarly, Singh and Singh (1973) obtained better results with hard wood cuttings than the other type of cuttings in case of lemon (*Citrus lemon* Burm). Aslo, Bandopadhyay *et al.* (1980) recorded the highest rooting in hard wood cuttings of three *Carissa* species.

But, Sifrat (1982) observed that in case of *Cotinus coggyria* Cultivar Royal purple, the terminal cuttings rooted better than the sub-terminal cuttings. Similarly Bhattacharjee and Balakrishna (1989) reported that cuttings gave cent per cent rooting as well as survival as compared to middle and basal cuttings of Bouganivillea.

Khanna and Arora (1984) reported that treatment of hard wood cuttings of amaltas (*Cassia fistula*) with higher concentrations of growth regulators (NAA and IBA 2500 ppm each) gave 60 per cent rooting.

Blazich and Acedo (1989) found that among the two types of cuttings of *Osmanthus heterophyllus* CultivarRotundifolius hard wood cuttings performed better than semi-hard wood cuttings.

2.1.2 Effect of water soaking as a pre-plant treatment

A general improvement in rooting by soaking the basal two nodes of olive (*Olea europea*) cuttings in water was noticed by Panneli *et al.* (1980) especially at times when natural rooting ability was high.

Bartolini *et al.* (1986) reported that soaking the bases of grape cuttings made from one year old wood of the cultivars 5 BB and 140 Raggeri in deionized water for 24 hours stimulated rooting.

The inhibitory effect on rooting of herbaceous cuttings of *Genista monosperma* Lan. due to the accumulation of 3-hydroxy mandelic acid (an endogenous phenol) in the basal tissues could be lessened by soaking cuttings in distilled water (Curir *et al.*, 1992).

2.1.3. Effect of Auxins on rooting of cuttings .

Among the synthetic root promoting chemicals indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) have been found to induce rooting in a variety of horticultural speices.

Shanmugavelu (1960) studied the effect of IBA on the rooting of cutting in *Hibiscus rosasinensis* and obtained 75 per cent rooting with 2000 ppm.

In case of stem cuttings of Bottle brush (*Callistemon lanceolatus*) the best result (80% rooting) was obtained with IBA at 500 and 1000 ppm at 60 day after planting (Rajput *et al.*, 1979).

Kwach and Chung (1980) treated the cuttings of 25 ornamental plant species with IBA and NAA under mist and found in many species, NAA promoted rooting compared to IBA at the same concentrations (200 ppm); dipping for 24 hours was more effective than the shorter dipping treatments.

Vadivel *et al.* (1981)have reported that in case of *Cinnamomum zeylanicum* cuttings, rooting was best (45%) in hard wood cuttings treated with IBA at 2500 ppm (control 5%) whereas in semi-hard wood cuttings, the best rooting (22.5%) was obtained with NAA at 5000 ppm when compared to control.

Basal cuttings of Carissa species treated with 500 ppm IBA gave 75 per cent rooting (Bandopadhyay *et al.*, 1982) and higher rooting in *Poinsettia pulcherrima* cuttings was also recorded by Gowda *et al.* (1982) at the same concentration.

Rao *et al.* (1982) experimented with cuttings of *Euphorbia caracasana* and observed that cuttings treated with 500 ppm NAA gave higher rooting (80%) than those treated with 10,000 ppm IBA (70%).

Nicholson (1984) observed 53 per cent rooting when cuttings of *Pieris floribunda* were soaked in IBA 400 ppm solution for 24 hours before planting.

Sherer *et al.* (1985) reported that among the six growth regulators tried at 0.005 to 0.01 per cent, the best root forming chemical was IBA, followed by NAA.

Guo et al. (1986) reported that cuttings of *Acanthopanax senticosus* treated with NAA at 50 ppm or 100 ppm rooted well (80.67 and 73.67%). Benyei (1987) observed that NAA treatment of *Hedera helix* resulted in the best rooting.

Branch cutting of *Woodfordia fruticosa* were treated by dipping them in 100 or 200 ppm IBA or NAA solutions for 12 or 24 hours and the best response of rooting was obtained in the 200 ppm 24 horus IBA treatment (Bahuguna et al., 1988).

Bisaria and Rao (1988) reported that in *Boehveria nivea* Gaud. that IBA at 100 ppm indued early differentiation of adventitious roots, increased number and length of roots and enhanced fresh and dry weight.

Khromova (1988) obtained the best rooting (94%) in *Schisandra chinensis* by treating spring cultivars with IBA at 0.02 per cent for 24 hours as compared to summer soft wood cuttings (20 to 63%).

Semi hard wood cuttings of Kathbael (*Limonia acidisima*) immersed for 24 hours in 50 to 100 ppm NAA gave good results with respect to rooting and field establishment (Poi and Mazumdhar, 1989).

Kaundal and Singh (1989) reported the higher rooting success in *Pyrus communis* cultivar Leconte with 200 ppm IBA treatment and correlated to high C:N ratio.

Kim *et al.* (1990) in their studies on the propagation of *Citrus junos* by soft wood cuttings found that IBA treatment had the greatest effect on percentage rooting and root length at 1000-4000 ppm.

Sadiq *et al.* (1991) while studying the effect of different concentration of IBA (50-400 ppm) on root initiation in semi hard wood cutting of peach cultivar Early Grande found that IBA at 400 ppm resulted in the highest average number of shoots (2), leaves (21.25), roots (11), lowest number of days to sprouting (14) and average shoot (7.5 cm) and root (3.75 cm) lengths.

The highest percentage of sprouting (96.5%), plant raised (4.65%), number of branches per plant (23.6) were obtained with basal cuttings with seradix-B (IBA) incase of cuttings of plum cultivar Kala Amritsari (Mehrotra, 1991).

Oosthuizen (1993) reported the pecan cuttings treated with IBA gave highest rooting percentage (97%) for cultivar Sioux.

Reddy *et al.* (1996) noticed that the maximum percentage of rooting and mean length were observed with IBA 1250 ppm in case of grape hybrids.

2.1.4 Auxin synergism

Synergistic effects of different auxins in general and IBA and NAA in specific on the rooting of cuttings have been reported by several workers.

Bose *et al.* (1972) observed the synergistic effect between auxin and phenolic compounds in the rooting of cuttings of many ornamental plants.

Bhujabal and Kale (1973) obtained maximum percentage of rooted cuttings, number of roots and highest root length in different rose root stocks with a mixture of IBA and NAA at 1000 ppm.

Bhattacharjee and Balakrishna (1992) observed the maximum per cent rooting (100%) and survival in stem cuttings of *Ixora singaporensis* with IBA and NAA at 4000 ppm each and IAA and IBA at 6000 ppm each. Nath (1992) found that semi hard wood cuttings of *Mussaenda phillippica* produced highest rooting percentage (90%), number of primary roots (25.0/cutting) length of the longest root (12.7 cm), root dry weight (172 mg/cutting) and survival percentage (90%) with 2000 ppm IBA treatment.

Dunn *et al.* (1996) stated that *Pistacia chinensis* cuttings produced more rooted cuttings at 5000 ppm of IBA and NAA each in combination than other treatment combinations.

2.1.5 Effect of growth retardants

Rooting enhancement activity of growth retardants is attributed to the fact that they lower the endogenous levels of gibberllins in the tissues of cuttings either by inhibiting the GA-biosynthesis or its deactivation. This mechanism coupled with the increase in the partitioning of assimilates or hormones to the base of cuttings have been suggested to be the reason for root promotion (Davis *et al.*, 1985).

Davis *et al.* (1985) reported that cuttings of *Plectranthus australia* and *Phaseolus vulgaris* when treated with paclobutrazol (for 24 to 40 hours) at very low concentration (3-6 mg/l) produced 100 and 85 per cent more roots than control, respectively. They also reported that paclobutrazol did not effect root length greatly although it reduced shoot length.

Davis (1986) obtained increased root formation with increasing paclobutrazol concentration upto 24 ppm in bean hypocotyls cuttings.

Marino (1986) observed that application of paclobutrazol (0.25 or 0.5 mg/l) markedly increased rooting in shoots of *Pyrus persica* and *P.kanvensis*. Roots from treated shoots were shorter and thicker than formed without paclobutrazol.

Asare-Boamah (1986) found that application of triadimefon promoted adventitious roots development in both intact and excised bean hypocotyls.

Bausher and Yelenosky (1987) observed in citrus that, at higher concentrations of paclobutrazol these was no secondary root formation and it resulted in progressive basal enlargement of the primary root.

Goffreda (1991) obtained 75 per cent rooting in semi hard wood cuttings of apricot when treated with paclobutrazol.

2.1.6 Establishment of rooted cuttings

The success of propagating plants by any method lies in how best they establish in the field after transplanting. Thus, a knowledge about their extent of establishment is important to obtain better productivity.

Gupta (1982) observed a maximum of 80 per cent rooting, and 90 per cent survival in the cuttings of *Legastroemia lancasteri* treated with 400 ppm IBA. The best rooting in mango (75%) and plant survival after one year (55%) was obtained with IBA at 10,000 ppm (Chattarjee, 1982).

Kempe Gowda (1984) reported that in rose cultivar Maria callas, there was 40 per cent field establishment of hard wood cuttings treated with 2000 ppm IBA whereas in Queen Elizabeth, semi hard wood cuttings treated with 2000 ppm NAA recorded 81.66 per cent establishment.

Poi and Mazumdar (1989) reported that in semi hard wood cuttings of kath bael treated with NAA at 50 and 100 ppm the survival percentage was 62.2 and 40 respectively.

Application of IBA at 300 ppm to Damask Rose cuttings resulted in the highest survival of rooted cuttings (48.97%) followed by 300 ppm NAA (41.11%). The basal woody cuttings were better than the middle semi-woody cutting (Shenoy, 1992).

2.1.7 Physiological and biochemical factors influencing rooting in cuttings

The rooting success in cuttings, irrespective of the pre-treatment is very much influenced by the physiological and biochemical status of the planting material used. The changes in the biochemical constituents brought about by the auxin treatment in the cuttings determine the case with which the rooting is accomplished. The role of auxins in initiation of rooting is attributed to their capacity to enhance hydrolysis by the nutritional reserves.

2.1.7.1 Role of auxins on rooting

Cooper (1938) reported in lemon and apple cuttings that the IBA applied at the base in solution caused the downward transport of a substance called rhizocaline which was necessary for root formation.

Auxins help in accumulation of metabolites, synthesis of new proteins, cell enlargement, cultivation and increase in nitrogen content leading to callus formation which in turn grows into roots in plum (Strydem and Hartmann, 1960).

Eriksen and Shafaat (1974) stated that auxins are of greater importance in the early stages of root primodia formation than during the later stages of this

process. Further, they observed in pea cuttings that auxins promote root formation process to proceed almost continuably without any interruption.

In a physiological study with carob, Fadl *et al.* (1979) attributed the beneficial effect of IBA on rooting of cuttings to the disappearance of inhibitors and appearance of growth stimulators.

2.1.7.2 Influence of carbohydrates and nitrogen in the rooting of cuttings

Studies conducted by many workers have revealed that the ratio between carbohydrates and nitrogen determines the rooting capacity of a cutting. For instance Haun and Cornel (1959) observed that a low level of nitrogen in Geranium species resulted in a significantly higher percentage of rooted cuttings.

Sen and Bose (1960) reported that root initation was directly proportional to the C:N ratio in cuttings of *Justicia*. They obtained enhanced rooting in cuttings with low nitrogen but more of total carbohydrates. In case of tea clones also higher sugar and lower nitrogen resulted in better rooting (Gabricludze *et al.*, 1975).

Rajanna (1981) recorded a higher carbohydrate nitrogen ratio in easy to root pomegranate Cultivar 'Kabul Red' cuttings than the difficult-to-root 'Bassein seedless'.

Purushotham *et al.* (1986) reported that cuttings of *Coffea arabica* Cultivar S-795 collected in August showed highest rooting percentage (66.73) which had the highest total sugar and lower starch contents.

2.1.7.3 Role of carbohydrates

Gosh and Basu (1974) noticed only a slight fall in concentration of total available carbohydrates up to root emergence stage, but subsequent fall in total carbohydrate was more with the onset of root emergence. This fall was greater in IBA and NAA treated cuttings than in control.

Prasad *et al.* (1980) observed the break down of starch and double sugars to release the reducing sugars. Further, they observed that the activities of amylase and invertase were more in regenerating part than in the non-generating part of the cutting.

Kumar et al. (1985) noticed higher levels of reducing and non-reducing sugars in easy to root *Ficus elastica* cultivar Decora than difficult to root cultivar Variegata. The cultivar Variegata had higher starch and lower phenolic contents than Cultivar Decora.

2.1.7.4 Role of phenolic compounds in the rooting of cuttings

The result of research work suggests that the phenolic compounds would prove useful to plant propagator in the relatively difficult to root plants. Phenolic compounds play an important role in regulation of growth. These compounds regulate the growth primarily affecting the compound on the decarboxylation of IAA.

Hess (1962) indicated that phenols act as auxin sunergists in root initiation later, Roy *et al.* (1972) reported that natural difference in rooting ability of *Phyton argentia* cuttings could be due to difference in phenolic compounds.

Bose et al. (1973) showed that easy-to-root materials of bougainvillea and hibiscus contained p-hydroxy benzoic, ferulic and p-coumaric acid but in difficult to root plant material only p-hydroxy benzoic acid was present in high concentration.

The failure of *Jasminum auriculatum* CultivarParimullai cuttings to root satisfactory when compared to easy to root *Jasminum sambac* cultivar Gundumullai was attributed to high contents of total phenols and ortho-di-hydroxy phenol in the former (Veeraragavatham *et al.*, 1983).

Curir *et al.* (1992) were of the opinion that the seasonal fluctuations in rooting ability of *Genista monosperma* Lam. were correlated with the ratio of two main endogenous phenols, 3-hydroxy mandelic acid and luteolin-7-0-glucoside, accumulating in the tissues, the first acting as an inhibitor and the second as a promoter of adventitious root development.

2.1.7.5 Role of root promoting co-factors in rooting of cuttings

These are naturally occurring substances in leaves that appear to act synergistically with indole acetic acid in promoting rooting. Hence, retaining leaves has greatly increased rooting per cent in a number of difficult-to-root species.

Girouard and Hess (1966) observed that 'Co-factor 4' had the highest root promoting activity and was present in leaves, stem and roots. 'Co-factor 1' was the second most active root promoter and was found to be present chiefly in the leaf tissues. Whereas Co-factor 2 and 3 had lowest activity and were found in most of the plant parts.

Adopting chromatography and mung bean bioassay technique, Hess (1966) detected differences in substances involved in stimulating root initiation from the methanol extracts of juvenile and matured tissues. The region of peak activity was referred to as 'rooting Co-factor'. These was synergistic reactions between rooting Co-factors and IAA in stimulating the root initiation. Further, a maximum difference in the level of 'rooting Co-factor' between juvenile and matured shoots was noted.

Vijaykumar (1973) concluded that the root promoting 'Co-factor 4' present in invigourated roots of guava, was essential for rooting.

While working on propagation of grape by cuttings Mokashi (1976) found that shoot extracts of 'Gulabi' cutting exhibited root promotion activity at all the Rf zones except at 0.2, while that of 'Thompson seedless' showed root inhibition at 0.1, 0.2 and 0.3. All the four root promoting co-factors were present in 'Gulabi' whereas 'Thompson seedless' lacked the co-factors 1 to 4 which are known to be most essential for rooting.

All the four root promoting Co-factors were present in easy to root 'Kabul Red' cultivar of pomegranate but 'Bassein seedless' lacked the Co-factor 1 and 4, which are known to be required for rooting (Rajanna, 1981).

