Sc

METHODS FOR CLONAL PROPAGATION OF PODOCARPUS FALCATUS (THUNB.) MIRB.

A THESIS SUBMITTED TO THE
SCHOOL OF GRADUATE STUDIES
OF ADDIS ABABA UNIVERSITY

IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE IN BIOLOGY

BY

KASSA SEMAGN

JUNE, 1993

ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

METHODS FOR CLONAL PROPAGATION OF PODOCARPUS FALCATUS (THUNB.) MIRB.

KASSA SEMAGN

JUNE, 1993

ACKNOWLEDGEMENT

I am indebted to express my deepest and sincere appreciation to my advisor, Dr Legesse Negash, for his enthusiastic effort, constructive guidance, encouragement and material support until the completion of this manuscript. I am also indebted to express my sincere appreciation to Ato Kifle Dagne for his kindest assistance in photographing plant tissues. My thanks go to Dr Ensermu Kelebsa, for his valuable suggestion and comment on the preparation of microscopic slides for anatomical investigation, and Dr Seyoum Mengistu, for his cooperation in allowing me to use an inverted microscope for anatomical investigations. I would also like to thank professor L. O. Bjorn, University of Lund, Sweden, for his constructive comments and suggestions.

My friends, Alemayehu Balcha and Worash Getaneh, are highly acknowledged for their moral and material support in the written up and preparation of this manuscript. Dr Zerihun Woldu, Ato Tufa Abate, Girmay Medhin and Temesgen Zewotir are also greatly acknowledged for their assistance in data analysis.

Special thanks are also due to Mami Asrat, Mihret Mokonnen, Alemnesh Abebe, Hawa Mohammed, Demekech Feleke, Dereje Ketema , Zelalem Abebe and Getachew Mekonnen who were consistently assisted me in the nursery and glasshouse work.

ILCA and AAU science libraries are greatly appreciated for their kindest

cooperation in providing me a lot of reprints and abstracts. I would like to thank the Swedish Agency for Research Cooperation with Developing Countries (SAREC) for financial support. Finally, I would like to thank all individuals who were directly or indirectly contributed to the completion of this thesis.

TABLE OF CONTENTS

LIST OF FIGURES

FIGURES PAGE

1. I N T R O D U C T I O N

The climatic climax vegetation of Ethiopia is classified into six major regions with respect to altitude (temperature) and mean annual rainfall. These are Afroalpine, Sub-afroalpine, Forest, Woodland and Savanna, Steppe, and Semi-desert regions (FAO, 1984).

The forest regions of Ethiopia cover a broad altitudinal range from 450 to 3500 m. The mean annual rainfall ranges from 200 to 2200 mm. At the beginning of this century, closed forests were reported to have covered about 37 per cent of the land area of Ethiopia . By the early 1950's, they had been reduced to about 16 per cent; and by the early 1980's they dropped to about 4 per cent. Satellite imagery around 1988 indicated the forest cover to be as low as 2.7 per cent. This means that over the last two decades the area of the forest has been declining at a rate of about 100,000 ha per year (or about 1 ha every 5 minutes !) (Davidson, 1988).

The remaining forest regions mainly include Coniferous forests (*Juniperus* forest and *Podocarpus* forest), *Hagenia* forest, *Aningeria* forest, *Olea* forest, *Arundinaria* forest and *Baphia* forest (Davidson, 1988). Coniferous forest composed primarly of *Podocarpus* spp. and *Juniperus* spp. soft woods, the most commonly used timber in Ethiopia, originally covered about 176,000 sq. km in Central, Eastern and Northern parts

of the country. This type of forest has been extensively removed and now only 0.9 % of the original remains, accounting for only 4 % of the total forest resource of the country (WCMC, 1991). Therefore, Ethiopia is facing very rapid deforestation and degradation of land resources (Davidson, 1988).

Podocarpus falcatus (Thunb.) Mirb. (syn. *Podocarpus gracilior* Pilg.) is distributed in the central part of the Northwest Highlands with one record in Tigray, common around Lake Tana, more rare on the largely deforested plateau, and rare or almost entirely absent from the Southwestern part of the Northwest Highlands. *Podocarpus* is common in the Southwestern part of the Southeast Highlands and around Harar, but does not occur in the mountain chain in Northern Somalia. Its distribution is limited at about 14°N and 42°E (Friis, 1992).

Podocarpus is found occasionally in Afromontane rain forest, but is particularly characteristic of undifferentiated Afromontane forest, where it is frequently one of the dominant species (*Podocarpus* forest), often persisting in relic forest patches (gully forests, church forests). The genus is frequently found as a single tree left in derived grassland or farm land in areas with sufficient rainfall. However, sometimes the genus seems to be cultivated and it can sometimes be difficult to distinguish between records of

cultivation and records of marginal distribution. The distribution of the genus with respect to altitude and rainfall ranges from 1550-2800 m and 1000-1500 mm per year, respectively (Friis, 1992).

The wood of *P. falcatus* is a high-class soft wood considerably superior to European deals, if suitably manufactured and conditioned. The wood is suitable for many purposes including panel framing and panels (figured stock), shop and counter fittings, display cabinets, drawer linings (especially on accounts of its smoothness), for handicraft work in schools and technical institutes, bakery boards and confectionery trays for which non-tainting wood is required; also for cupboards, shelving or fittings where a bright, clean-coloured wood is desirable. From box-jointing tests, the wood appears to be suitable for the packing of fruit and the carriage of food stuffs, especially as its colour and smoothness permit containers to be painted and stencilled without difficulty. Boards have a tendency to warp, which may be regarded as the greatest fault of the timber, but this may be counteracted by suitable conversion and seasoning. Tests on its resistance to acids indicate that the wood is likely to be suitable for battery separators. A test for the manufacture of plywood from *P. falcatus* also indicated that plywood made from it is suitable for general utility purposes (Dale and Greenway, 1961).

Currently, *P. falcatus* is facing three serious problems: 1) it is being selectively logged for timber and furniture, 2) there is only one seed-based method for the propagation of the tree (Legesse Negash, 1992), and 3) knowledge concerning the physiological requirements for a successful establishment of *P. falcatus* seedlings is lacking. In Ethiopia, therefore, this valuable tree species is nearly endangered.

The main objective of this investigation is, therefore, to develop an alternative method for the rapid propagation of *P. falcatus* through rooting of branch cuttings.

2. L I T E R A T U R E R E V I E W

Plant propagation involves the control of two basically different types of developmental life cycles, sexual and asexual. The sexual cycle utilizes seed propagation by which new individual offspring plants are created whose characteristics reflect the genetic contribution of the parents. The asexual cycle, on the other hand, utilizes various vegetative methods of propagation such as cuttings, layering, grafting, separation, division, budding, micro-propagation and others. The use of vegetative propagation methods enable to preserve the unique characteristics of any individual parent plant in the offspring plants and, in addition, the genotype of the source plant can be preserved intact (e.g. Hartmann and Kester, 1975).

In propagation by cuttings, a portion of stem/branch, root, or leaf is cut from the parent plant, after which this plant part is placed under certain favourable environmental conditions and induced to form roots and shoots, thus producing a new independent plant (Hartmann and Kester, 1975).

Stem/branch cutting is the most important type of cutting and rooting of stem/branch cuttings is an important means for vegetative propagation practised in forestry and horticulture

for mass production of improved materials within a short period of time (Nanda and Anand, 1970). Bonga and Durzan (1987) have used the following terms to distinguish, and establish equivalence between the processes of driving plants from seeds and from cuttings in *vivo*:

Seed---------->Germinant---------->Seedling

Cutting------->Rooted cutting-------->Steckling

2.1. Factors which affect rooting of stem/branch cuttings

It is well recognized that rooting of cuttings is determined by a complex interplay of both external and internal factors (e.g. Eliasson, 1980 ; James, 1983; Nordstrom and Eliasson, 1984). The most important external factors are light (e.g. Hartmann and Kester, 1975; Ooishi *et al.*, 1981, 1982; Hosoi and Ooishi, 1987; Bissaria, 1988; Hansen, 1989; Svenson and Davies, 1989), temperature (e.g. Gislerod, 1984; Reddy and Singh, 1987; Bissaria, 1988; Davis *et al.*, 1991), aeration (e.g. Ivanova, 1981; Wally *et al.*, 1981; Gislerod, 1984; Malik and Harnard, 1984; Economou and Paul, 1986; Davis *et al.*, 1991), moisture (e.g. Hartmann and Kester, 1975; Bissaria, 1988; Bissaria and Rao, 1988), the physical and chemical properties of the rooting medium (e.g. Hartmann and Kester, 1975; Economou and Paul, 1986) and cutting pretreatment (e.g. Hartmann and Kester, 1975; Reddy and Singh, 1987; Hansen, 1989).

Among the internal factors are plant growth regulators (e.g. Friedman *et al.*, 1979; Bandzaitene, 1981; James, 1983; Kaundal and Bindra, 1986; Wiesman *et al.*, 1988), physiological conditions and nutrition of the stock plants (e.g. Hartmann and Kester, 1975; Ivanova, 1981; Leakey and Coutts, 1989), the age of the stock plants (e.g. Kennedy and Selby, 1985; Hong *et al.*, 1986; Kwon *et al.*, 1987; Moon *et al.*, 1987; Sunil, 1990), season (e.g. Klahr and Still, 1980; Ivanova, 1981; Wally *et al.*, 1981; Kukuchi *et al.*, 1983; Thompson, 1986; Moon *et al.*, 1987), type and size of wood selected (e.g. Miller *et al.*, 1982; Moon *et al.*, 1987; Davis *et al.*, 1991), position of the cutting (e.g. Hartmann and Kester, 1975; Cid *et al.*, 1982; Bissaria, 1988), inherent root forming capacity and premature leaf abscission (e.g. Sykes and Williams, 1959).