Khan (1983) reported that the root promoting Co-factor 1 and 4 were essential for inducing roots in cuttings of sandal wood.

A positive correlation was found between rooting ability of avocado cutting and mung bean root promoters at Rf 0.9-1.0 is chromatography of methanol extracts in isopropanol; water (8:2 v/v) (Raviv and Reuveni, 1984).

Purushotham *et al.* (1986) observed a positive correlation between the rooting percentage and levels of Co-factors 2, 3 and 4 in cuttings of *coffee arabica* variety S-795.

Gowda *et al.* (1990) recorded the presence of two inhibiting zones at Rf 0.3 and 0.7 and promotion of roots at the presence of rooting co-factors 1, 3 and 4 (Rf 0.1, 0.6 and 0.8) respectively. Inhibitors at, Rf 0.3 indicated the absence of 'Co-factors 2' in the fresh tissue extracts of the rose cultivar American heritage.

MATERIAL AND METHODS

III MATERIAL AND METHODS

The details of the material used and techniques adopted during the course of study on 'Influence of growth regulators on the rooting of different types of cuttings in *Bursera penicillata* (DC). Engl.' are described below.

The present investigation was carried out during 2000-2001 at the 'Sanjeevini Vatika' Herbal Garden located at E-block, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore.

3.1 Geographical location and climate

The experiment was conducted at the Sanjeevini Vatika which is attached to the Division of Horticulture, situated at an altitude of 930 m from mean sea level, with a latitude of 12 ^{O}18' North and a longitude of 77^{O}35' East. The seasons are very distinct, June to September is rainy season, October to January winter period followed by summer months during February to May. Generally extremities of temperature and humidity are not observed and a monthly mean relative humidity ranges from 50-80 per cent. The monthly mean data on minimum and maximum temperature, rainfall and humidity for the period of study are presented in Appendix-I.

3.2 Influence of growth regulators on the rooting of different types of cuttings in *Bursera penicillata* (DC) Engl.

3.2.1 Preparation of cuttings

The cuttings from a healthy mother plant of approximately 10 years old were collected from Horticulture Division, Gandhi Krishi Vignana Kendra Campus, Bangalore. One year old uniform shoots at the base and current season growth at the upper portion were selected. The cuttings prepared from base of the shoots were termed as hard wood cuttings, middle portion of the shoots were taken

as semi-hard wood cuttings and that of apical portion were referred as terminal cuttings.

3.2.2 Preparation of rooting media

Sieved sand was used as the rooting media. Before planting, the sand was thoroughly drenched using captan (0.2%). It was then filled in seed pans leaving a gap of 2 cm from the top. The prepared cutting were planted in seed pan after dipping in captan solution (2 g/l).

3.2.3 Design and layout of the experiment

Design	:	Factorial Completely Randomized Design
Number of main treatments	:	3
Number of sub-treatments	:	11
Number of replications	:	3
Number of cuttings/seed pan	:	15
Total number of cuttings	:	3 x 11 x 3 x 15 = 1485
Method of treatment	:	Soaking for 18 hours

a) Main treatments : Type of cuttings

a) Hard wood cuttings/basal cuttings
b) Semi-hard wood cuttings/middle cuttings
c) Terminal cuttings

b) Sub-treatments : Growth regulator treatments

T_1 : cuttings treated with IBA at 250 ppm concentration
T_2 : cuttings treated with IBA at 500 ppm concentration
T_3 : cuttings treated with IBA at 750 ppm concentration
T_4 : cuttings treated with NAA at 250 ppm concentration
T_5 : cuttings treated with NAA at 500 ppm concentration

T_6 : cuttings treated with NAA at 750 ppm concentration

T_7 : cuttings treated with IBA + NAA at 250 ppm concentration

T_8 : cuttings treated with IBA + NAA at 500 ppm concentration

T_9 : cuttings treated with IBA + NAA at 750 ppm concentration

T_{10} : cuttings treated with distilled water

T_{11} : Control

3.2.4 Preparation of growth regulator solutions

The aqueous solution of IBA was prepared by dissolving the required quantity of IBA in a small quantity of acetone and the required volume was made up by adding distilled water.

NAA was produced by dissolving the required quantity of NAA in sodium hydroxide and required volume made up by adding distilled water.

In case of IBA+NAAthe required quantities of IBA+NAA were dissolved in equal amounts with acetone and NaOH respectively, the required volume was then made up with distilled water.

3.2.5 Treatment of cuttings with growth regulators and planting

The basal portion of the cuttings (about 2.3 m length) were dipped in the growth regulator solutions for 18 hours and then planted in the rooting medium in seed pan with two basal nodes buried inside the medium. For the control, the cuttings were directly planted without any treatment. All the seed pans were placed in the intermittent mist chamber.

Two sprays of 0.1 per cent Bavistin was drenched after planting to prevent fungal infection.

3.2.6 Observations recorded

The cuttings were allowed to root under intermittent mist, the cuttings were carefully removed from pans along with media and dipped in water to remove the rooting media. The following observations on shoot and root parameters were recorded.

3.2.7 Shoot parameters

1. Time taken for first sprouting

Five labelled cuttings were observed daily under each replication and the number of days required for sprouting was recorded and the mean was computed.

2. **Number of sprouts**

The total number of sprouts of five cuttings in each replication was counted and the mean was computed.

3. Length of the longest sprout per cutting

The length of the longest sprout of five labelled cuttings was measured in each replication and its mean days expressed in cm.

4. Number of leaves per rooted cutting

The number of leaves in the five labelled cuttings were counted in each replication and the mean was computed.

3.2.7 Root parameters

1. Number of roots per cutting

The number of adventitious roots (except lateral roots) in the five labeled cuttings were counted in each replication and the mean was computed.

2. Length of the longest root

The length of the longest root of five labelled cuttings was measured and mean was computed and expressed in cm.

3. Percentage of cuttings rooted

The percentage was calculated by taking the ratio of number of rooted cuttings to total number of cuttings planted per pan and it was multiplied by 100.

4. Fresh and dry weight of roots per cutting

The fresh root portion of the five labelled cuttings from each replication was made use of. These root sample were later dried in a hot air oven at 60^{O}C to attain a constant weight. The roots were then weighed and the mean was computed and expressed in g.

3.2.7.3 Field establishment of rooted cuttings

At the completion of the experiment after 70 days the rooted cuttings were carefully removed from the seed pan and planted individually in polythene bags containing mixture of sand, FYM and soil in 2:1:1 ratio. The polythene bags were kept in partial shade and watered on alternate days till the completion of experiment. The survival percentage was recorded 30 days after transplanting.

3.4.7.4 Biochemical analysis

Biochemical analysis was done for total sugars, reducing sugars, non reducing sugars, starch, nitrogen, total carbohydrates and total phenols. At the time of preparation of cuttings, a sample of 100 g each of the plant material from the basal 1-1.5 cm portion of the basal, middle and terminal cuttings was collected and oven dried and ground to fine powder for the purpose of all biochemical estimation. Similarly, another lot of 100 g each of the plant sample at the time of

taking rooting observations was collected for analysis and the fresh sample of 100 g was also collected for determining the rooting co-factors.

The methods used for the estimation of different biochemical constituents are given below.

3.2.7.4.1 Total sugars

For estimation of total sugars 500 mg of oven dried finely ground sample was extracted successively thrice using 80 per cent ethanol. A known volume (1 ml) of this extract was taken in test tube and the alcohol was evaporated. Distilled water was added and the volume made up to a known volume (10 ml). The total sugar content in the alcohol free extract was determined after hydrolyzing a known volume in this extract with 1N HCl on a hot water bath for 20 minutes at $50^{O}C$ and after neutralizing it with 1N NaOH and the total sugar was estimated by anthrone method. (Sadasivam and Manickam, 1992). The results were expressed in percentage on dry weight basis.

3.2.7.4.2 Reducing sugars

Reducing sugars in the extract was determined by adopting the anthrone method (Sadasivam and Manickam, 1992). The results were expressed in percentage on dry weight basis.

3.2.7.4.3 Non-reducing sugars

Non-reducing sugars were computed by deducting the value of reducing sugars from the amount of total sugars. The results were expressed in percentage on dry weight basis.

3.2.7.4.4 Estimation of starch

The starch content in the shoot samples was estimated by adopting the anthrone method suggested by Sadasivam and Manickam (1992). The results were expressed in percentage on dry weight basis.

3.2.7.5 Estimation of Nitrogen

The nitrogen basis of the dried powdered sample was determined by following microkjeldhal method as outlined in Sadasivam and Manickam (1992) and the nitrogen content was expressed in percentage on dry weight basis.

2.2.7.4.6 Estimation of total carbohydrates

The value obtained for total sugars and starch were arithmetically summed up to get the content of total available carbohydrates. The results were expressed in percentage on dry weight basis.

3.2.7.4.7 Carbohydrate to nitrogen ratio (C/N ratio)

The value of the total carbohydrate present in the shoot was divided by total nitrogen to obtain the C:N ratio.

3.2.7.4.8 Estimation of total phenols

The amount of total phenols was estimated by Folinciacalteaus regent (FCR) method (Sadasivam and Manickam,1992) the results were expressed in mg per g on dry basis.

3.2.7.4.9 Root promoting co-factors

The root promoting co-factors present in the shoot was assayed using mung bean (*Phaseolus aurens* Roxb.) bioassay technique as described by Hess (1961).

Shoot samples were collected at the time of making the cuttings and immediately brought to the laboratory in an ice box. They were thoroughly washed first in running tap water, and then in distilled water. Excess water was removed by pressing them gently in between two layers of filter papers and then air dried. The shoots were chopped into small pieces and a representative sample of 100 grams each was weighed. The prepared samples were stored in a deep freezer at -23^{O}C until analysis.

The frozen sample was macerated with ice cold methanol in a pre-chilled wareing blender. The macerated material was submerged in excess of the extracting solvent and allowed to stand at -23^{O}C for 20 hours. At the end of the extraction period, the methanolic extract was filtered and washed with an excess of methanol. These washings were added to the original filterate and the whole extract evaporated to dryness in a flash evaporator under reduced pressure at 35 to 40^{O}C. For analysis, the extracts were concentrated to a final volume of 2ml by adding methanol to the dried residue. Growth active substances were separated by paper chromatography.

Chromatography

A volume representing 250 mg dry weight of the extracted tissues was strip loaded as a line on a 5 cm wide strip of whatman No.3 mm chromatography paper. The chromatogram was developed using the descending chromatographic technique in isopropanol: water (8:2 v/v) solvent system. The chromatograms were developed unidirectionally for 18 hours until the solvent front was about 30 cm from the origin. The chromatograms were then taken out from the chamber and dried in forced air for at least 30 minutes. The dried chromatogram were cut in to 10 transverse strips of equal length of 3 cm each representing one co-factor from 1 to 10. Each strip was then subsequently bio-assayed by the mung bean rooting test.

The paper strip above the origin served as control. Each chromatogram strip was transferred to a shell vial, containing 4 ml of 5 x 10^{-6} m IAA and 1 ppm boron.

Bio-assay

Ten mung bean (*Phaseolus aurens* Roxb.) cuttings containing primary leaves, epicotyl and three centimeters of hypocotyl tissue were placed in growth chamber. The chamber was kept illuminated for 16 hours a day with a light intensity of approximately 200 foot candles and at a temperature of 20^{O}C. The IAA present in the vials and the substances leached from the chromatogram sections were taken up by the cuttings within 12 hours. Glass distilled water was added upto a level of the cotyledonary nodes and maintained throughout the period of assay. At the end of sixth day, the cuttings were removed and the number of roots per cutting was counted. The mung bean bioassay test for each test sample was replicated thrice. Based on suggestions of Hess (1961). The approximate R_f values of the four rooting co-factors separated by using the isopropanol and water (8:2 v/v) solvent system were used for identifying the presence of various co-factors on the chromatograms of different shoot extracts.

Rooting co-factor	**R_f values**
1	0.1
2	0.3
3	0.6
4	0.8

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The results of the present investigation on the influence of growth regulators on rooting in different types of cuttings of *Bursera penicillata* (DC). Engl. are presented in this chapter under the following headings.

4.1.1 Shoot parameters

4.1.1.1 Days taken for first sprout to appear

The data pertaining to days taken for the first sprout to appear as influenced by the type of cutting or wood and growth regulators are presented in Table-1.

A significant effect of the type of wood, was noticed on the mean number of days taken for the first sprout to appear. The hard wood cuttings recorded the minimum number of days (16.78) to sprout as compared to semi-hard cuttings (17.96) and terminal soft wood (19.33) cuttings.

The growth regulators also significantly influenced the number of days taken for the first sprout to appear. Earliest sprouting was observed in the cuttings treated with IBA at 250 ppm (13.50 days) which was *on par* with IBA at 500 ppm (15.9 days) and 250 ppm NAA (15.10 days).

In case of IBA + NAA, the sprouting observed in cuttings treated with 250 ppm (16.88 days) was *on par* with 750 ppm IBA (18.7 days) and 750 ppm NAA (17.11 days).

The maximum number of days (24.33) for the first sprout to appear in the cuttings was in untreated (control) which was *on par* with cuttings treated with distilled water (23.33 days).

Table-1: Effect of growth regulators and type of wood on days taken for first sprout to appear in *Bursera penicillata* (DC.) Engl.

Treatments	Basal	Middle	Terminal	Mean
IBA 250 ppm	12.00	13.33	15.33	13.5
IBA 500 ppm	14.66	17.00	16.33	15.9
IBA 750 ppm	15.33	20.00	21.00	18.7
NAA 250 ppm	12.66	15.66	17.00	15.10
NAA 500 ppm	13.00	17.33	18.00	16.11
NAA 750 ppm	14.00	16.33	21.00	17.11
IBA + NAA 250 ppm	17.66	16.33	16.66	16.88
IBA + NAA 500 ppm	14.00	18.00	19.33	17.11
IBA + NAA 750 ppm	20.66	19.33	18.00	19.33
Distilled water	23.67	21.33	25.00	23.33
Control (untreated)	25.00	23.00	25.00	24.33
Mean	16.78	17.96	19.33	
	F-test	**SEm±**	**SED**	**CD at 5%**
Type of wood	*	0.471	0.66	1.52
Growth regulator Concentration	*	0.90	1.27	2.92
Interaction of wood and growth regulator	*	1.56	2.20	5.05

There was a significant interaction effects between the type of wood and growth regulators. In case of hard wood cuttings, the minimum number of days (12.00 days) for the first sprout to appear was recorded in IBA at 250 ppm treatment which was *on par* with 250 and 500 ppm NAA treated cuttings. While, IBA at 500 ppm treated cuttings required 14.66 days for first sprout to appear and it was followed by IBA at 750 ppm (15.33 days) and IBA + NAA at 250 ppm (17.66 days) and 500 ppm (14.00 days). which were *on par* with each other. The maximum number of days (25.00 days) were recorded for first sprout to appear was recorded in cuttings untreated (control) which was *on par* with cuttings treated with distilled water (23.67 days).

4.1.1.2 Number of sprouts per cutting

The data regarding the mean number of sprouts produced per cuttings as influenced by the type of wood and growth regulators is presented in Table-2.

The number of sprouts produced was significantly influenced by the type of wood used. The hard wood cuttings recorded significantly higher number of sprouts (4.10) than semi-hard wood cuttings (3.52) and terminal soft wood cuttings (2.75).

All the growth regulator concentrations significantly differed with regard to number of sprouts produced. However, cuttings treated with 250 ppm NAA recorded the maximum (6.17) which was followed by 250 ppm IBA (4.86) and 500 ppm NAA (4.14).