2.1.1. External factors

i) Light

Light is the source of energy for photosynthesis. Results of various workers (e.g. Ooishi *et al.*, 1981,1982; Hosoi and Ooishi, 1987; Svenson and Davies, 1989) indicated that since the rate of apparent photosynthesis during root initiation is low, root initiation probably is little affected by photosynthesis. However, photosynthesis has an important role in the development of roots after initiation. On the other hand, etiolation was found to be conducive for root

initiation of cuttings (Leopold, 1955; Kawase, 1965; Krul, 1968; Hartmann and Kester, 1975).

ii) Temperature

Daily air temperatures of 21 to 27°C and night temperature of 15°C are satisfactory for rooting of cuttings of most species, although some species root better at lower temperatures (Hartmann and Kester, 1975). In cutting beds, some type of thermostatically controlled heat, applied below the cuttings, is beneficial in maintaining the temperature at the base of the cuttings higher than that of the shoot (e.g. Reddy and Singh, 1987; Davis *et al.*, 1991).

iii) Rooting media

Among the media used for rooting stem cuttings and other organs of plants are soil, sand, peat moss, perlite, compost, shredded bark, sphagnum moss, vermiculite and sawdust. The use of mixtures of some of the media, instead of any one of them alone seems to favour the development of normal roots. Sand and sand mixed media provide adequate amount of moisture and aeration which favours rooting of cuttings of a large number of plant species (Hartmann and Kester, 1975).

iv) Moisture

One of the most pressing problems confronting the plant propagators is that of maintaining the cuttings on the

greenhouse bench without wilting until such time that roots may be produced. Various methods have been used to assure an environment that will reduce wilting such as the use of closed cases, bell-jars, shades and cloth covers (O'Rourke, 1949).

Traditionally it has been a standard practice in propagating frames and greenhouses to sprinkle the cuttings frequently, as well as the walls and floor, so as to maintain a high humidity. Automatically operated devices which disperse a fog-like mist are sometimes used in greenhouses. These methods of humidification gives a beneficial effect primarily in increasing the amount of water vapour in the air (Sykes and Williams, 1959; Hartmann and Kester, 1975). To reduce transpiration of the leaves on cuttings to a minimum, the vapour pressure of the water in the atmosphere surrounding the leaves should be maintained nearly equal to the water vapour pressure in the intercellular spaces within the leaf (Hartmann and Kester, 1975).

2.1.2. Internal factors

i) Plant growth regulators

One of the most important factor in root induction is the use of synthetic plant growth regulators (e.g. Friedman *et al.*, 1979; James, 1983). Auxins are the most important synthetic plant growth regulators used in forestry and horticulture for

the induction of root formation in cuttings (Wiesman *et al.*, 1988). It is well accepted and has been subsequently confirmed many times that auxins, endogenous or exogenously applied, are the requirement for the initiation of adventitious roots in cuttings. It has also been shown that the division of the first root initial cells are dependent upon either applied or endogenous auxins (Haissig, 1972). Auxins are the only applied phytohormones that consistently enhance root primordium development, at least in naturally responsive (i.e. easy-to-root) tissues (Haissig, 1974b).

According to Hartmann and Kester (1975), plants could be divided into three groups with regard to their relation to substances involved in adventitious root initiation. The first groups are those plants in which the tissues provide all the various native substances including auxin, essential for root initiation. When cuttings are made and placed under proper environmental conditions, rapid root formation occurs. The second groups are those plants in which the naturally occurring rooting co-factors are present in ample amounts but auxin is limiting. With the application of auxin, rooting is greatly increased. The last groups are those plants that lack the activity of one or more of the internal co-factors although natural auxin may or may not be present in abundance. The external application of auxin to the last groups of plants gives little or no response.

Several compounds, natural or synthetic, are known under the name auxin. Some of these are indole compounds such as indole-3-acetic acid, indole-3-acetaldehyde, indole-3-acetonitrile, indole-3-butyric acid, indole-3-propionic acid and indole-3-pyruvic acid (Wareing and Phillips, 1970); others are phenoxy compounds such as para-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid; and still others are naphthol compounds (e.g. α-naphthaleneacetic acid) (Leopold, 1955).

ii) Rooting co-factors (Auxin synergists)

The rooting ability of cuttings depends on some factors named "rooting co-factors" or auxin synergists which are produced in plants mainly in the leaves (Hartmann and Kester, 1975). Successful rooting of cuttings is dependent on the presence of co-factors which, in combination with auxin, enable cuttings to root. Difficult-to-root cuttings fail to root because they lack the necessary co-factors and/or such cuttings contain inhibitory substances in amounts high enough to mask the effect of promotive substances present (Mostaffa and Hartmann, 1967). The authors found endogenous rooting co-factors to be greatest in easy-to-root clones followed by intermediate-to-root and difficult-to-root cuttings, respectively.

The role played by synergists in rooting of cuttings is not clearly understood but the assumption is that synergists may:

(1) protect the root inducing auxins from destruction by the enzyme IAA oxidase (Gorter, 1962, 1969), (2) activate or enhance the conversion of tryptophan to IAA (Gorter, 1969), (3) slow the conjugation of auxins with other compounds (Hess, 1965), and (4) have a kind of preparatory action, which makes more cells able to react to the auxin (Gorter, 1969).

iii) Nutrition of the stock plants

Like all growth processes, development of root primordia demands nutrients (Pearse, 1943). The rooting of tomato stem cuttings, for example, is influenced by the relative proportion of carbohydrates, nitrogenous compounds and vitamins. Tomato stem cuttings containing high carbohydrates and low nitrogen produced many roots but only feeble shoots, whereas those greenish stems that contain ample carbohydrates and high nitrogen produced fewer roots but stronger shoots. The green, succulent stems, very low in carbohydrates but high in nitrogen all decayed without producing either roots or shoots (Kraus and Kraybill, 1918: cited in Hartmann and Kester, 1975)). This indicated that high carbohydrate and low nitrogen level of the stock plants is the best for good root formation (Hartmann and Kester, 1975). However, results of Leakey and Coutts (1989), using *Triplochiton scleroxylon* stem cuttings, indicated that rooting ability was not related to the initial carbohydrate content, suggesting that rooting is

dependent on carbohydrates formed after severance.

iv) The age of the stock plants

The age of the stock plants is an important factor in rooting of difficult-to-root plants, while it has a relatively little effect on easy-to-root plants (Kennedy and Selby, 1985; Hong *et al.*, 1986; Kwon *et al.*, 1987; Moon *et al.*, 1987). Experiments with apple, pear, Eucalyptus, and Douglas fir (Hartmann and Kester, 1975), and other species have shown that the ability of cuttings to form adventitious roots decreased with increasing age of the plants from seeds.

2.2. Histological origins of root primordia

Adventitious roots of gymnosperms and dicotyledons arise either from preformed root primordia developed during branch or stem formation, or in attached twigs (layers) or severed twigs and leaves (cuttings) that would not have differentiated root primordia during normal development. The latter type of root primordia is known as "induced" (Haissig, 1974a).

It has been difficult to ascertain when induced primordia initiate and how long it takes for development, even in apparently uniform groups of cuttings, because these primordia usually do not initiate at the same time or at predictable sites. Root primordia initiation has been known

to take months (or not even occur) in some difficult-to-root woody cuttings, whereas it has begun within a few days in more easily rooted cuttings (Haissig, 1974a).

An examination of data on the anatomy of root formation and development in stems indicated that at least four distinct and at times discontinuous, stages are involved in the formation of roots by a cutting, namely, (a) the initiation of groups of meristematic cells, (b) the differentiation of these tissues into recognizable root primordia, (c) the extension and the emergence of roots, involving the rupturing of epidermal surface and perhaps other stem tissues, and (d) the development of roots outside the cuttings (Argles, 1959).

3. MATERIALS AND METHODS

3.1. The glasshouse and its environmental condition

This experiment was conducted in a glasshouse at the Addis Ababa University, Science Faculty campus from mid-May, 1992 to the beginning of April, 1993. The working area and height of the glasshouse were 5.32 m^2 and 2.60 m, respectively. Cuttings in the glasshouse were protected from high temperature and light intensity by a shade which was prepared by making a wooden bed at a height of 1.10 m from the floor. The bed was then covered with branches (leaves) of *Phoenix reclinata* Jacq. The mean temperature of the glasshouse below the shade ranged from 13°C (mornings) to 30°C (noons and early afternoons). The relative humidity (RH) of the glasshouse was kept within 75-85% by spraying water using a three-arm sprinkler. The mean quantum flux density of the glasshouse below the shade was 88.6 $\pm$ 8.2 μ mol m^{-2} s^{-1}. RH was measured using an MK3 Automatic Porometer (Delta-T Devices, Cambridge CB5 OEJ, England). Light was measured using Quantum Radiometer (LI-189, LI-COR, Lincoln, Nebraska, USA).

3.2. Rooting medium

The rooting medium that was used during this investigation was sand. It was sieved using 1.75 mm mesh size and washed in running water until most of the soil particles and weed seeds were removed. About 1.5 kg of the washed sand was filled in

each plastic pot (diameter, 100 mm; depth, 130 mm) a few days before use. The pots were frequently watered using a three arm sprinkler until they were used for planting cuttings. The base and sides of the pots were pierced with needle for drainage and aeration.

3.3. Plant material

The plant material that was used as a source of cuttings was conveniently divided into three age classes:

a) The youngest age class (1.5-2.0 years old saplings)

These source (stock) saplings were raised *in vitro* from seeds, following the method described by Legesse Negash (1992), and were planted in plastic pots (diameter 100 mm; depth 200 mm) filled with ca 950 gram of soil. [The soil in this experiment refers to soil collected from underneath trees of *P. falcatus*, *Juniperus procera* Endl. and *Olea europaea L. sbsp. cuspidata* (Wall. ex Dc.) Cifferi]. The seedlings were grown on beds at the nursery of the Department of Biology. Partial shade was provided at 0.65 meter height using branches of *Phoenix reclinata*. Possible damage of the seedlings by aphids was protected by spraying diluted solution of malathion whenever needed.

b) The intermediate age class (4-5 years old saplings)

These stock plants were raised from seeds and planted either directly on the soil or large plastic pots (diameter 180 mm, depth 300 mm) filled with soil.

c) The oldest age class (ca 25-30 years old trees)

These stock plants were obtained from the Addis Ababa University, Science Faculty campus near Saba student dormitory.

3.4. Preparation of cuttings

Seven hundred twenty firm primary branches of 100 to 220 mm in length and 20 to 30 mm in diameter were randomly collected from the youngest age class. Three hundred sixty similar cuttings were also randomly collected from each of the intermediate and oldest age classes. [Firmness of branches due to high carbohydrate storage, rather than due to tissue maturation, was tested using 0.2 % potassium iodide solution]. The number of branch cuttings collected from a single source plant of the youngest and intermediate age classes ranged from 5-15. A cut was made at the base of the branch with a sharp, sterile razor blade or a scalpel knife. Immediately after the cut, the base of the cutting was soaked in water to minimize the entrance of air into the xylem elements. The leaves near the base of the cutting were removed in order to avoid leaf decomposition during the rooting period. The cuttings were then divided into three groups with respect to their length, 100-140, 141-180 and 181-220 mm, to make the number of cuttings treated at each concentration of a PGR more homogenous.

3.5. Preparation of plant growth regulators

Three plant growth regulators, namely, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA), and one vitamin (nicotinic acid) were used to investigate whether root initiation in branch cuttings of *P. falcatus* is stimulated by these chemicals or not. All the chemicals were "plant tissue culture tested" and were purchased in powder form from Sigma Chemical Company (ST. Louis, MO, USA). A stock solution of each plant growth regulator was prepared by dissolving the required amount of the powder in 15-20 drops of 1M NaOH. This solution was diluted with distilled water and was neutralized with 15-20 drops of 1M HCl. The pH of the solution was adjusted to 4.50 $\pm$ 0.10 using a Gallenkamp pH meter. The concentrations of the plant growth regulators used were 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} and 10^{-3} M. These concentrations were prepared by serial dilution from the 10^{-3} M stock solution. Distilled water was used as a control. A stock solution of nicotinic acid (NA) was also prepared to be used in combination with IAA, IBA and NAA at a concentration of 0.5 ppm.

3.6. Treatment of cuttings with plant growth regulators

Fifteen randomly selected branch cuttings (5 from each of 100-140, 141-180 and 181-220 mm in length) collected from the intermediate and oldest age classes were soaked in 70 ml of the various concentrations of IAA, IBA and NAA solutions

alone. Soaking was done in 400 ml beakers for 24 hours. Similarly, fifteen randomly selected cuttings collected from the youngest age class were soaked for 24 hours in each of the concentrations of IAA, IBA and NAA either alone or in combination with 0.5 ppm NA. At the end of the soaking period, ca 40-70 mm of the base of the 15 treated cuttings at each concentration of the PGRs and the control were randomly inserted in three plastic pots filled with the rooting medium (5 cuttings per pot). The pots were then randomly arranged in the glasshouse under the shade. Twenty ml of Hoagland mineral solution was added to each pot at a weekly interval throughout the experimental period.

During the experiment, the cuttings were sprinkled with tap water at least twice a day for a total of at least 5 minutes per day depending on the temperature and the RH of the glasshouse. Watering was done by connecting one end of a hose to a water pipe and the other end of the hose to a three arm sprinkler.

3.7. Data collection and analysis

Data were collected for all the treated and control cuttings. The number of rooted cuttings were counted 4 and 6 months after treatment. However, data on the number and length of roots per rooted cutting were collected only 4 months after treatment. During data collection, the rooting medium was

gently splashed with running water to expose the roots with as little damage as possible. After data collection, the rooted cuttings were planted in plastic pots (depth 130 mm, diameter 100 mm) filled with a 3:1 mixture of soil and manure. Surviving but unrooted cuttings at the time of the first observation were replanted in the rooting medium to be assessed for the second time six months after treatment.

Verifications for significant differences among treatments were made by computing the standard deviations for each category of data.

3.8. Growth measurements

P. falcatus was propagated both by seeds and branch cuttings at the same time and growth in height of seedlings and stecklings was compared by randomly selecting 120 seedlings and 120 stecklings. The seedlings were obtained from *in vitro* germinated seeds using the method described by Legesse Negash (1992). Both the seedlings and stecklings were planted in plastic pots filled with a 3:1 mixture of soil and manure. The pots containing the seedlings and stecklings were arranged randomly at the nursery. Growth in height was measured for six months at a monthly interval.

3.9. The Histology (Anatomy) of branch cuttings

Histological investigations on the processes of callus

formation and root initiation were made in cuttings obtained from the youngest age class. Cuttings sampled for sectioning were fixed in formalin, acetic acid and ethyl alcohol (70%) in a ratio of 5:5:90 v/v from 5-15 days. The cuttings were dehydrated in a t-butyl alcohol series and were embedded in paraffin wax (melting point 52°C). Microtome sections were cut at 10-12 μm thickness. Sections were double stained in safranine-fast green and mounted in Canada balsam (Jensen, 1962). Selected sections were photographed at x100 magnification.

4. R E S U L T S

4.1. Callus formation and root initiation

The time required for callus formation and root initiation of branch cuttings of *P. falcatus* was highly variable. Some cuttings developed calli and initiated roots within a relatively short period of time (1 month) while others needed very long time (about 5 to 6 months). Although the first swelling of the base of some cuttings collected from the youngest and intermediate age classes was observed one month after treatment, well developed calli were observed in most cuttings from 1.5 to 2.0 months (Fig. 1A). Root primordia were observed after callus formation; and the first adventitious roots emerged at the base of some cuttings from 2-3 months after treatment (Fig. 1B). Most cuttings showed well developed roots within 3-4 months (Fig. 1C). Root emergence was very quick once root primordia were visible around the callus. The first emerged roots were mostly white and very brittle.

Morphologically, roots emerged from the base and nodes (leaf scars) of the cuttings (Fig. 2). However, most roots emerged at the base of the cuttings rather than from the nodes.

4.2. Effect of age of stock plants on rooting

The effect of age of stock plants on rooting percentage,

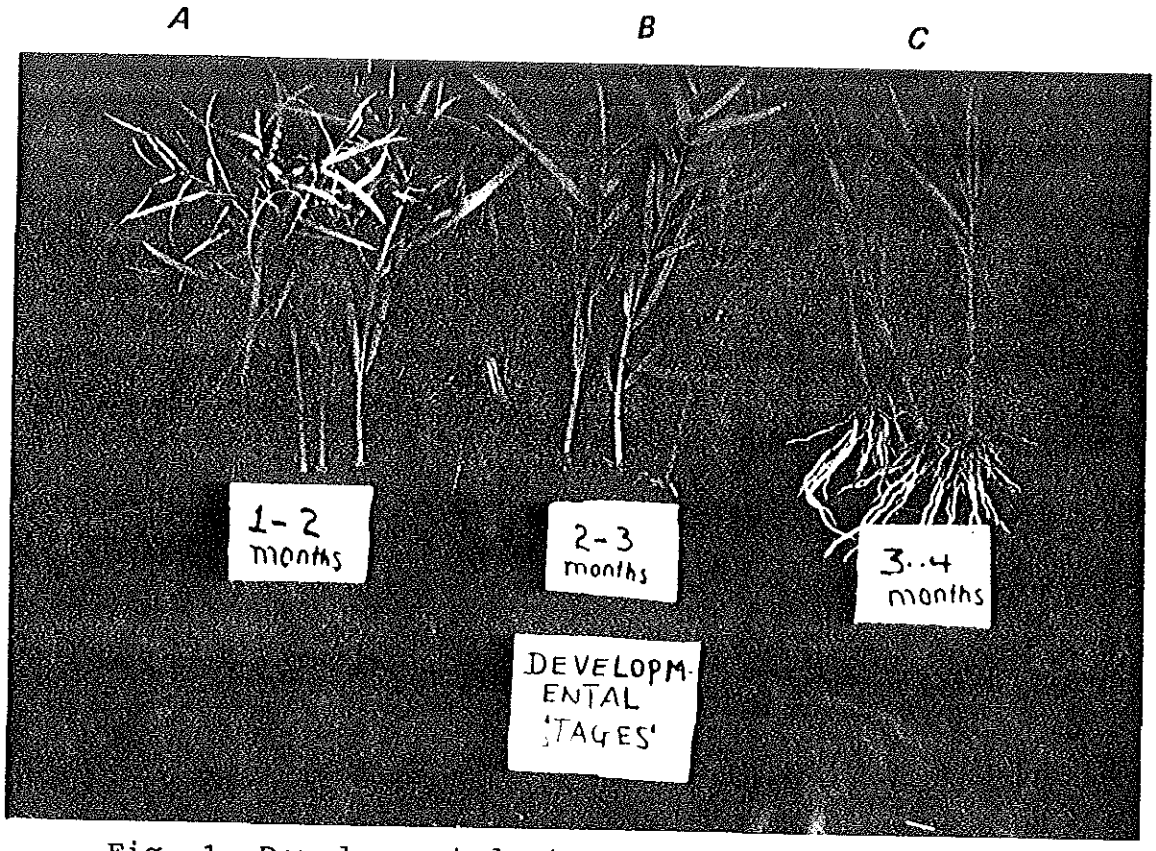


Fig. 1. Developmental stages of adventitious roots.