The control treatment recorded least number of sprouts (1.12) which was *on par* with distilled water treatment (1.54) treated cuttings.

Table-2: Effect of growth regulators and type of wood on number of sprouts per cutting in *Bursera penicillata* (DC.) Engl.

Treatments	Number of sprouts per cuttings			Mean
	Basal	Middle	Terminal	
IBA 250 ppm	7.16	5.46	3.66	4.86
IBA 500 ppm	6.20	3.80	3.43	3.93
IBA 750 ppm	4.26	4.06	2.50	3.26
NAA 250 ppm	5.46	6.96	4.40	6.17
NAA 500 ppm	4.50	3.86	3.16	4.41
NAA 750 ppm	3.20	3.13	2.83	3.41
IBA + NAA 250 ppm	3.66	3.60	3.10	4.06
IBA + NAA 500 ppm	3.43	2.83	2.36	2.82
IBA + NAA 750 ppm	2.50	1.96	2.53	2.46
Distilled water	4.40	1.73	1.43	1.54
Control (untreated)	3.16	1.33	0.86	1.12
Mean	4.10	3.52	2.75	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	0.095	0.30	
Growth regulator Concentration	*	0.182	0.59	
Interaction of wood and growth regulator	*	0.316	1.02	

A significant interaction effect was also observed between the type of wood and growth regulator concentrations for the number of sprouts (7.16) in the hard wood cuttings treated with 250 ppm IBA, followed by IBA 500 ppm (6.20) which was *on par* with 250 ppm NAA (5.46). The least number of sprouts (2.50) was noticed in 750 ppm IBA + NAA combination. But, failed to differ significantly with 750 ppm NAA (3.2), 250 ppm (3.66) and 500 ppm (3.43) IBA + NAA combination and control (3.16).

In case of semi-hard wood cuttings, NAA 250 ppm also recorded the maximum number of sprouts (6.96) per cutting followed by IBA 250 ppm (5.46). While the cuttings treated with of 750 ppm IBA + NAA produced the least number of sprouts (1.96) per cutting than other levels. However, it failed to differ significantly with 500 ppm IBA (3.80) and 500 ppm NAA (3.86). The similar trend was also noticed in terminal soft wood cuttings with NAA 250 ppm giving maximum number of sprouts (4.40) and followed by 250 ppm IBA (3.66).

4.1.1.3 Length of the longest sprout

Data on the mean length of longest sprout per cutting as influenced by type of wood and growth regulators are presented in Table-3.

Growth of sprout as indicated by mean length of longest sprout at 70 days after planting was significantly affected by the type of wood. Sprouts arising from the hard wood cuttings was longest (3.24 cm) as compared to sprouts from semi-hard wood cuttings (3.09 cm) and terminal soft wood (2.47 cm) cuttings.

The different concentrations of growth regulators influence significantly the growth of longest sprout. The cuttings dipped in solutions of 250 ppm IBA resulted in the maximum length of sprouts (4.78 cm) per cutting than its all levels which were *on par* with each other. The cuttings treated with NAA at 250 ppm

Table-3: Effect of growth regulators and type of wood on length of the longest sprout per cutting in *Bursera penicillata* (DC.) Engl.

Treatments	Length of the longest sprout per cutting (cm)			Mean
	Basal	Middle	Terminal	
IBA 250 ppm	5.53	4.80	4.03	4.78
IBA 500 ppm	3.96	4.66	2.66	3.73
IBA 750 ppm	3.40	3.56	2.13	3.03
NAA 250 ppm	4.80	4.60	3.56	4.32
NAA 500 ppm	3.96	3.90	2.73	3.53
NAA 750 ppm	3.36	2.96	2.40	2.91
IBA + NAA 250 ppm	2.80	2.30	2.90	2.66
IBA + NAA 500 ppm	2.20	2.40	2.50	2.36
IBA + NAA 750 ppm	2.16	1.90	2.20	2.08
Distilled water	1.80	1.73	1.16	1.56
Control (untreated)	1.66	1.23	1.00	1.30
Mean	3.24	3.09	2.47	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	0.05	0.16	
Growth regulator Concentration	*	0.100	0.32	
Interaction of wood and growth regulator	*	0.174	0.56	

recorded the maximum length (4.32 cm) of sprouts than other levels which were *on par* with 500 ppm IBA (3.73 cm) and 500 ppm NAA (3.53 cm). Where as, all levels of IBA + NAA were *on par* with each other.

The interaction effect of type of wood and different growth regulators differ significantly. In case of hard wood cuttings, the longest sprout (5.53 cm) was observed in cuttings treated with 250 ppm IBA which was *on par* with the 250 ppm NAA (4.80 cm). But NAA 500 ppm and IBA 500 ppm failed to differ significantly as they produced same length in the cuttings sprout (3.96 cm) whereas, all the concentrations of IBA + NAA treatments were *on par* with each other. The lowest length of sprout was recorded in case of control (1.66 cm) which was not found significant with the cuttings treated with distilled water (1.80 cm).

Among the interaction effects, the longest sprout (4.80 cm) was observed in case of semi-hard wood cuttings treated with 250 ppm IBA followed by 500 ppm IBA (4.66 cm). However, all the levels of IBA + NAA combinations except 750 ppm (1.90 cm) failed to differ among themselves significantly. In case of terminal soft wood cuttings, a similar trend was noticed, with 250 ppm IBA producing the longest sprout (4.30 cm) followed by 250 ppm NAA (3.56 cm). While, all the levels of IBA + NAA combinations failed to differ significantly and were *on par* with each other.

The minimum sprout length (1.23 cm) was recorded in control which was *on par* with cuttings treated with distilled water (1.73 cm).

4.1.4 Number of leaves per cuttings

The data pertaining to the mean number of leaves per cuttings as influenced by type of wood and growth regulators are furnished in Table-4.

Table-4: Effect of growth regulators and type of wood on number of leaves per rooted cutting in *Bursera penicillata* (DC.) Engl.

Treatments	Number of leaves per rooted cuttings			Mean
	Basal	Middle	Terminal	
IBA 250 ppm	11.86	8.66	9.70	10.07
IBA 500 ppm	10.50	7.46	9.03	9.00
IBA 750 ppm	8.56	7.16	7.26	7.76
NAA 250 ppm	11.10	9.16	11.23	10.50
NAA 500 ppm	9.83	8.56	10.23	9.54
NAA 750 ppm	9.33	8.10	10.10	9.17
IBA + NAA 250 ppm	9.03	7.83	6.73	7.86
IBA + NAA 500 ppm	8.00	6.90	6.90	7.26
IBA + NAA 750 ppm	7.86	6.00	5.90	6.58
Distilled water	6.90	4.76	4.60	5.42
Control (untreated)	6.0	3.90	2.56	4.15
Mean	9.02	7.13	7.66	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	0.09	0.29	
Growth regulator Concentration	*	0.17	0.55	
Interaction of wood and growth regulator	*	0.29	0.96	

The different types of cutting were found to differ significantly from each other with regard to the number of leaves per cutting. The maximum number of leaves (9.02) were observed in hard wood cuttings followed by semi hard wood (7.13) and terminal soft wood cuttings (7.66).

The different concentrations of growth regulators with in the type of wood found significant for the number of leaves per cutting. The maximum number of leaves (10.50) was recorded in the treatment 250 ppm NAA followed by 250 ppm IBA (10.07). In case of IBA + NAA combination, cuttings treated with 250 ppm resulted in maximum number of leaves (7.86) per cutting which was *on par* with IBA 750 ppm and NAA 750 ppm. Decrease in the number of leaves per cutting was observed with the increase in the concentration of IBA, which was same in case of NAA and IBA + NAA combination.

The interaction effects between type of wood and growth regulators differed significantly with regard to number of leaves per cutting, in hard wood cuttings IBA 250 ppm (11.86) treatment results in the maximum number of leaves followed by 250 ppm NAA (11.10), while minimum number of leaves (6.0) was recorded in the untreated cuttings followed by (6.90) in cuttings treated with distilled water.

In case of semi-hard wood cuttings, the maximum number of leaves (9.16) was recorded in the NAA 250 ppm treated cuttings followed by 250 ppm IBA (8.66) and 500 ppm (8.56) NAA, whereas, IBA + NAA at 250 and 500 ppm recorded 7.83 and 6.90 respectively which were *on par* with each other. And the highest number of leaves (11.23) recorded in the terminal soft wood cuttings was with the treatments of NAA at 250 ppm closely followed by 500 ppm NAA (10.23). IBA at 250 ppm also gave fairly higher number of leaves (9.70), whereas IBA + NAA at 250 ppm and 500 ppm recorded 6.73 and 6.90 respectively which

were *on par* with each other. However, higher concentration of IBA and NAA (750 ppm) further decreased the number of leaves.

While the minimum number of leaves was observed in control (3.90) when compared to other levels.

4.1.2 Root parameters

4.1.2.1 Percentage of cuttings rooted

The data on the percentage of rooted cuttings at the end of 70 days as influenced by the type of wood and different growth regulator solutions are presented in Table-5 and illustrated through figure 1.

The type of wood had a significant effect on percentage of rooting. The maximum rooting (56.48%) was obtained in hard wood cuttings than the semi-hard wood cuttings (51.09%) and the terminal soft wood cuttings (24.69%).

The influence of growth regulators on rooting was also found to be significant. All the growth regulators used increased rooting significantly over the control. The maximum rooting was (68.88%) was recorded in 250 ppm IBA and was followed by (59.0%) by 250 ppm NAA which was closely followed by IBA 500 ppm (58.44%) and combination of IBA + NAA at 250 ppm (46.0%) which were *on par* with each other. While the cuttings without any treatment (control) recorded the least rooting (20.0%).

Among the various interaction effects, the hard wood cuttings treated with IBA 250 ppm produced the maximum rooting (84.66%) followed by 500 ppm IBA (75.0%). In case of NAA, cuttings treated at 250 ppm recorded 68 per cent rooting which was *on par* with IBA + NAA combination at 250 ppm (63.0

Table-5: Effect of growth regulators and type of wood on rooting percentage in *Bursera penicillata* (DC.) Engl.

Treatments	Rooting percentage (70 days after planting)			Mean
	Basal	Middle	Terminal	
IBA 250 ppm	84.66	77.00	45.00	68.88
IBA 500 ppm	75.00	64.66	35.66	58.44
IBA 750 ppm	70.66	54.33	29.66	51.55
NAA 250 ppm	68.00	79.66	34.33	59.00
NAA 500 ppm	67.66	51.66	23.66	47.66
NAA 750 ppm	64.33	55.00	21.66	47.00
IBA + NAA 250 ppm	63.00	54.66	20.33	46.00
IBA + NAA 500 ppm	45.00	35.00	19.00	33.00
IBA + NAA 750 ppm	33.00	38.33	18.00	29.77
Distilled water	28.33	28.33	14.30	23.66
Control (untreated)	21.66	28.33	10.0	20.00
Mean	56.48	51.09	24.69	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	1.42	4.61	
Growth regulator Concentration	*	2.72	8.82	
Interaction of wood and growth regulator	*	4.72	15.2	

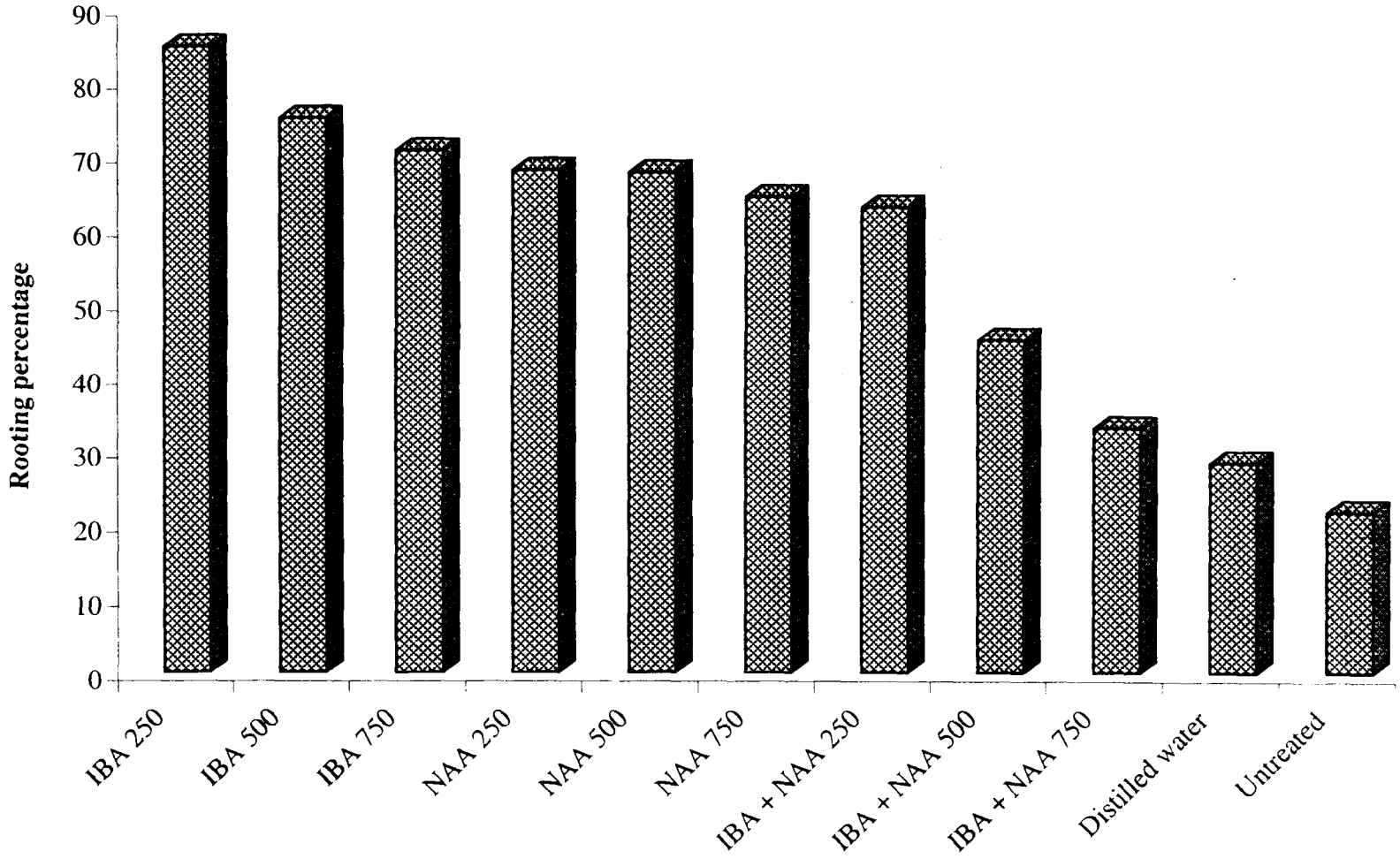


Fig 1 : Effect of IBA, NAA and IBA + NAA concentration (ppm) on percentage of rooting in basal woody portion cuttings of *Bursera penicillata* (DC) Engl