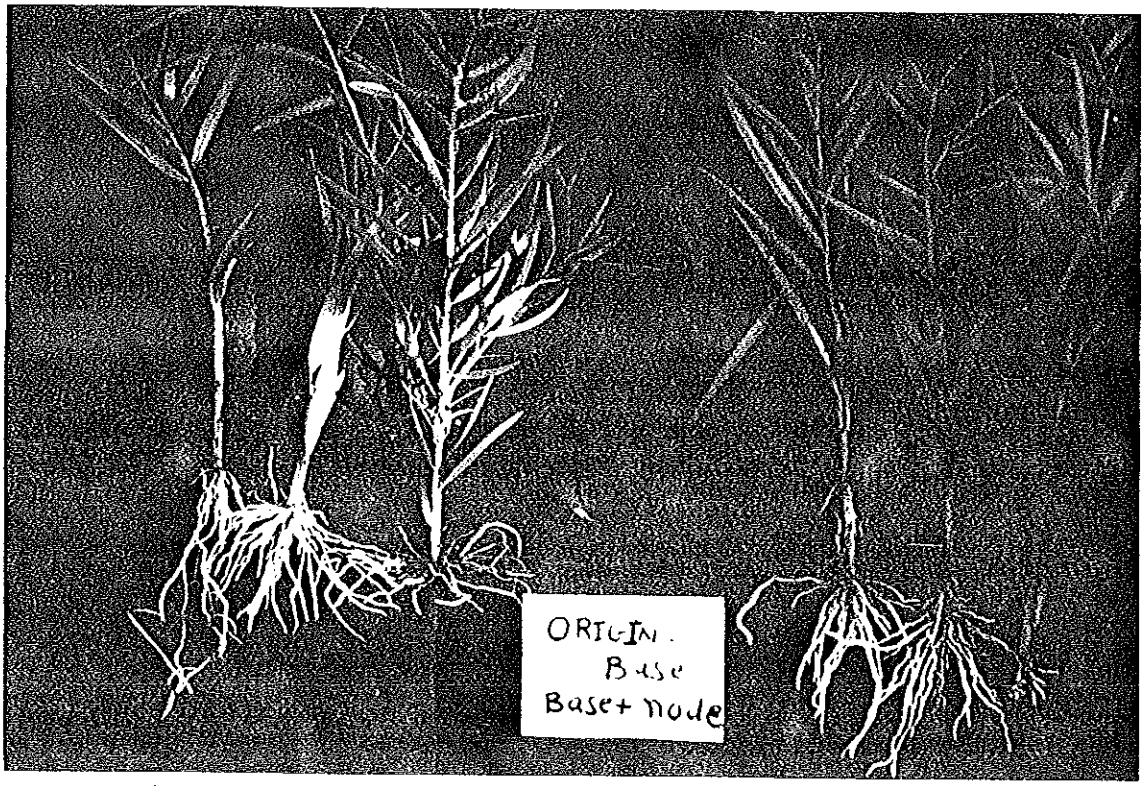


Fig. 2. Morphological origins of adventitious roots.

overall mean number and length of roots per rooted cutting is shown in Figure 3. Cuttings collected from the youngest age class gave significantly higher percentage (77.8 ± 23.9 %) of rooted cuttings (number of rooted cuttings/number of planted cuttings expressed as a %) six months after treatment as compared to those collected from the intermediate (29.7 ± 18.7 %) and oldest (13.9 ± 14.9 %) age classes. Also, the rooting percentage of cuttings relatively increased by extending the rooting duration from 4 to 6 months (Fig. 3).

The overall mean number of roots differentiated per rooted cutting collected from the youngest age class (17.9 ± 6.6) is significantly higher than the overall mean number of roots differentiated by the oldest (3.1 ± 5.3) age class (Fig. 3). Also, Cuttings collected from the youngest age class produced significantly longer roots (22.2 ± 6.1 mm) than those collected from the oldest age class (3.9 ± 6.1 mm)(Fig. 3).

4.3. Rooting responses of age classes to PGRs

The overall effect of PGRs on rooting percentage is shown in Figure 4. PGRs did not significantly increase the rooting percentage of cuttings collected from the youngest and intermediate age classes as compared to the control. However, significantly higher rooting percentage was obtained when cuttings collected from the oldest age class were treated with IBA as compared to the control.

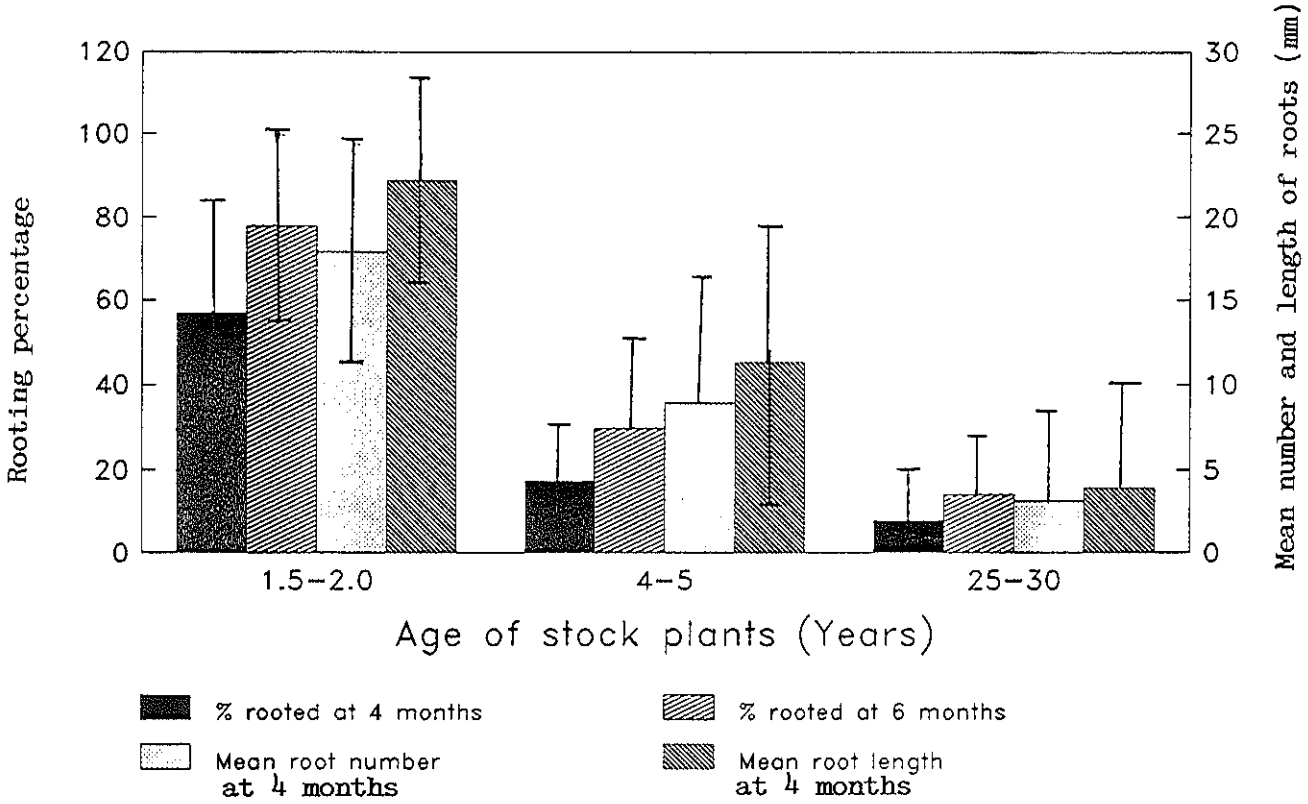


Fig. 3. Effect of age of stock plants on rooting percentage, mean number and length of roots per rooted cutting. Bars indicate ± SD.

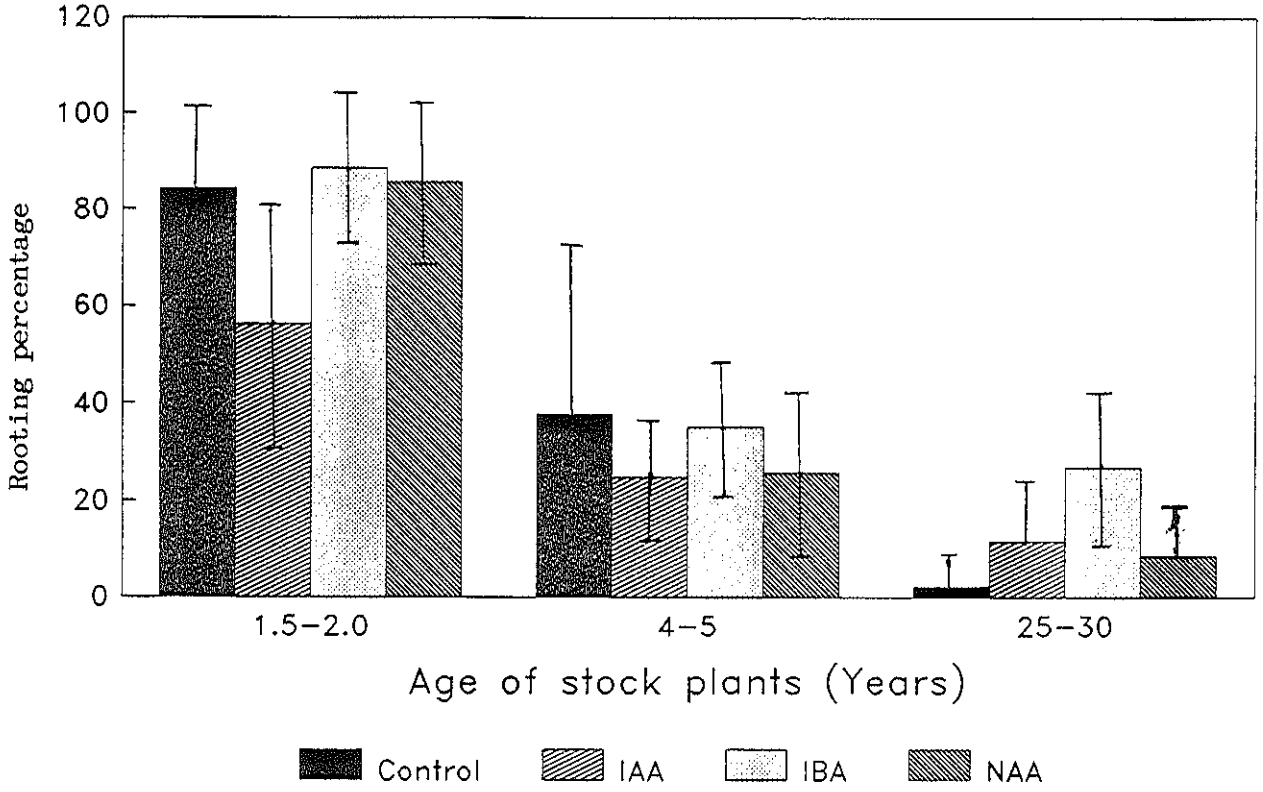


Fig. 4. Effect of age of stock plants and PGRs on rooting percentage 6 months after treatment. Bars indicate ± SD.