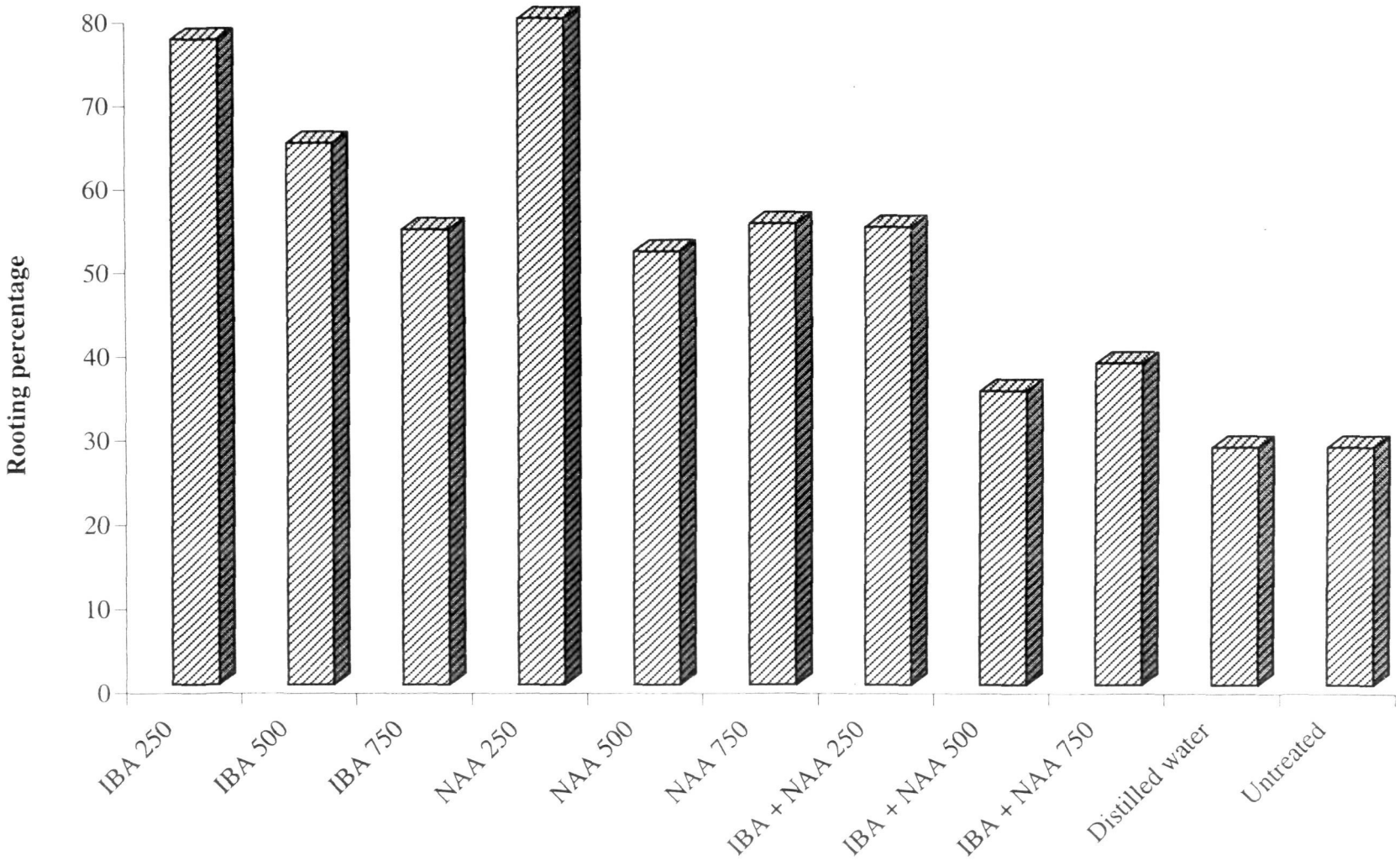


Fig 2 : Effect of IBA, NAA and IBA + NAA concentration (ppm) on percentage of rooting in middle semi - woody portion cuttings of *Bursera penicillata* (DC) Engl

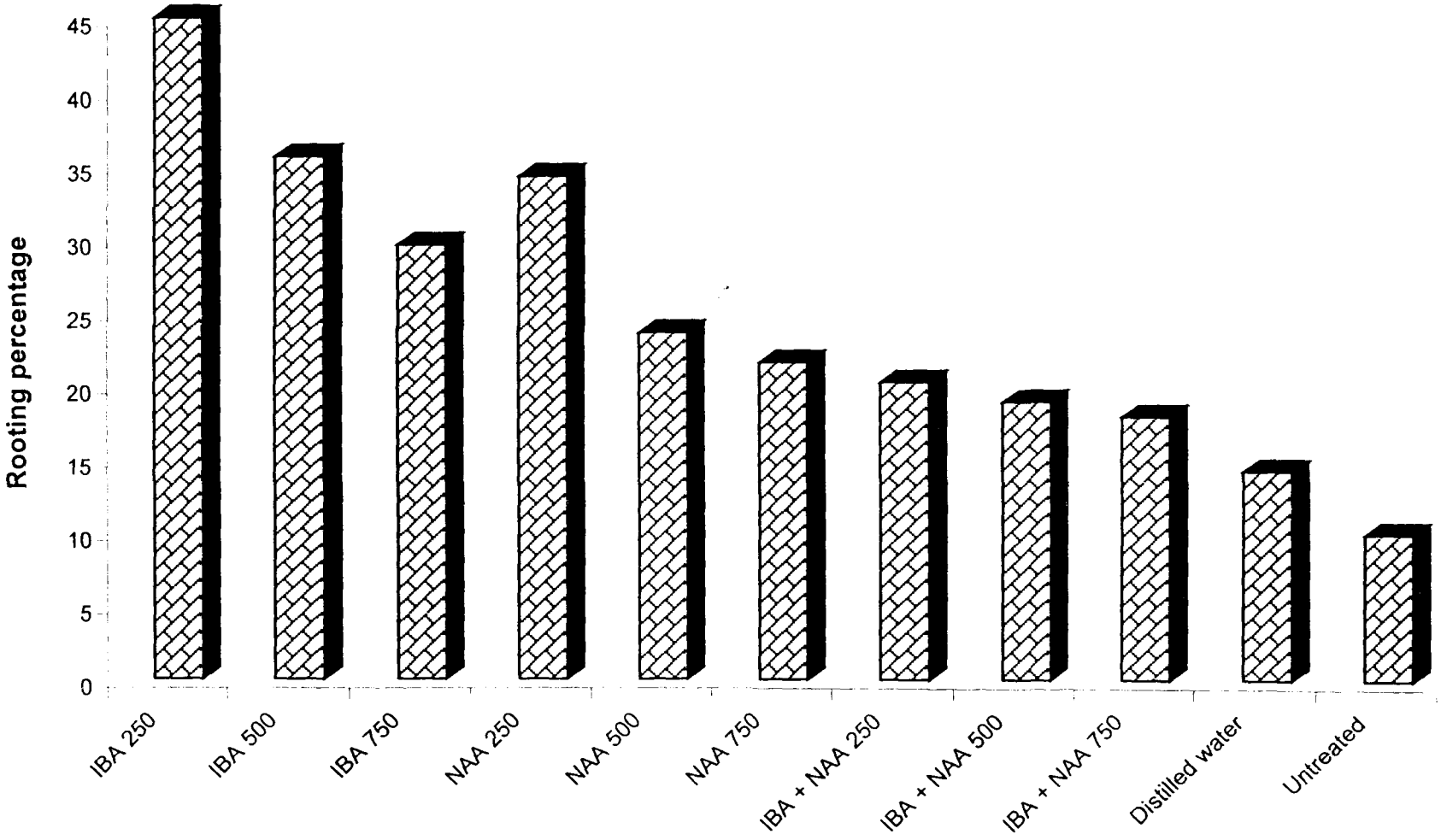


Fig 3 : Effect of IBA, NAA and IBA + NAA concentration (ppm) on percentage of rootig in terminal soft woody portion cuttings of *Busera penicillata* (DC) Engl.

The maximum rooting in semi-hard wood cuttings was recorded when they were treated with IBA 250 ppm (77.0%) followed by 250 ppm NAA (79.66%). A similar trend was also noticed in the terminal soft wood cuttings with IBA 250 ppm giving maximum rooting (45.0%) and followed by NAA 250 ppm (35.66%).

4.1.2.2 Number of roots per cutting

The data pertaining to the mean number of roots per cutting as influenced by the type of wood and different growth regulators are presented in Table-6.

Hard wood cuttings produced 11.73 roots per cutting as compared to semi-hard wood (9.27) and terminal soft wood (6.12) cutting.

All the growth regulators used increased the number of roots per cutting significantly over control. The maximum number of roots (18.56) was recorded in cuttings treated with 250 ppm IBA followed by the same concentration of NAA (14.42) and IBA+NAA at 250 ppm recorded (7.78) which were *on par* with each other whereas, minimum number of roots was noticed in control (2.31).

The interaction between type of wood and growth regulator concentration was also significant. Application of IBA at 250 ppm resulted in the maximum number of roots (27.83) in hard wood cuttings followed by NAA at 250 ppm (18.6) and 500 ppm IBA (17.36) which were *on par* with each other.

In case of semi-hard wood cuttings treated with IBA at 250 ppm recorded a maximum number (17.93) followed by NAA at 500 ppm and 250 ppm recorded (14.66 and 14.60 respectively) which were *on par* with each other. While very least number of roots was observed in control (2.43). And in terminal soft wood cuttings the maximum roots (10.06) were obtained with NAA 250 ppm which was closely followed by 250 ppm IBA (9.93).

Table-6 Effect of growth regulators and type of wood on number of roots per cutting in *Bursera penicillata* (DC.) Engl.

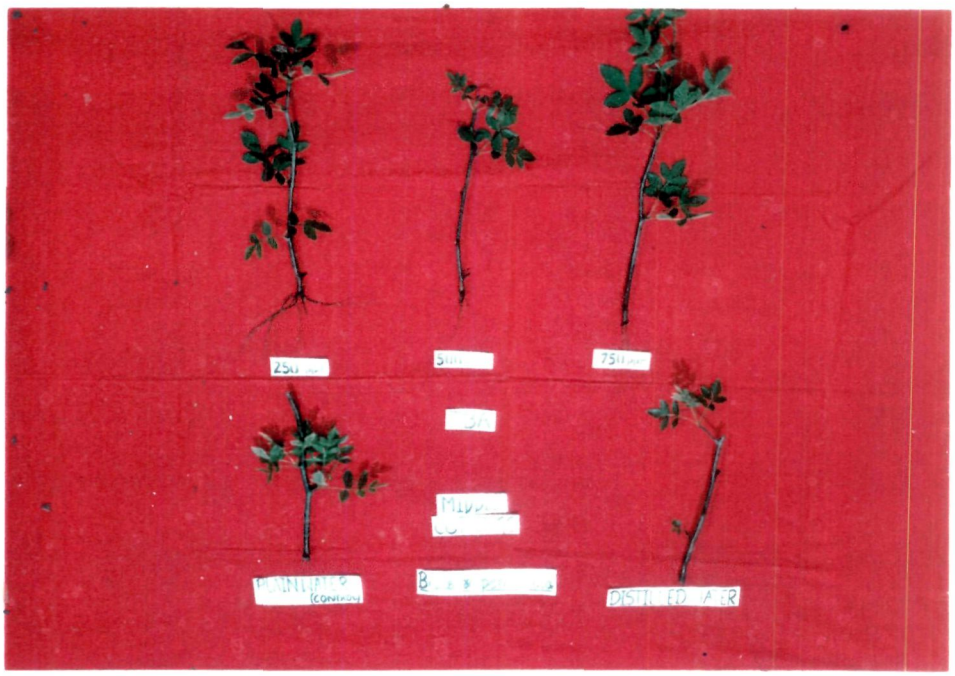
Treatments	Number of roots per cuttings (70 days after planting)			Mean
	Basal	Middle	Terminal	
IBA 250 ppm	27.83	17.93	9.93	18.56
IBA 500 ppm	17.36	10.66	9.00	12.34
IBA 750 ppm	11.06	9.76	7.03	9.28
NAA 250 ppm	18.60	14.60	10.06	14.42
NAA 500 ppm	9.80	14.66	9.50	11.34
NAA 750 ppm	8.70	10.40	8.03	9.04
IBA + NAA 250 ppm	9.96	6.96	6.43	7.78
IBA + NAA 500 ppm	8.23	6.73	2.76	5.71
IBA + NAA 750 ppm	7.36	4.80	2.83	5.00
Distilled water	6.13	3.63	1.16	3.64
Control (untreated)	3.90	2.43	0.6	2.31
Mean	11.73	9.27	6.12	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	0.14	0.46	
Growth regulator Concentration	*	0.27	0.88	
Interaction of wood and growth regulator	*	0.47	1.53	

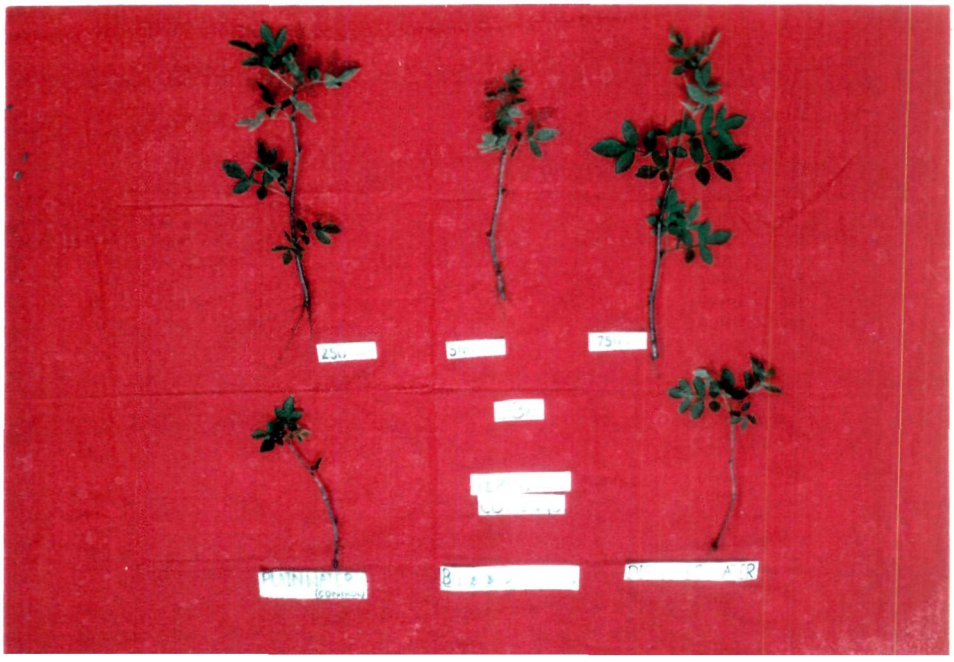
Plate 1. Effect of IBA on the rooting of basalwoody portion cuttings of *Busera penicillata* (DC) Engl.

Plate 2. Effect of IBA on the rooting of middle-semi woody portion cuttings of *Busera penicillata* (DC) Engl

Plate 3. Effect of IBA on the rooting of terminal softwoody portion cuttings of *Busera penicillata* (DC) Engl


CUTTINGS





4.1.2.3 Length of the longest root per cutting

The data on the mean length of the longest root per cutting are presented in Table-7. The different types of wood differed significantly with respect to length of root.

The longest (8.59 cm) root was produced in hard wood cuttings than semi hard wood cuttings (4.35 cm) and terminal soft wood cuttings (1.81).

The different growth regulator levels significantly influenced the length of root. The cuttings treated with 250 ppm IBA and 500 ppm IBA or 250 ppm NAA were the most effective in producing the longest root (7.95, 6.54 and 6.37 cm) respectively per cutting. However, which were *on par* with each other. In case of IBA + NAA at 250 ppm the maximum (5.13 cm) root length was recorded than other levels, but the minimum root length was observed incase of control (2.23 cm).

The interaction between the type of wood and growth regulators were also found to be significant. Incase of hard wood cuttings treated with 250 ppm NAA recorded the maximum (11.13 cm) root length and was followed by 250 ppm IBA and 500 ppm NAA (10.70 and 10.60 respectively) which failed to differ significantly with each other and IBA + NAA at 250 ppm recorded(10.46).

In case of semi-hard wood cuttings, the treatment of IBA 250 ppm recorded the maximum (8.46 cm) root length followed by 500 ppm IBA (6.70 cm) and 750 ppm IBA or 250 ppm NAA (5.86 and 5.20 cm) respectively and in terminal soft wood cuttings, the lower level of IBA 250 ppm (4.70 cm) was found to be better. The IBA at 500 ppm and NAA at 250 ppm recorded almost similar results.

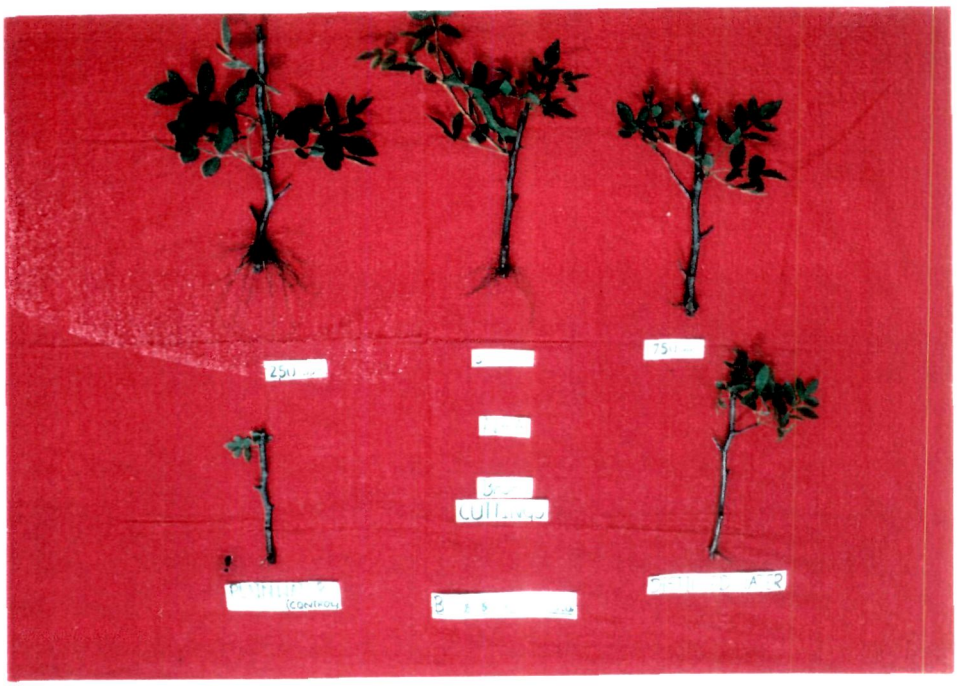
Table-7: Effect of growth regulators and type of wood on length of the longest root per cutting in *Bursera penicillata* (DC.) Engl. (70 days after planting)

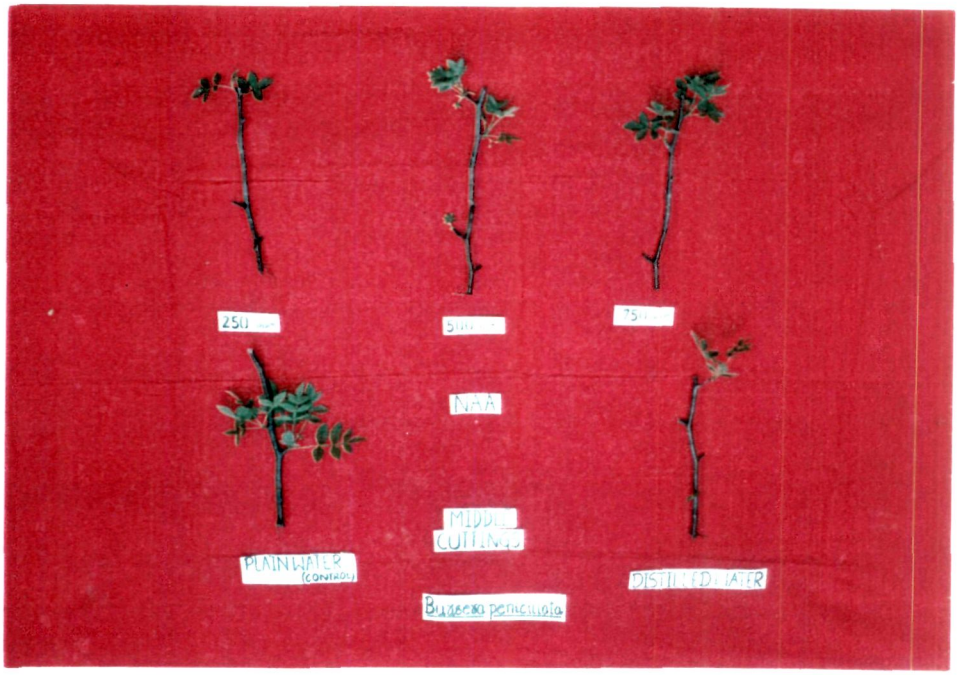
Treatments	Length of the longest root per cuttings (cm)			Mean
	Basal	Middle	Terminal	
IBA 250 ppm	10.70	8.46	4.70	7.95
IBA 500 ppm	10.00	6.70	2.93	6.54
IBA 750 ppm	9.56	5.86	1.46	5.63
NAA 250 ppm	11.13	5.20	2.80	6.37
NAA 500 ppm	10.6	3.10	1.80	5.16
NAA 750 ppm	9.33	3.60	0.76	4.56
IBA + NAA 250 ppm	10.46	3.63	1.30	5.13
IBA + NAA 500 ppm	7.50	3.13	1.26	3.96
IBA + NAA 750 ppm	6.00	2.13	1.10	3.27
Distilled water	5.50	3.20	1.20	3.30
Control (untreated)	3.73	2.30	0.66	2.23
Mean	8.59	4.35	1.81	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	0.118	0.38	
Growth regulator Concentration	*	0.22	0.73	
Interaction of wood and growth regulator	*	0.39	1.27	

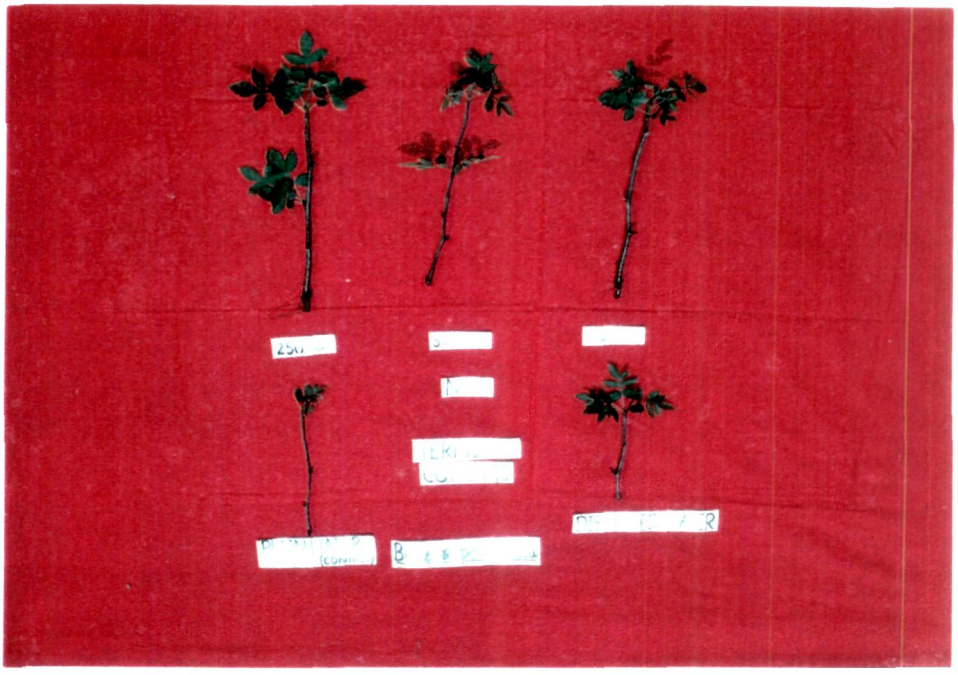
Plate 4. Effect of NAA on the rooting of basal woody portion cuttings of *Busera penicillata* (DC) Engl

Plate 5. Effect of NAA on the rooting of middle-semi woody portion cuttings of *Busera penicillata* (DC) Engl.

Plate 6. Effect of NAA on the rooting of terminal softwoody portion cuttings of *Busera penicillata* (DC) Engl.


250
CUTTINGS
(CONTROL)


250
500
750
NAA
MIDDLE
CUTTINGS
PLAIN WATER
(CONTROL)
DISTILLED WATER
Bougainvillea peruviana


250

The minimum (2.30 cm) root length was recorded in control which was *on par* with distilled water treatment cuttings (3.20 cm).

4.1.2.4 Fresh weight of roots

The data pertaining to mean fresh weight of roots produced per cutting as influenced by the type of wood and growth regulators are given in Table-8.

Among the different types of cuttings the maximum fresh weight of roots (0.099 g) per cutting was recorded in case of hard wood cuttings which was significantly differ from semi-hard wood cuttings (0.044 g) and terminal soft wood (0.024 g) cuttings.

The growth regulator treatments also differed significantly with each other. The maximum fresh weight of roots was recorded with IBA 250 ppm (0.093 g) which was *on par* with NAA at 250 ppm (0.089 g) and in case of IBA + NAA, the maximum fresh weight (0.058 g) was recorded at 250 ppm followed by (0.055 g) at 250 ppm IBA + NAA.

The interaction effects found to be significant, the maximum fresh weight (0.179 g) was recorded incase of hard wood cuttings treated with IBA 250 ppm followed by NAA at 250 ppm (0.159 g) and in all the levels of IBA + NAA treatments failed to differ significantly. However, the minimum fresh weight was recorded in control (0.043 g).

Among the interaction effects semi-hard wood cuttings recorded the maximum fresh weight when treated with IBA 250 ppm and NAA 250 ppm (0.065 g and 0.064 g respectively) followed by 500 ppm IBA (0.053 g) which were *on*

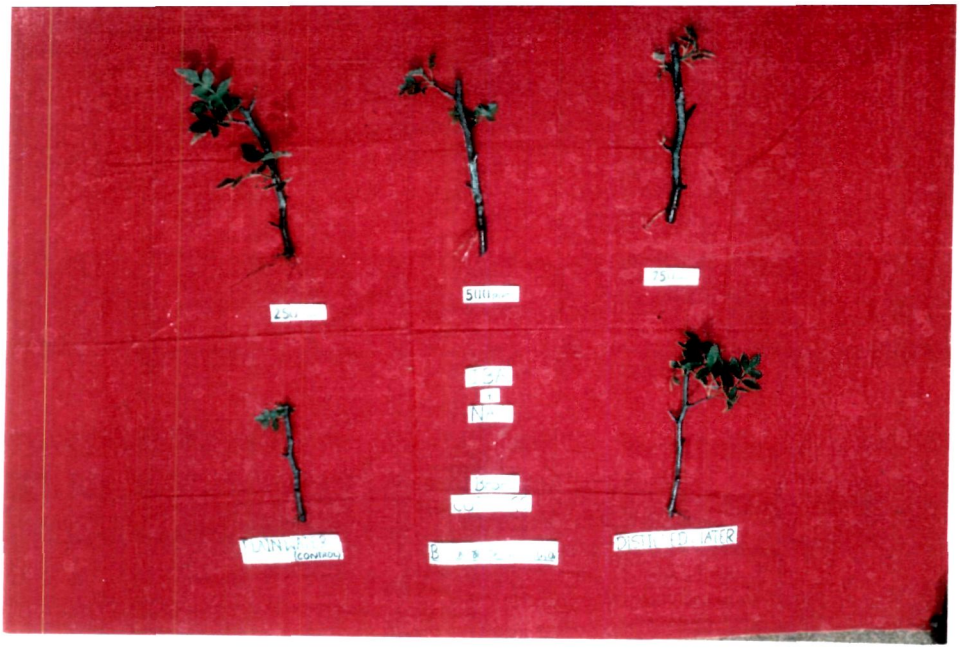
Table-8: Effect of growth regulators and type of wood on the fresh weight of roots in *Bursera penicillata* (DC.) Engl.

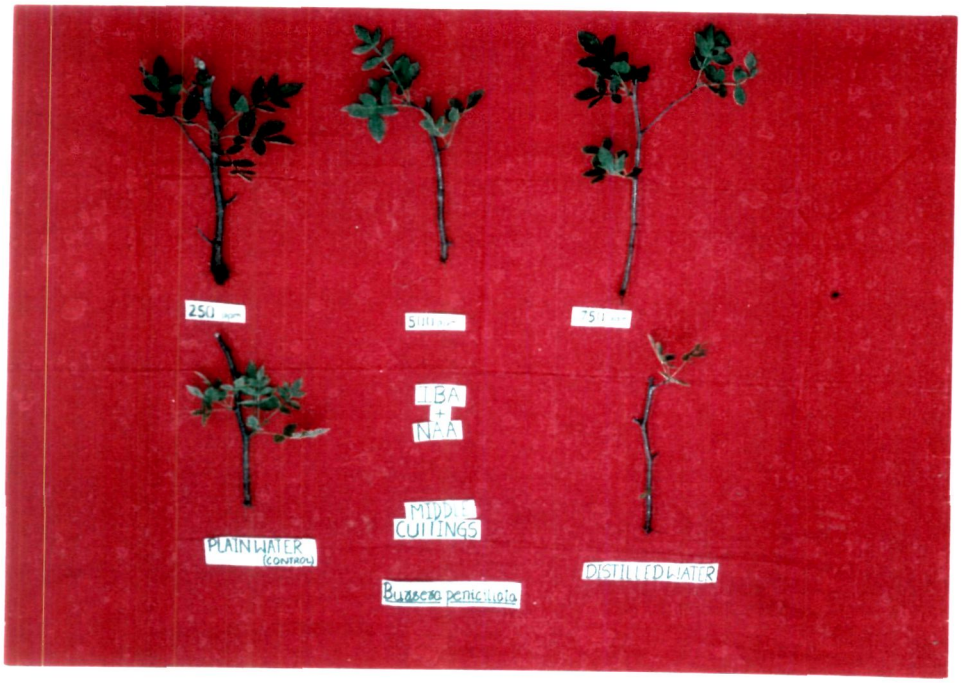
Treatments	Fresh weight of the roots (g)			Mean
	Basal	Middle	Terminal	
IBA 250 ppm	0.179	0.065	0.035	0.098
IBA 500 ppm	0.120	0.053	0.024	0.066
IBA 750 ppm	0.081	0.036	0.020	0.046
NAA 250 ppm	0.159	0.064	0.043	0.089
NAA 500 ppm	0.091	0.036	0.032	0.053
NAA 750 ppm	0.106	0.034	0.028	0.056
IBA + NAA 250 ppm	0.093	0.045	0.028	0.055
IBA + NAA 500 ppm	0.077	0.053	0.018	0.049
IBA + NAA 750 ppm	0.098	0.058	0.018	0.058
Distilled water	0.044	0.022	0.011	0.026
Control (untreated)	0.043	0.018	0.011	0.024
Mean	0.099	0.044	0.024	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	0.002	0.0094	
Growth regulator Concentration	*	0.005	0.018	
Interaction of wood and growth regulator	*	0.009	0.031	

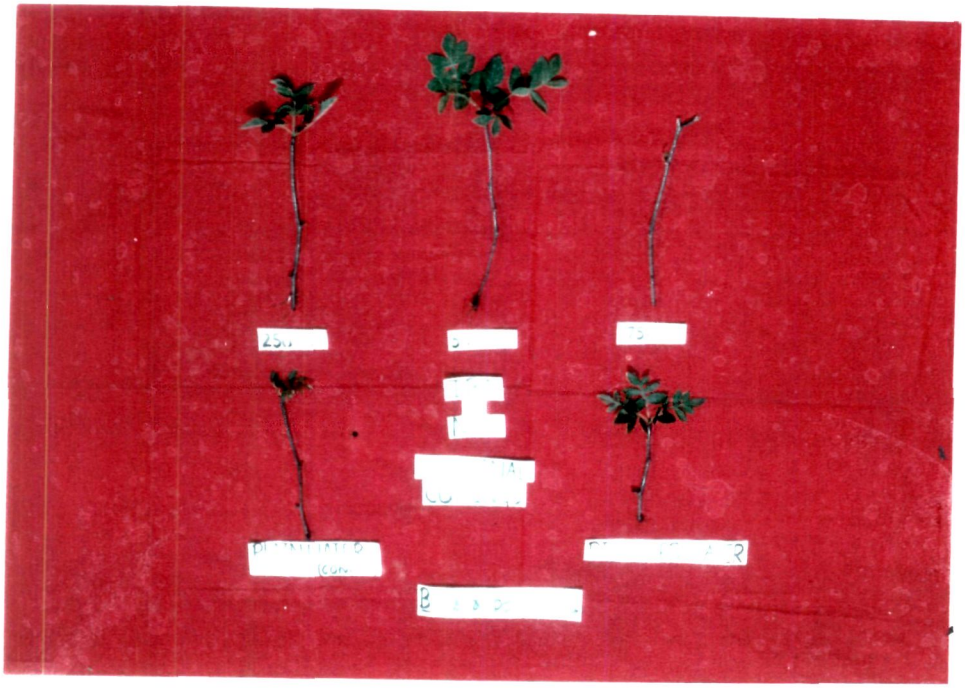
Plate 7. Effect of IBA + NAA on the rooting of basalwoody portion cuttings of *Busera penicillata* (DC) Engl

Plate 8. Effect of IBA + NAA on the rooting of middle-semi woody portion cuttings of *Busera penicillata* (DC) Engl.