The effect of the various concentrations of PGRs on rooting percentage of cuttings collected from the youngest age class is shown in Figure 5. The rooting percentage obtained after treatment with 10^{-4} M IAA was significantly lower than the control and the other concentrations of IAA. The next lowest rooting percentage for IAA treated cuttings was recorded at 10^{-6} M (Fig. 5A). IBA treated cuttings gave significantly higher rooting percentage (100 %) at 10^{-9} and 10^{-6} M than the control, 10^{-8}, 10^{-7} and 10^{-4} M (Fig. 5B). Also, significantly higher rooting percentage was obtained when similar cuttings were treated with 10^{-7} and 10^{-6} M NAA and the control than 10^{-9} M NAA (Fig. 5C).

The overall effect of PGRs on mean number of roots per rooted cutting was not significantly different from the control (Fig. 6). None of the cuttings collected from the oldest age class and treated with water (control) were rooted until the end of the fourth month after treatment (Fig. 6 and Fig. 8)).

The highest and the lowest mean number of roots per rooted cutting collected from the youngest age class were differentiated when cuttings were treated with 10^{-4} M IAA and the control, respectively. In addition, the mean number of roots differentiated by the control was significantly lower than those treated with the various concentrations of IAA (Fig. 7A). The mean number of roots differentiated after

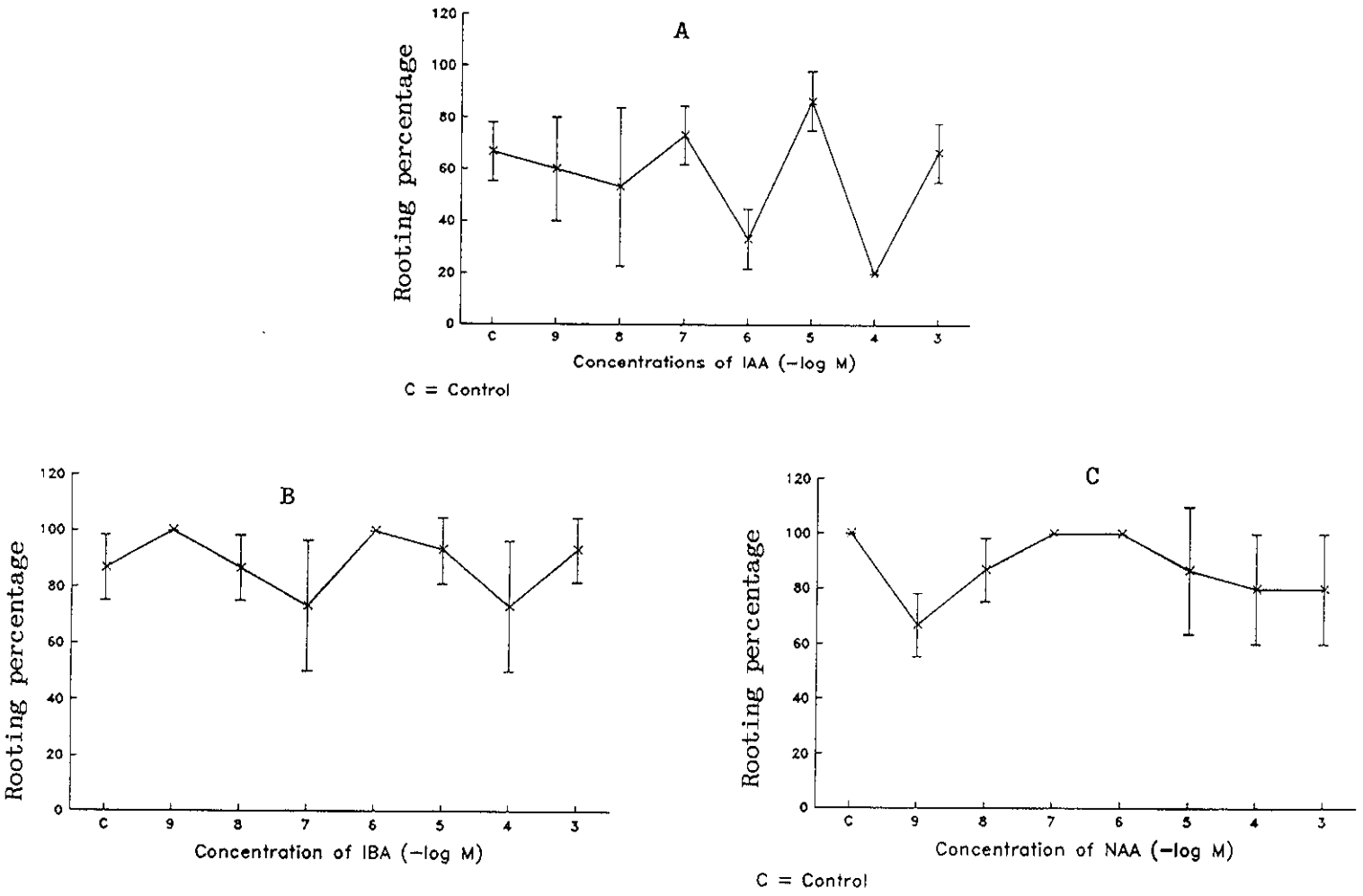


Fig. 5. Effect of various concentrations of PGRs on rooting percentage in cuttings collected from the youngest age class and 6 months after treatment. Bars indicate ± SD.

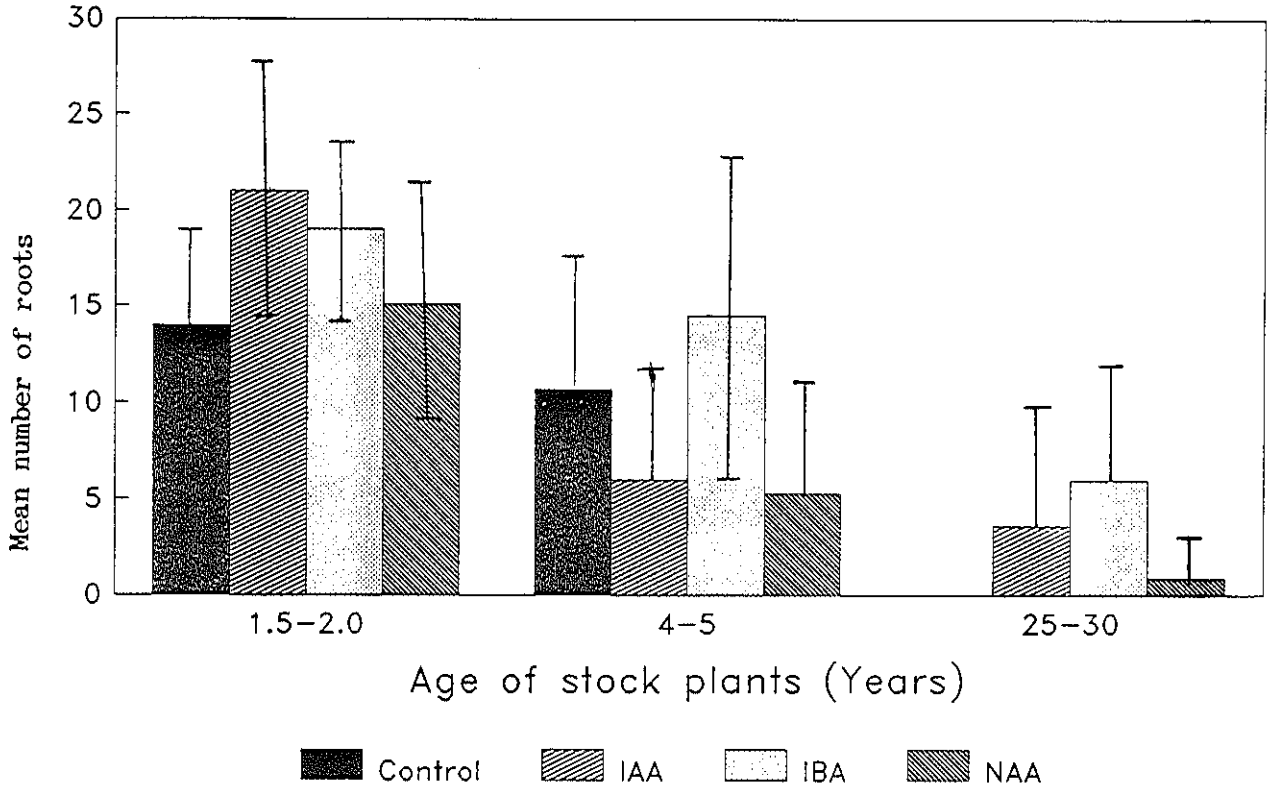


Fig. 6. Effect of age of stock plants and PGRs on mean number of roots per rooted cutting 4 months after treatment. Bars indicate ± SD.