Plate 9. Effect of IBA + NAA on the rooting of terminal softwoody portion cuttings of *Busera penicillata* (DC) Engl.


25%
75%
IBA + NAA
(CONTROL)
DISTILLED WATER


25%
75%
IBA + NAA
MIDDLE CUTTINGS
PLAIN WATER (CONTROL)
DISTILLED WATER


25%
75%
IBA + NAA

par with each other. While the least fresh weight (0.018 g) was recorded in control and in case of terminal soft wood cuttings NAA at 250 ppm recorded maximum fresh weight of root (0.043 g) which was followed by 250 ppm IBA and 500 ppm NAA (0.035 g and 0.032 g respectively) which were *on par* with each other and cuttings treated with distilled water and untreated recorded the same weight (0.011 g).

4.1.2.5 Dry weight of roots per cutting

The dry weight of hard wood (0.069 g), semi-hard wood (0.026 g) and terminal soft wood cuttings (0.018 g) significantly differed and presented in Table-9.

Even among the different growth regulators, significant differences were observed with respect to the mean dry weight of root produced per cutting. Application of NAA 250 ppm recorded the highest dry weight of root (0.062 g) followed by 250 ppm IBA and 500 ppm IBA dry weight (0.057g and 0.048 g respectively). However, cuttings treated with 250 ppm IBA + NAA, 500 ppm NAA and 750 ppm had the same dry weight of (0.040 g).

All the growth regulator treatments differed significantly from the control. The minimum dry weight of roots (0.016 g) was recorded in cuttings untreated (i.e. control).

The interaction effect between type of wood and growth regulator treatments were found to be significant. Among the various combinations, the maximum dry weight was recorded in hard wood cuttings treated with 250 ppm NAA (0.119 g) followed by 250 ppm IBA (0.117 g). Both in case of IBA and NAA the definite decreasing trend was showed with increasing the concentration,

Table-9 Effect of growth regulators and type of wood on the dry weight of roots in *Bursera penicillata* (DC.) Engl.

Treatments	Dry weight of roots (g)			Mean
	Basal	Middle	Terminal	
IBA 250 ppm	0.117	0.034	0.020	0.057
IBA 500 ppm	0.097	0.028	0.018	0.048
IBA 750 ppm	0.061	0.022	0.011	0.031
NAA 250 ppm	0.119	0.037	0.030	0.062
NAA 500 ppm	0.076	0.022	0.033	0.043
NAA 750 ppm	0.074	0.019	0.027	0.040
IBA + NAA 250 ppm	0.072	0.028	0.021	0.040
IBA + NAA 500 ppm	0.041	0.026	0.013	0.026
IBA + NAA 750 ppm	0.052	0.035	0.015	0.034
Distilled water	0.033	0.017	0.009	0.019
Control (untreated)	0.024	0.015	0.009	0.016
Mean	0.069	0.026	0.018	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	0.001	0.004	
Growth regulator Concentration	*	0.002	0.008	
Interaction of wood and growth regulator	*	0.004	0.014	

while minimum dry weight (0.024 g) was recorded in case of control when compared to distilled water treated cutting (0.033 g).

In case of semi-hard wood cutting NAA at 250 ppm treated cuttings recorded the highest dry weight (0.037 g) followed by 750 ppm IBA + NAA (0.035 g) and this was *on par* with 250 ppm IBA (0.034 g) and 500 ppm IBA (0.028 g). The similar trend was, also noticed in case of terminal soft wood cuttings with 250 ppm NAA recording maximum dry weight (0.030 g) followed by 750 ppm NAA (0.027 g) while the control and distilled water treatments recorded same dry weight (0.009 g).

4.1.3 Field establishment of rooted cuttings

The data on the per cent field establishment of the transplanted cuttings is furnished in Table-10.

Field establishment of transplanted rooted cuttings was significantly influenced by type of wood. The per cent establishment of rooted hard wood cuttings was significantly higher (36.96%) over the semi-hard wood (16.59%) and terminal soft wood (3.08%) cuttings.

All the growth regulator concentrations used recorded the higher yield establishment of rooted cuttings over control (5.28%). Application of IBA at 250 ppm resulted in the maximum field establishment (44.45%) followed by 250 ppm NAA (32.80%). Incase of combination of IBA + NAA at 500 ppm recorded maximum field establishment (17.63%) over 250 ppm (17.42%) which was *on par* with each other.

The interaction effects between the type of wood and growth regulator treatments of this experiment were found significant for the field establishment of

Table-10: Effect of growth regulators and type of wood on number of the survival Percentage in *Bursera penicillata* (DC.) Engl.

Treatments	Survival percentage			
	Basal	Middle	Terminal	Mean
IBA 250 ppm	78.33	46.33	8.10	44.45
IBA 500 ppm	42.66	19.33	3.16	21.72
IBA 750 ppm	35.00	14.00	2.66	17.22
NAA 250 ppm	70.33	21.06	7.00	32.80
NAA 500 ppm	26.66	18.83	2.33	15.87
NAA 750 ppm	22.63	15.16	1.70	13.16
IBA + NAA 250 ppm	33.70	16.00	2.56	17.42
IBA + NAA 500 ppm	38.16	12.66	2.06	17.63
IBA + NAA 750 ppm	29.74	11.00	1.36	14.03
Distilled water	16.16	6.5	1.50	8.05
Control (untreated)	13.16	1.8	0.86	5.28
Mean	36.96	16.59	3.08	
	F-test	**SEm±**	**CD at 5%**	
Type of wood	*	0.58	1.90	
Growth regulator Concentration	*	1.12	3.64	
Interaction of wood and growth regulator	*	1.94	6.30	

rooted cuttings. A maximum field establishment (78.33%) was recorded in hard wood cuttings treated with 250 ppm IBA while minimum (13.16%) was recorded in control over all the levels of growth regulators in hard wood cuttings.

In case of semi hard wood and terminal soft wood cuttings, the cuttings treated with IBA 250 ppm recorded the highest field establishment (46.33% and 8.70% respectively) over all the treatments followed by 250 ppm NAA (21.06% and 7.0% respectively).

4.4 Biochemical studies

Data on biochemical composition (on % dry weight basis) of different types of cuttings of *Bursera penicillata* (DC) Engl. one given in table-11 and depicted in figure.

4.1.4.1 Total sugar content

It is evident from the Table-11 that hard wood cuttings of *Bursera penicillata* (DC) Engl. had a higher amount of total sugars (6.25%) as compared to semi-hard wood and terminal soft wood (6.02% and 5.85% respectively) cuttings.

4.1.4.2 Starch content

Of the different types of cuttings of *Bursera penicillata* (DC) Engl. Higher amount of starch was found in hard wood cuttings (2.59%) than in semi-hard wood cuttings (2.48%) and terminal soft wood (2.30%) cuttings.

4.1.4.3 Nitrogen content

In *Bursera penicillata* (DC) Engl. the nitrogen content was found higher in terminal soft wood cuttings (0.79%) compared to semi hard wood cuttings (0.648%) and hard wood (0.588%) cuttings.

Table-11: Bio-chemical composition of different portions of cuttings (Hard wood, Semi hard wood and Soft wood cuttings) of *Bursera penicilata* (DC) Engl. (% dry weight basis)

Biochemical composition	Portion cuttings		
	Hard wood	Semi-hard wood	Soft wood
Starch	2.59	2.48	2.30
Total sugars	6.25	6.02	5.85
Nitrogen	0.58	0.64	0.79
C/N ratio	18.04	15.74	12.36
Total phenols (mg/g dw.p)	1.40	1.42	2.40

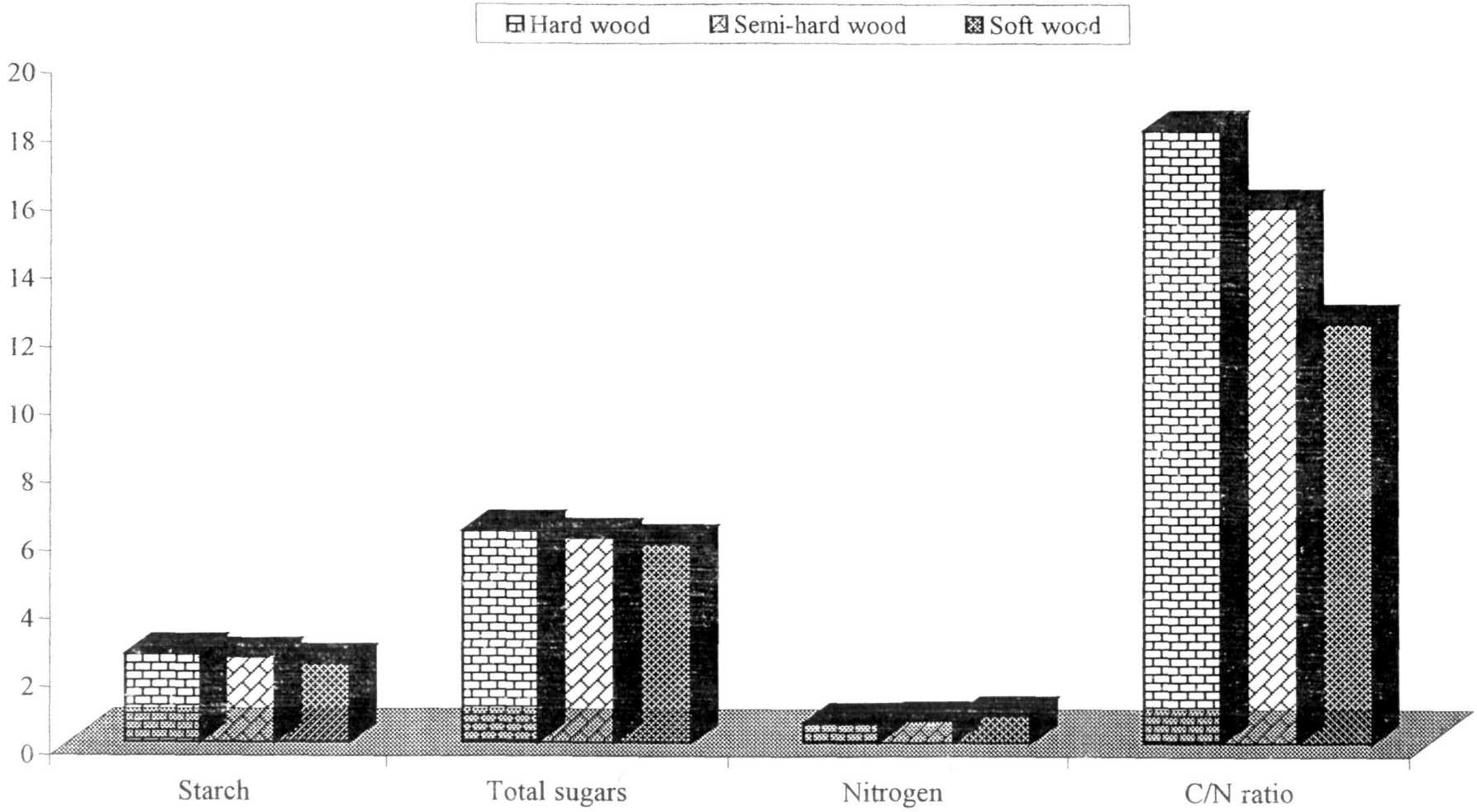


Fig 4 : Histograms showing different biochemical composition of the three types of cuttings of *Bursera penicillata* (DC) Engl

4.1.4.4 Carbohydrate / Nitrogen ratio

From the table- 11 it is evident that in hard wood cuttings of *Bursera penicillata* (DC) Engl. the C/N ratio was found highest (18.04) followed by semi-hard (15.74) and terminal soft wood (12.36) cutting.

4.1.4.5 Total phenols

The data pertaining total phenols in different types of cuttings of *Bursera penicillata* (DC) Engl. are presented in table-11.

It is evident from the data that terminal soft wood cuttings exhibited the highest amount of total phenols (2.4 mg/dry weight) followed by semi-hard (1.428 mg/ dry weight) and hard wood (1.404 mg/ dry weight) cuttings.

4.1.4.6 Root promoting co-factors

Root promoting co-factors occurring in the three types of *Bursera penicillata* (DC) Engl. were separated on chromatograms by descending chromatography technique using iso-propanol and water (8:2 v/v) as solvent system. The shoot extracts were assayed for the presence of different root promoting co-factor by mung bean rooting test. The results are presented in the form of histograms (Fig. 6, 7 and 8) the horizontal line indicating promotion and below, inhibition of root formation.

It is quite evident that there was a wide difference in the activity of the root promoting co-factors in the three different types of cuttings in *Bursera penicillata* (DC) Engl.