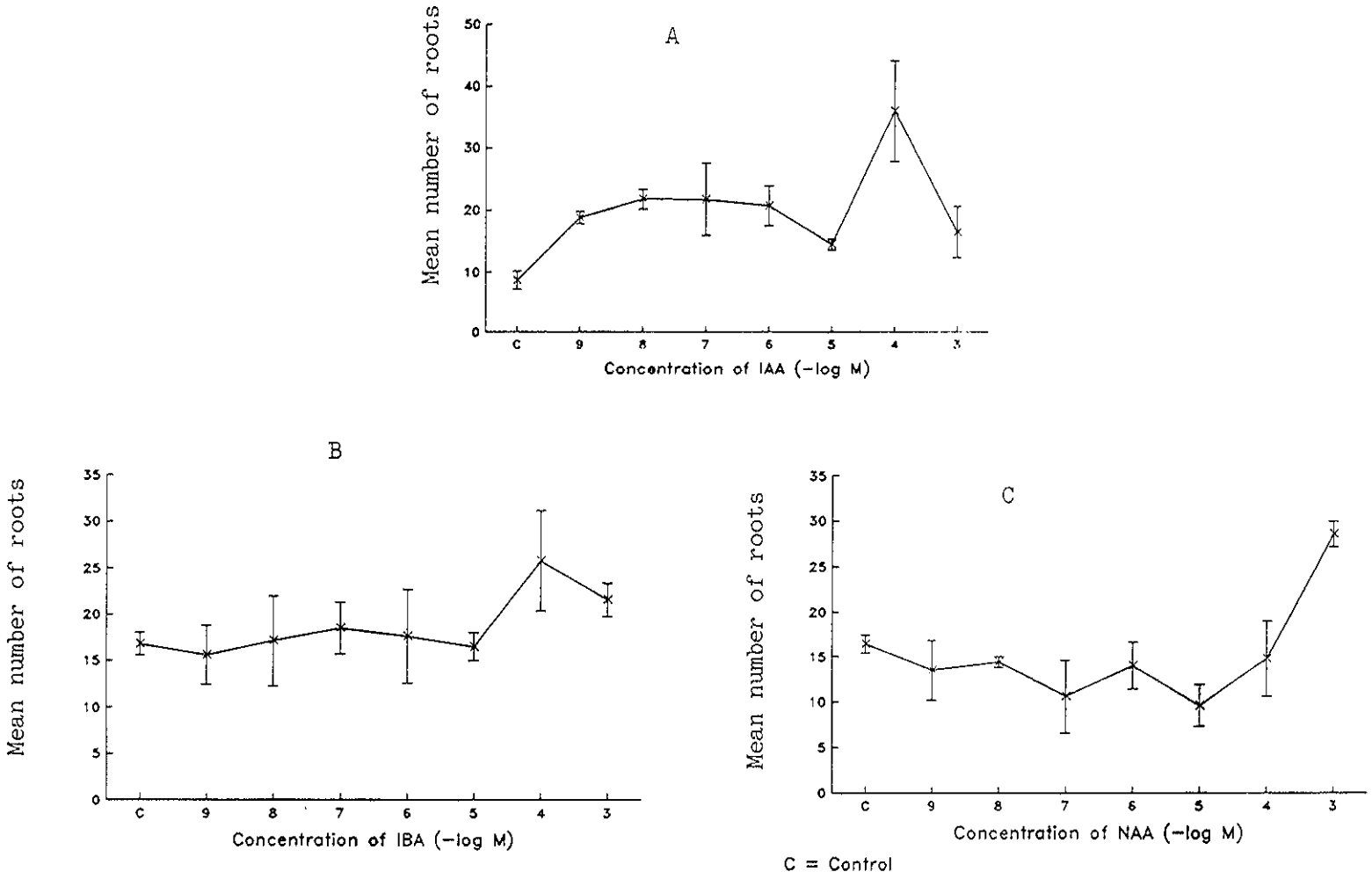


Fig. 7. Effect of various concentrations of PGRs on mean number of roots per rooted cutting collected from the youngest age class and 4 months after treatment. Bars indicate ± SD.

treating cuttings with 10^{-9} and 10^{-5} M IBA as well as its control was significantly lower than those treated with 10^{-4} and 10^{-3} M IBA (Fig. 7B). Significantly higher mean number of roots for NAA treated cuttings were recorded at 10^{-3} M than at the other concentrations of NAA. Also, the mean number of roots differentiated after treatment with 10^{-5} M NAA was significantly lower than those treated with 10^{-8} M NAA and the control (Fig. 7C).

The overall effect of PGRs on the mean length of roots differentiated per rooted cutting collected from the youngest and intermediate age classes was not significantly different from the control (Fig. 8). On the other hand, significantly higher mean length of root (8.8 mm) was recorded when cuttings collected from the oldest age class were treated with IBA as compared to the control (Fig. 8).

Cuttings collected from the youngest age class and treated with 10^{-8} and 10^{-3} M IAA produced significantly longer roots than the other concentrations of IAA (Fig. 9A). When similar cuttings were treated with the various concentrations of IBA and NAA, significantly longer roots were recorded at 10^{-4} M (Fig. 9B and 9C). However, the morphological appearance of roots differentiated per rooted cutting collected from the youngest age class and treated with most concentrations of the three PGRs was more or less similar (e.g. Fig.10).

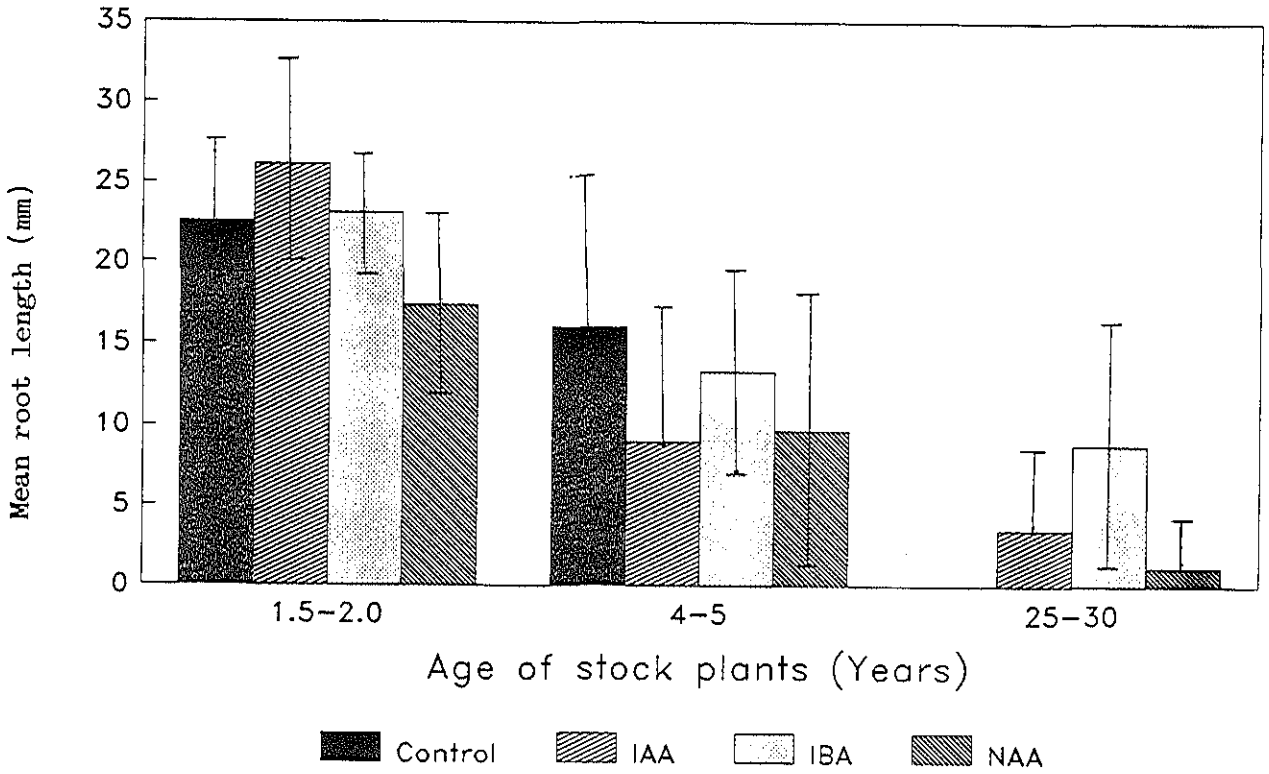


Fig. 8. Effect of age of stock plants and PGRs on mean length of roots per rooted cutting 4 months after treatment. Bars indicate ± SD.

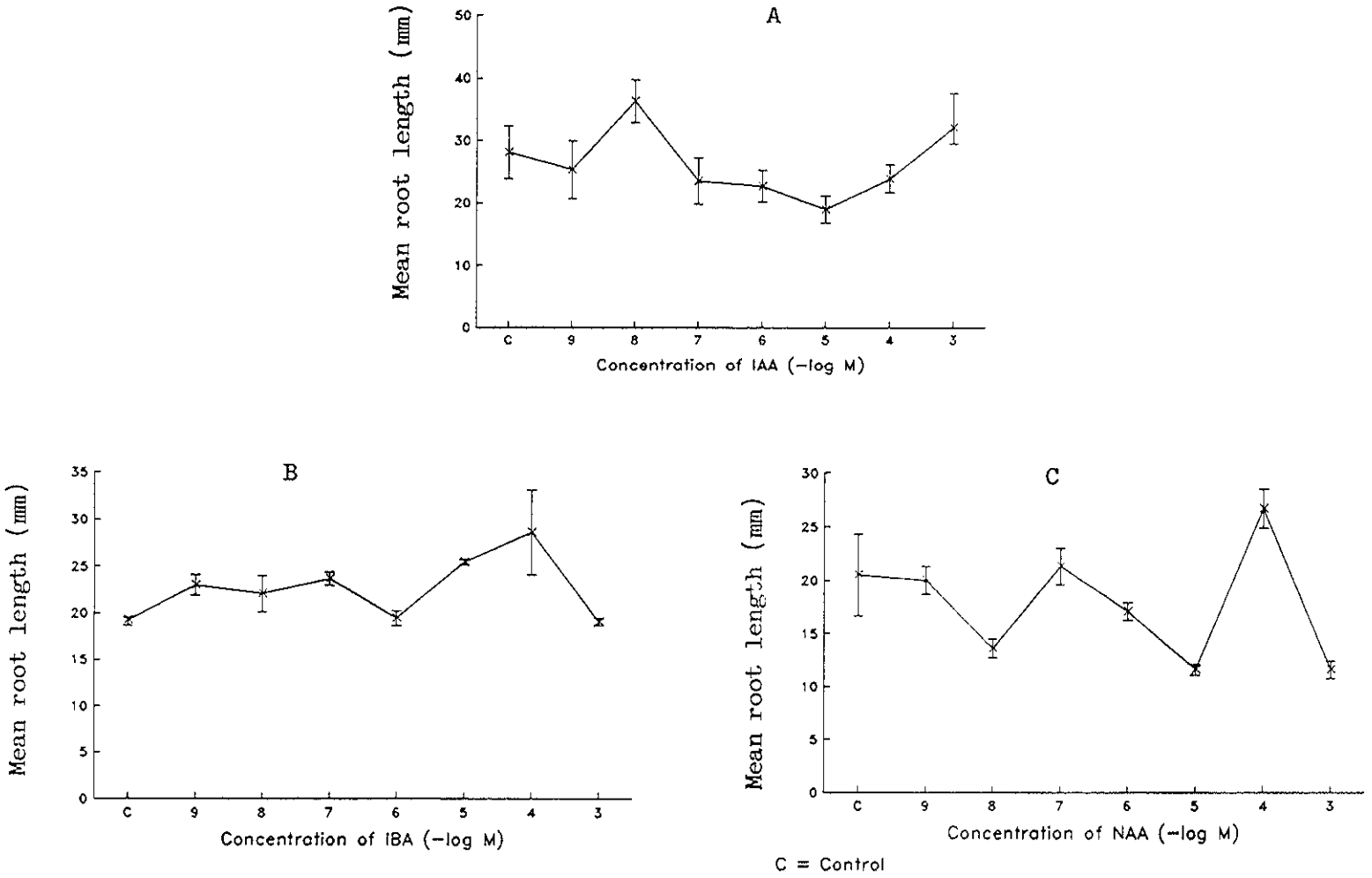


Fig. 9. Effect of various concentrations of PGRs on mean length of roots per rooted cutting collected from the youngest age class and 4 months after treatment. Bars indicate ± SD.

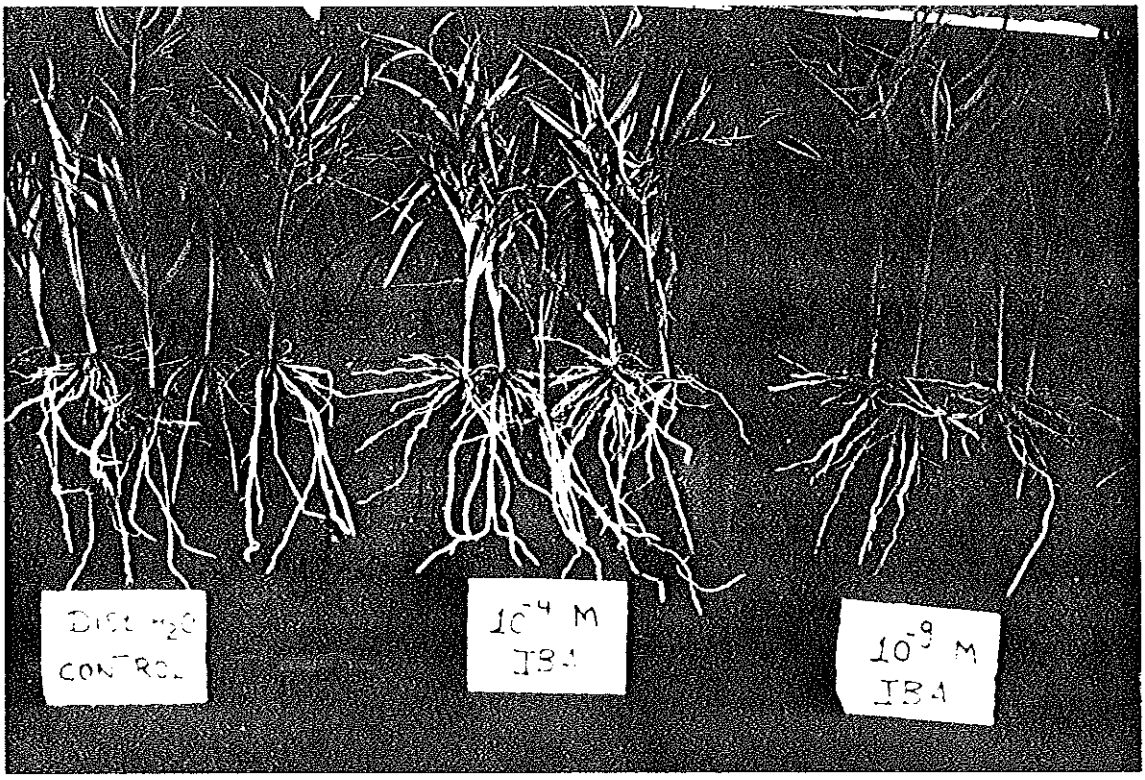
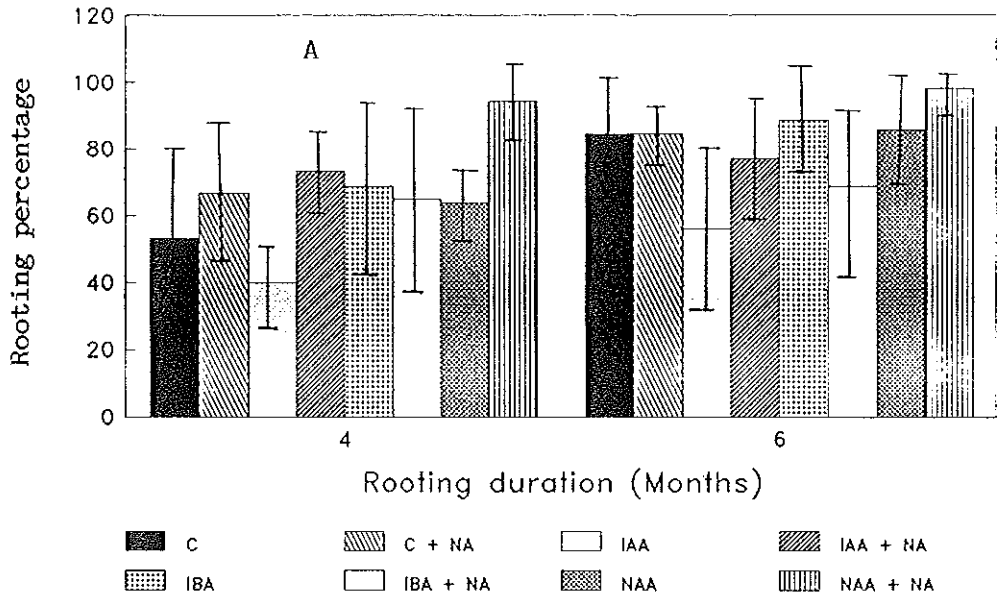


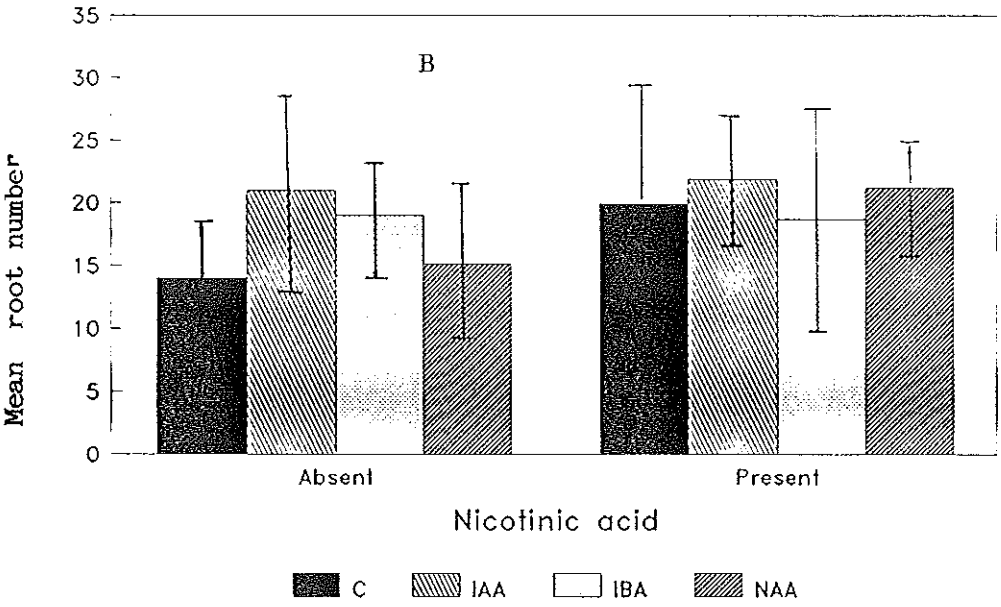
Fig. 10. Morphological appearance of adventitious roots 4 months after treatment with water and IBA.