The tissue extract of hard wood cuttings the root promoting co-factors 1, 2, 3 and 4 (corresponding to 0.1, 0.3, 0.6 and 0.8 respectively according to Hess, 1961a) were present whereas the tissue extract of semi-hard wood cuttings, the

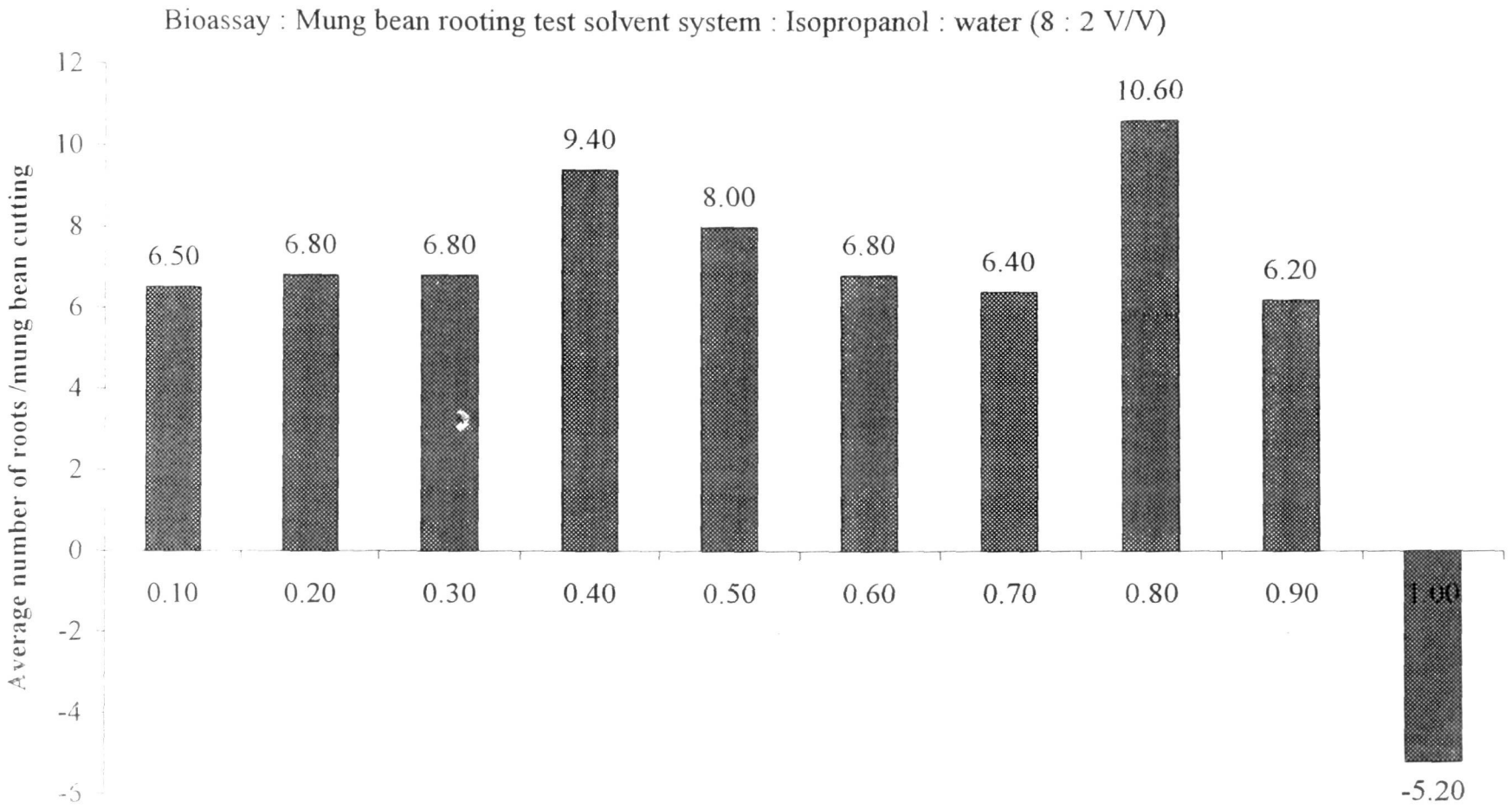


Fig 5 : Histograms showing the biological activity of root promoting co-factors extracted from the basal woody portion cuttings of Bursera penicillata (DC) Engl

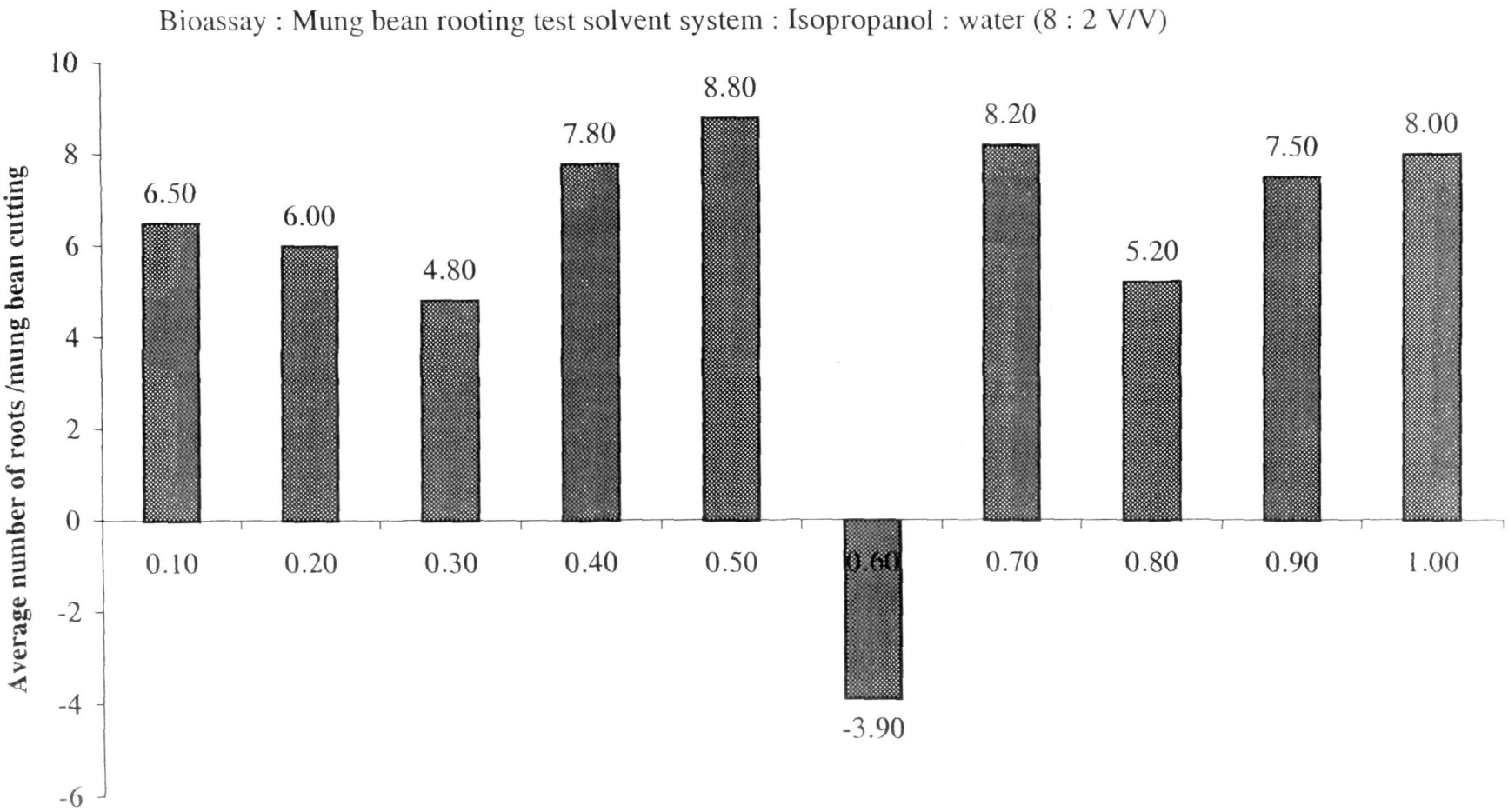


Fig 6 : Histograms showing the biological activity of root promoting co-factors extracted from the middle semi-woody portion cuttings of *Bursera penicillata* (DC) Engl

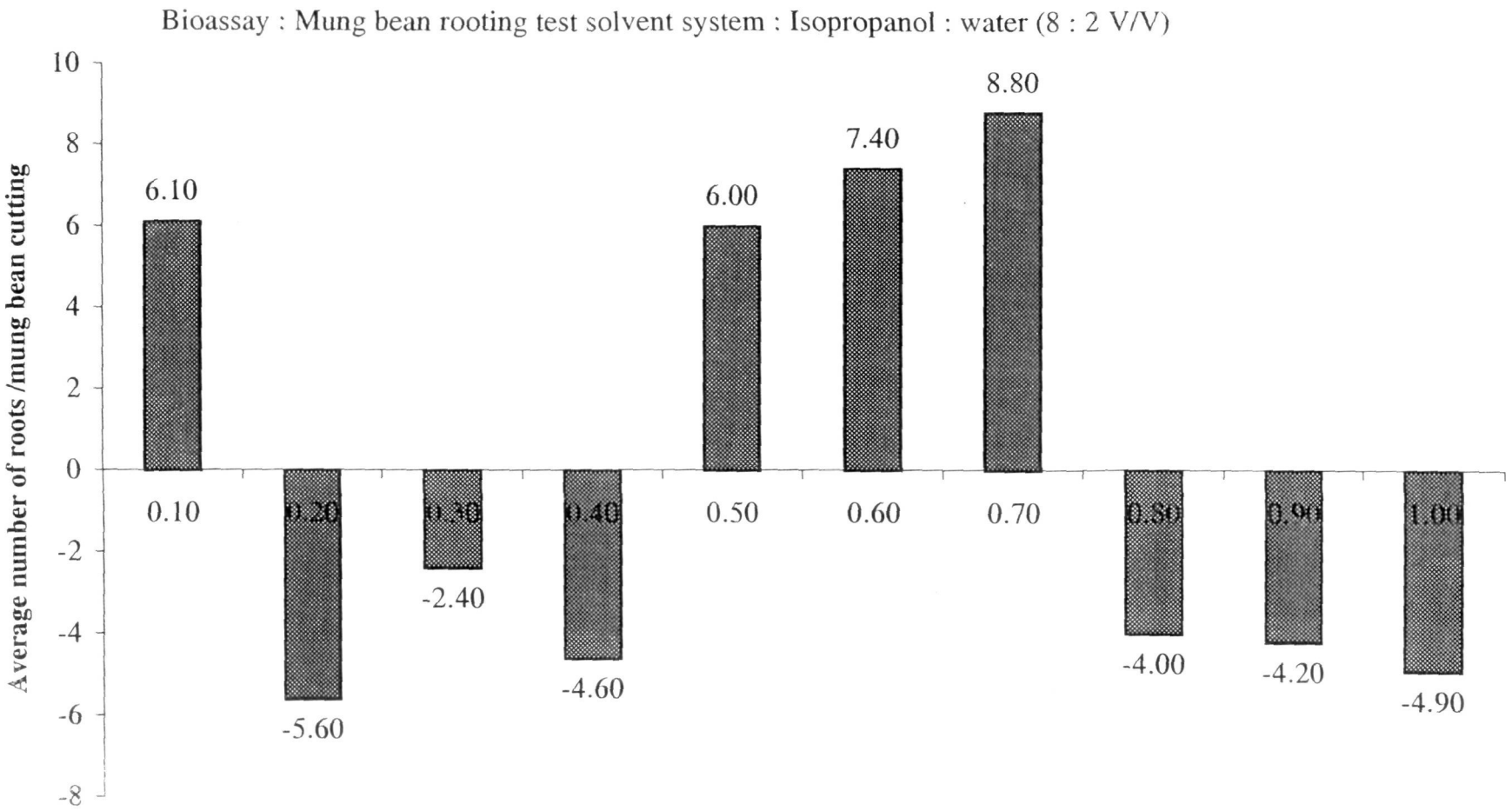


Fig 7 : Histograms showing the biological activity of root promoting co-factors extracted from the terminal soft wood portion cuttings of *Bursera penicillata* (DC) Engl

other root promoting co-factor 1, 2 and 4 were present. The terminal soft wood cuttings had only root promoting co-factor No.3 in little amount 'co-factor No.1' (corresponds to R_F 0.1) were present in very negligible amount.

DISCUSSION

V DISCUSSION

The *Bursera penicillata* (DC). Engl. is generally propagated by cuttings. However, the cuttings not only take a long time to root, but also exhibit sparse and erratic rooting, resulting in poor field establishment. In the present investigation an attempt was therefore made to determine the type of wood most suitable for cutting propagation, and also study the influence of several growth regulators on rooting of cuttings taken from different portions of shoot. Further, studies on the biochemical compositions of the different portions of the cuttings as well as studies on the survival of the rooted cuttings made. The significance of the results are discussed in the following pages.

5.1.0 Shoot parameters

5.1.1.1 Days taken for first sprout to appear

Significant difference were observed with respect to days taken for the first sprout to appear in different type of cuttings. Hardwood cuttings took minimum number days (16.78) whereas, compared to semi hard cuttings. The earlier sprouting in hard wood cuttings could be due to the higher reserved carbohydrates, sugars and starch content in the cuttings as compared to semi-hard wood cuttings.

The different growth regulators also influence the number of days taken for sprouting. The earliest sprouting was observed with 250 ppm IBA treatment (13.50 days). The number of days required for first sprout to appear was higher with an increase in the concentration of IBA, NAA and their combinations. Distilled water treatment and control took the maximum number of days (23.33 and 24.33 days, respectively) to sprout when compared to other treatments. It may be due to the accumulation of endogenous phenols in the basal tissues could be lessened by soaking cuttings in distilled water. These findings are in conformity with Curir *et al.* (1992)

Interaction effects were significant with regard to the time required for sprouting. Hardwood cuttings treated with IBA at 250 ppm resulted the minimum number of days (12.00) for sprouting. Earliness in sprouting may be due to better utilization of stored carbohydrates, nitrogen and other factors with the aid of auxins. Whereas control and distilled water treatment took maximum time for sprouting (25.0 and 23.67 days, respectively).

5.1.2 Number of sprouts

The maximum number of sprouts per cuttings was obtained in hard wood cuttings (4.10) being rich in reserve carbohydrate when compared to the other two cuttings.

All the growth regulators tried influenced the number of sprouts per cuttings. The maximum number of sprouts per cuttings (6.17) was observed with NAA at 250 ppm. Shenoy (1992) and Simly (1994) have also obtained similar results with NAA in *Rosa damascena* and *Cinnamomum zeylanicum* cuttings respectively.

Among the interaction effects, the maximum number of sprouts (7.16) was found in hard wood cuttings treated with IBA at 250 ppm. This is due to better utilization of stored carbohydrate, nitrogen and other factors with the aid of auxins. These findings are in conformity with Purohit and Shekharappa (1994) in pomegranate. The next best treatment was NAA at 250 ppm in case of semi-hard wood cuttings which recorded 5.46 sprouts per cutting. The results reported by Singh *et al.* (1986) in citrus, Shenoy (1992) in *Rosa damascena,* Simly (1994) in *Cinnamomum zeylanicum* cuttings confirmed the same.

5.1.3 Length of the longest sprout

Hard wood cuttings sprouted earlier and exhibited the maximum length of sprouts (3.24 cm) compared to semi-hard wood (3.09 cm) and terminal soft wood (2.47 cm) cuttings. This may be due to better physiological maturity and higher amount of reserve food materials in the hard wood cuttings. Moore and Ink (1964) in Blue berry has reported that basal cuttings produced the maximum shoot growth.

With regard to the growth regulators effects, IBA at 250 ppm resulted in the maximum length of sprouts (4.78 cm) which was followed by NAA at 250 ppm (4.32 cm).

With regard to interaction effects, the maximum length (5.53 cm) of sprout was obtained in hard wood cuttings treated with 250 ppm IBA followed by the NAA at 250 ppm (4.80 cm). This is due to earliest sprouting which was noticed in hard wood cuttings of these treatments and may be due to better utilization of stored carbohydrates, nitrogen and other factors with the aid of growth regulators on the different types of cuttings.

5.1.4 Number of leaves

Hard wood cuttings recorded the maximum number of leaves (9.02) as compared to semi-hard wood and terminal soft wood cuttings (7.13 and 7.66 respectively). It may be due to higher stored materials like carbohydrates present in hard wood cuttings and also due to the fact that the member and length of sprout were higher in hard wood cuttings.

The growth regulators tried, have significantly increased the number of leaves per cutting. The treatment of NAA at 250 ppm produced a maximum (10.50) number of leaves than control. This could be due to the longer sprouts and

higher number of sprouts noticed in cuttings treated with these particular treatments.

With regard to interaction effects, hard wood cuttings treated with IBA 250 ppm recorded the maximum number of leaves (11.86) per cuttings. Production of more number of sprouts and longer sprouts per cutting in hard wood cuttings might have helped in this regard.

5.2 Root parameters

5.2.1 Percentage of cuttings rooted

The results presented in the table-5 indicate that hard wood cuttings produced the highest percentage of rooting (56.48%) as compared to semi-hard wood (51.09%) and terminal soft wood (24.69%) cuttings. This difference may be due to the different physiological status of the cuttings. As seen in the biochemical studies , hard wood cuttings of siamese rough bush had higher amount of total carbohydrates, C:N ratio and rooting co-factors than the semi-hard wood cuttings. The results are in comfirmity with the findings of Roy *et al.* (1973), Singh and Singh (1973) in *Citrus limon* – burm, Blazich and Acedo (1989) in *Osmanthus heterophyllus* and Simly (1995) in *Cinnamomum zeylanicum.*

The growth regulators were also found to enhance rooting with the treatment of IBA at 250 ppm recording the highest (68.88%) percentage which was *on par* with NAA 250 ppm (59.0%) and IBA + NAA at 250 ppm (46%). An increase in rooting percentage by IBA has been reported by Guo et al. (1986) in *Acanthopanax senticosur*, Benyei (1987) in *Hedera helix*, Simly (1995) in *Cinnamomum zeylanicum* and synergistic effects of NAA and IBA has been reported by Bhattacharjee and Balakrishna (1992) in *Ixora singaporensis*. Dunn et al. (1996) in *Pistacia chinensis*. This may be attributed to the root stimulating and promoting effects of IBA.

Among the interaction effects hard wood cuttings treated with IBA 250 ppm resulted in the maximum (84.66) percentage of rooting followed by 500 ppm IBA (75.0%). The beneficial effects of exogenous application of auxins in enhancing rooting in hard wood cuttings has been reported by many workers Guo *et al.* (1986) Puri and Shamet, (1986) Poi and Mazumdar, (1989) in Bael Oosthuigen (1993) in Pecan, Kumar and Farooqi (1996) in Garden Rue.

5.2.2 Number of roots

Hard wood cuttings were found to be superior to semi-hard wood and terminal soft wood cuttings with respect to number of roots produced per cuttings. Hard wood cuttings produced on an average of 11.73 roots per cuttings, whereas it was only 9.27 roots in semi-hard wood and 6.12 in terminal soft wood cuttings. This again could be due to stored carbohydrates content, rooting co-factors as well as physiological maturity of the planting material. An increase in number of roots towards the base of the shoot is in agreement with the findings of Poulsen and Anderson (1980) in *Hedera helix*.

As far as the growth regulators are concerned, IBA at 250 ppm (18.56) and NAA at same concentration (14.42). This observation supports the earlier findings of Dettweiler (1942) who opined that the application of growth regulators cause greater meatabolic activity and mobilization of sugars and nitrogenous substances from stems and leaves which help in the initiation of root primordia in cuttings. The better activity of IBA may be due to its slower translocation property or its destruction is relatively slowly by auxin degrading enzyme system. Such results are comparable to those of Bhujbal and Kale (1975), Bahuguna *et al.* (1988), Daoud *et al.* (1989) and Simly (1994).

With regard to interaction effects, hard wood cuttings treated with 250 ppm IBA produced the maximum (27.83) number of roots followed by same concentration of same growth regulator (17.93) in semi hard wood cuttings. It is generally accepted that auxin has a central role in the initiation and development of adventitious roots (Bisaria and Rao, 1988).

Higher number of primary roots over the control may be due to enhanced hydrolysis of carbohydrates caused by the treatment with auxin..

5.2.3 Length of the longest root per cuttings

Development of extensive root system is important for success in propagation by cuttings. The maximum length (8.59 cm) was observed in hard wood cuttings while it was least (1.81 cm) in terminal soft wood cuttings.

In the present investigation, application of IBA at 250 ppm was the most effective treatment recording the maximum root length (7.95 cm) followed by treatments of 500 ppm IBA and 250 ppm NAA (6.54 cm and 6.37 cm, respectively). Similar effect of growth regulators in enhancing the length of roots has been reported by Rajanna (1981) in pomegranate, Sherer *et al.* (1985) in grape vine and Bisaria and Rao (1988) in *Boehmeria nivea* Gaud. Reddy *et al.* (1996) horticultural crops. The average in length of roots in cuttings treated with growth regulators may be perhaps be due to the enhanced hydrolysis of carbohydrates, accumulation of metabolites at the site of application of auxins, synthesis of new proteins, cell enlargement and cell division induced by the auxins.

In case of interaction effects, NAA at 250 ppm recorded the maximum (11.13 cm) root length in hard wood cuttings followed by IBA 250 ppm (8.46 cm) in semi-hard wood cuttings and in terminal soft wood cuttings lower level of IBA 250 ppm (4.70 cm) was found to be better. This development of larger roots may

be attributed to the early initiation of roots, cuttings in the above treatments with regard to IBA and NAA only optimum levels instead of lower and higher concentration.

These results commensurate with the findings of Bisaria and Rao (1988) in *Boehemeria nivea* Gaud and Sadiq (1991) in peach.

5.2.4 Fresh weight and dry weight of roots

Cuttings taken from different types of wood differed significantly with respect to fresh and dry weight of roots. Roots of hard wood cuttings recorded the maximum fresh (0.099 g) and dry (0.069 g) weight as compared to semi-hard wood cuttings which recorded a fresh and dry weight of (0.044 g and 0.026 g respectively) and terminal soft wood cuttings recorded (0.024 and 0.018 g respectively). The better performance of hard wood cuttings could be due to the presence of more mature tissue of high carbohydrate and nitrogen, relationship or phenols or presence of more rooting co-factors.

Growth regulators also significantly affected the fresh and dry weight with IBA (at 250 ppm) treatment recorded the highest fresh weight (0.093 g) followed by NAA (at 250 ppm) treatment (0.089 g) which was also recorded the highest dry weight (0.062 g) followed by 250 ppm IBA treatment as against control which was recorded a 0.099 and 0.069 g of fresh and dry weight respectively. The effects of auxins observed in the present investigation with respect to the mean fresh and dry weight of roots per cuttings are in agreement with the findings of Bisaria and Rao (1988) in *Boehmeria nivea* Gaud, Shenoy (1992) in Rose, Kumar and Farooqi (1996) in Garden Rue.

From the interaction effects, it can be seen that, hard wood cuttings treated with IBA 250 ppm recorded the maximum fresh weight (0.179 g) followed by 250

ppm NAA (0.159 g). The minimum (0.018 g) was recorded in untreated (control) semi hard wood cuttings as against highest in 250 ppm IBA or 250 ppm NAA treatments (0.065 g or 0.064 g) and in terminal soft wood cuttings maximum fresh weight was recorded in 250 ppm NAA (0.043 g). The increase in fresh weight may be inferred due to the better physiological maturity of hard wood cuttings along with the mobilization of secondary metabolites towards better root formation with aid of growth regulators.

In regard to the dry weight of roots, hard wood cuttings treated with 250 ppm NAA recorded the maximum dry weight (0.119 g) followed by 250 ppm NAA treated semi-hard wood cuttings (0.037 g) and in terminal soft wood cuttings the same growth regulator recorded maximum dry weight (0.030 g) at same concentration. Similar results were obtained by Bisaria and Rao (1988) in *Boehmeria nivea* Gaud, Kumar and Farooqi (1996) in Garden Rue.

5.3 Establishment of rooted cuttings

There was a significant difference between the type of wood used with respect to the establishment after transplanting. Having recorded highest fresh and dry weight of roots and maximum stored carbohydrates, the physiologically mature hard wood cuttings were able to survive better (36.96%) than semi hard wood (16.59%) and terminal soft wood (3.08%) cuttings.

Owing to the positive influence on rooted shoot growth, the growth regulator tried in these experiment, recorded a relatively high success rate of establishment of rooted cuttings on transplanting cuttings treated with IBA at 250 ppm recorded the highest percentage of establishment (44.45%) followed by 250 ppm NAA (32.80%). The results in the present investigations are similar to those of Gupta (1982) in *Legastromia lancosteri* and Kumar and Farooqi (1996) in Garden Rue.

With regard to the interaction effects also, hard wood cuttings treated with 250 ppm IBA recorded the highest (78.33%) establishment of rooted cuttings followed by IBA at 250 ppm treated semi hard wood and terminal soft wood (46.33% and 8.70%, respectively). The highest survival of rooted cuttings in hard wood cuttings may be attributed to high C:N ratio, optimum concentration of IBA and NAA (at 250 ppm) and high content of carbohydrate reserve per cutting which resulted in maximum number of roots, root length and higher fresh and dry weight of roots per cutting. These findings are in confirming with Kempegowda (1984) in Rose Poi and Mazumdhar (1989) in Bael, Shenoy (1992) in Rose and Kumar and Farooqi (1996) in Garden Rue.

5.4 Biochemical studies

5.4.1 Total sugars

Hard wood cuttings had a slightly higher content of total sugars (6.25%) as compared to semi-hard wood cuttings (6.02%) and terminal soft wood cuttings (5.85%). This relatively higher sugar content found in hard wood cuttings and semi-hard wood cuttings was perhaps favourable for rooting. Thus, one of the reason for the failure of the terminal soft wood cuttings to root healthy was probably related to their low sugar content. Stoltz (1968), Gabricludze *et al.* (1975) and Kumar *et al.* (1985) also opined not a high regeneration capacity of cuttings was associated with a higher level of soluble sugars.

5.4.2 Starch content

A slightly higher starch content was seen in hard wood cuttings (2.59%) as compared to semi-hard (2.48%) and terminal soft wood cuttings (2.30%) such higher levels of sprout in the hard wood and semi hard wood cuttings could have brought abut favourable conditions for root initiation Mokashi (1978) and Kumar *et al.* (1985). Also favouring for more number of roots, high rooting percentage,

longest roots as well as a high fresh and dry weight of the roots than terminal soft wood cuttings.

5.4.3 Nitrogen content

The terminal soft wood cuttings contained relatively higher amounts of nitrogen (0.79%) as compared to semi-hard wood (0.64%) and hard wood (0.58%) cuttings. The relatively lower levels of nitrogen present in hard wood cuttings might have helped rooting.

The report of Haun and Cornell (1951) suggests that low level of nitrogen increases the rooting co-factors activity to produce better rooting. Also, with less nitrogen, the C/N ratio is higher and this may be conducive for better rooting. Similar observations have been reported by Vijay Kumar (1973), Gabricludze *et al.* (1975) and (1976).

5.4.4 Carbohydrate to nitrogen (C/N) ratio

Hard wood cuttings had a higher C/N ratio (18.04) as compared to semi-hard wood (15.74) and terminal soft wood (12.36) cuttings, suggesting that higher C/N ratio favours rooting in *Bursera pencillata* (DC) Engl.

Sen and Bose (1960) found that higher C/N ratio increased rooting activity in Justicia cuttings. Lin (981) also reported a positive correlation between high C/N ratio and high root ability.

5.4.5 Total phenols

Terminal soft wood cuttings contained higher amount of phenols (2.40 mg/g d.wt) as compared to either semi hard wood (1.42 mg/g d.wt.) (or) hard wood (1.40/g d.wt.) cuttings. These results may suggest that the relatively higher content of total phenols present in the terminal soft wood cuttings did not aid better rooting. In fact it is not just phenols in higher amounts which may not

induce better rooting; carbohydrates, auxins and root promoting 'co-factors' are also required in enough quantities to cause rooting. For their formation of auxin - phenol complex is believed to be important; this complex acts on the cell micro structure, thus indicating division and differentiation into root initials. In the presence of excess endogenous free auxin in the cuttings base, the phenolic cofactor complexes to form the rooting stimulus. However, in case of free auxin depletion, the phenolic cofactors acts as free and exerts its inhibiting effect on both division and differentiation, thus it retards rooting (Fadl and Hartmann, 1967). Veerangaratham *et al.* (1983) and Simly (1994) and Sumy (1996) have also obtained similar results with *Jasminium auriculatum* cv.Parimulai, *Cinnamomum zeylanicum* and *Lawsonia inermis* cuttings respectively.

5.5 Future line of work

Future research on the propagation of *Bursera penicilata* (DC) Engl can focus on the following lines.

- Study the season wise influence on rooting of cuttings.
- Treatment of cutting with PBZ and TRF in combination by slow as well as quick-dip methods and the effect on rooting.
- Pre-treatments such as girdling and etiolation in combination with root promoters and their effect on rooting.
- Characterization of the phenolic constituents and their possible interaction with auxins.
- Development of protocal for tissue culture.

SUMMARY

VI SUMMARY

The investigation on the influence of growth regulators on rooting in different types of cuttings in *Bursera penicillata* (DC) Engl. was carried out during 1999-2000 at Sanjeevini Vatika of the Horticulture Research Station, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bangalore.

The salient findings of the study are summarized below:

Experiment-I: Influence of growth regulators on the rooting in different types of cuttings in *Bursera penicillata* (DC) Engl.

Among the three types of cuttings tried, hard wood cuttings performed better than 'semi-hard wood and terminal cuttings with respect to days taken for first sprout to appear, number of sprouts, length of the longest sprout, number of leaves per cutting, rooting percentage, number of roots per cutting, length of the longest root per cutting, fresh and dry weight of roots and field establishment of rooted cuttings.

With regard to growth regulators used, the maximum rooting percentage was recorded in the treatments of 250 ppm IBA (68.88%), NAA 250 ppm (59.0%) IBA 500 ppm (58.44%) and 250 ppm IBA + NAA (46.0%) over control (20.0%).

Among the interaction effects, hard wood cuttings treated with IBA at 250 ppm recorded the maximum rooting percentage (84.66%) and fresh weight (0.179 g) and survival percentage (78.33) while 250 ppm NAA recorded the longest root (11.13 cm) and dry weight (0.119 g) over control.

Among the different levels of combination of IBA and NAA, treatment with 250 ppm IBA + NAA resulted in better rooting percentage (63%) more number of roots (9.96), longest root length (10.46 cm), higher fresh and dry weight of root and better sprout over control.

Initially hard wood cuttings contained higher reserved carbohydrates, total sugars and starch content in the cuttings as compared semi hard wood cuttings and terminal cuttings which may be reason for better rooting and better establishment.

Mung bean bioassay of tissue extracts from the three different types of cuttings indicated that hard wood portion contained all the four rooting co-factor NO.1, 2, 3 and 4 semi hard wood cuttings contained co-factors No.2 and 3 in little amount. This may be the probable reason for poor rooting in semi-hard wood cuttings in all the treatments and also in terminal cuttings.

Rooted hard wood cuttings, which had received IBA treatment at 250 ppm concentration survived better and established well in the field.

In over all conclusion, it may be stated, the hard wood cuttings treated with 250 ppm IBA may be used for multiplication of plant materials of *Bursera penicillata* (DC) Engl. to obtain the higher rooting and survival percentage under intermittent mist condition.

Alternatively, the semi-hard wood cuttings may also be used for multiplication under mist. Here also the application of IBA at 250 ppm is recommended for induction of the maximum roots, however terminal soft wood cuttings treated with IBA at 250 ppm also gave fair results.

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* Original not seen.