4.4. Effect of nicotinic acid on rooting

Figure 11A indicates the percentage of rooted cuttings collected from the youngest age class and treated with PGRs either alone or in combination with 0.5 ppm NA. Significantly higher rooting percentage four months after treatment (94.3 ± 11.2 %) was obtained when cuttings were treated with a combination of NAA and NA than when similar cuttings were treated with IAA (40.0 ± 11.9 %) and NAA (63.8 ± 11.5 %) alone. By Extending the rooting duration to 6 months, 98.1 % of NAA plus NA and 85.7 % NAA treated cuttings were rooted. The rooting percentage obtained from NAA plus NA treated cuttings 6 months after treatment was significantly higher than those cuttings treated with IAA alone. Also, significantly higher rooting percentage (73.3 ± 10.3 %) four months after treatment was obtained when IAA was used in combination with NA than alone (40 ± 11.9 %). Six months after treatment, 56.2 % of IAA and 77.1 % of IAA plus NA treated cuttings were rooted. On the other hand, the use of NA in combination with IBA relatively decreased rooting percentage from 88.6 % when it was used alone to 68.6 % when it was combined with NA (Fig. 11A).

The mean number of roots differentiated per rooted cutting collected from the youngest age class and treated with PGRs either alone or in combination with nicotinic acid is given in Figure 11B. The mean number of roots differentiated per





C = Control

Fig. 11. Effect of PGRs either alone or in combination with nicotinic acid on cuttings collected from the youngest age class. A) Rooting percentage B) mean root number per rooted cutting.

Bars indicate ± SD.

rooted cutting after treatment with PGRs alone was not significantly different from those treated with a combination of PGRs and NA.

The morphological appearance of roots differentiated after treatment with a combination of NA and the various concentrations of IAA and NAA was more or less similar at most concentrations. However, the appearance of roots after treatment with a combination of IBA and NA relatively differ from those treated with a combination of NA and IAA or NAA (e.g. Fig. 12).

4.5. Growth of seedlings and stecklings

Time course for the growth of seedlings and stecklings is shown in Figure 13. Growth of stecklings was significantly higher than that of the seedlings. Growth in stecklings increased quite rapidly two months after transplantation as compared to that of the seedlings.

4.6. Histological origin of root primordia

The non-meristematic cells of the pith, phloem, and cortex tissues (Fig. 14) were dedifferentiated to meristematic state at least 15 days after the cuttings were inserted in the rooting medium. The root initials activated from the pith (Fig. 15), phloem and cortex (Fig. 16) gave rise to callus. Callus was formed after the dedifferentiation of cells. Root primordia were developed from the callus (Fig. 17).

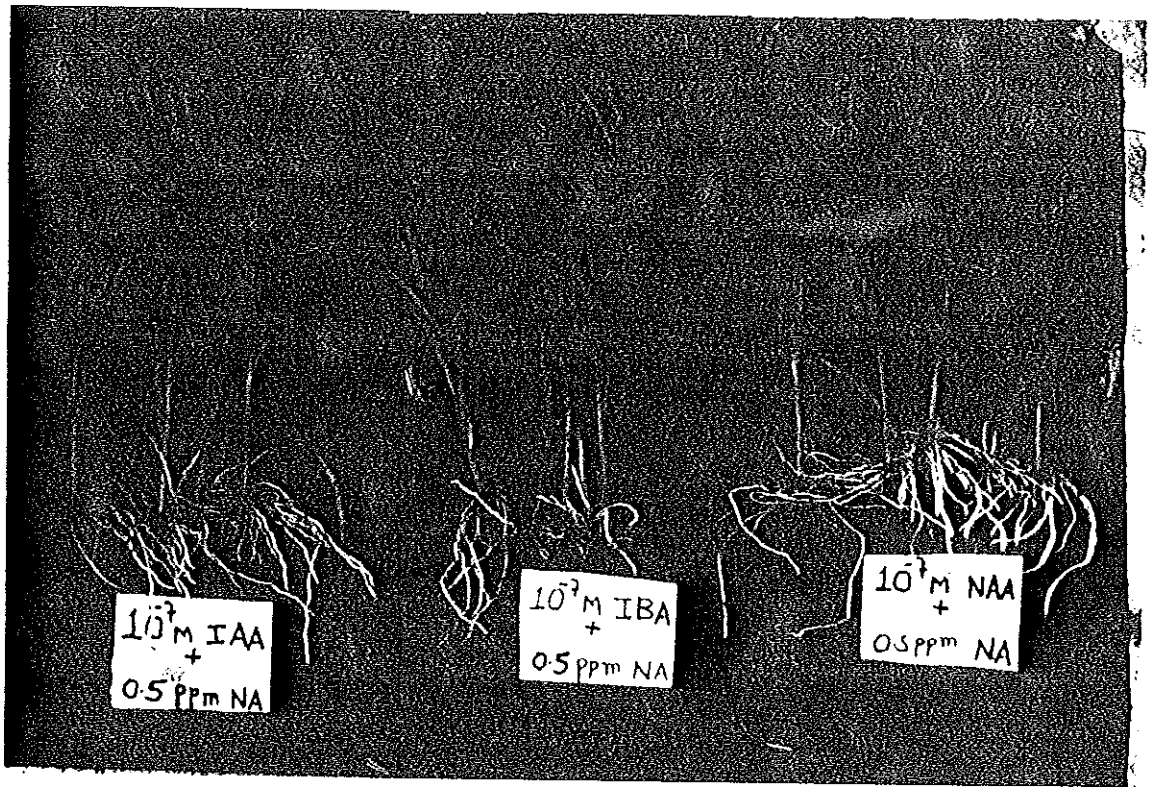


Fig. 12. Morphological appearance of adventitious roots 4 months after treatment with a combination of 0.5 ppm NA and 10^{-7} M PGRs.

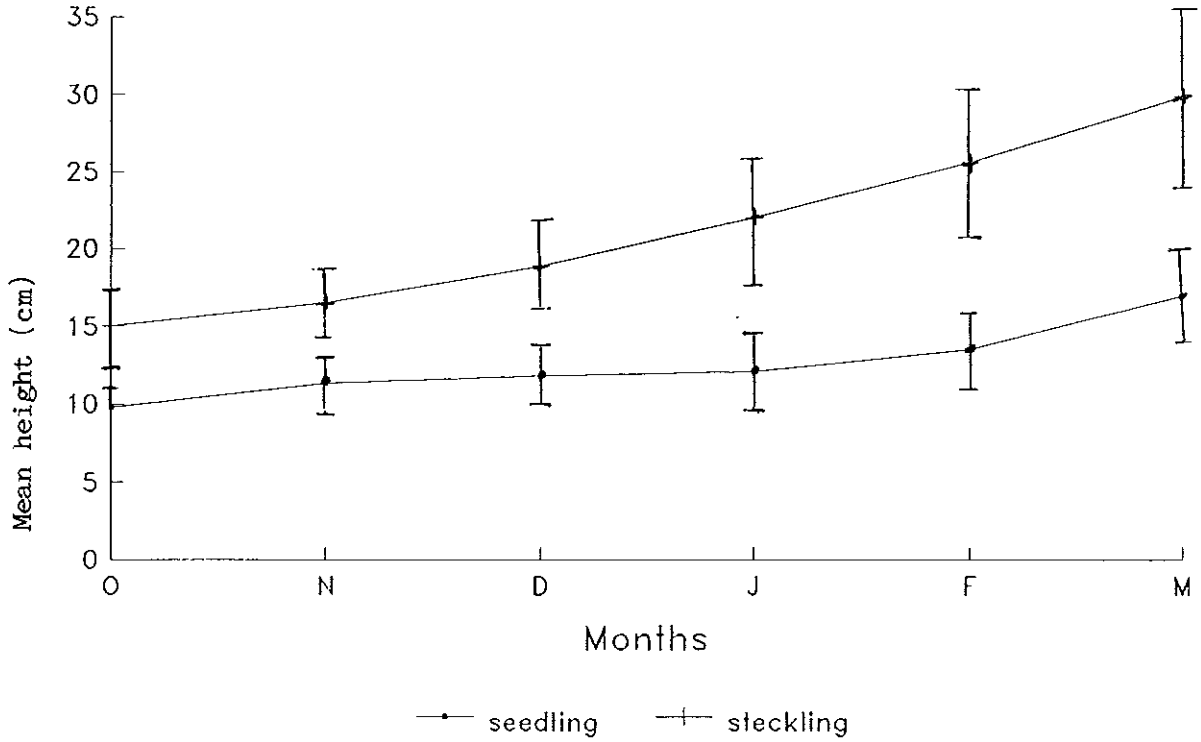


Fig. 13. Time course for the growth of seedlings and stecklings. Bars indicate ± SD.

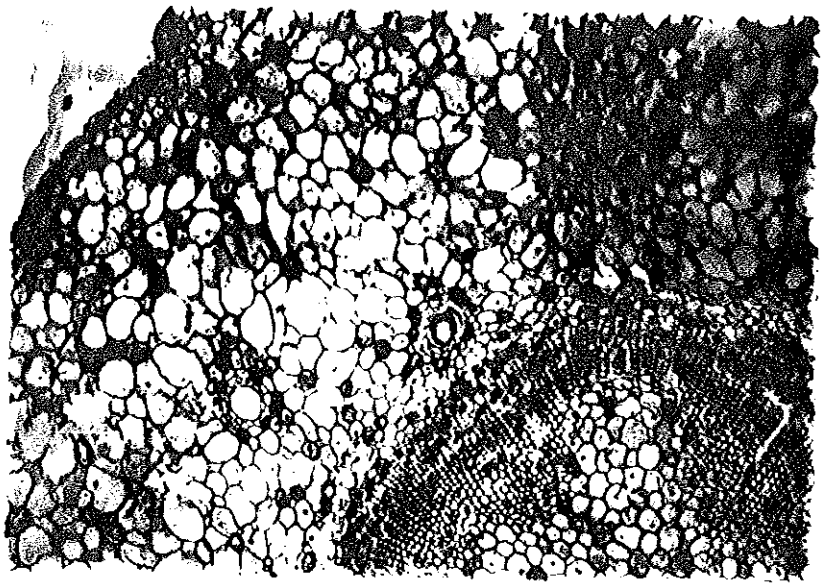


Fig. 14. Transverse section of base of branch cutting showing the structure of tissues before undergoing dedifferentiation.

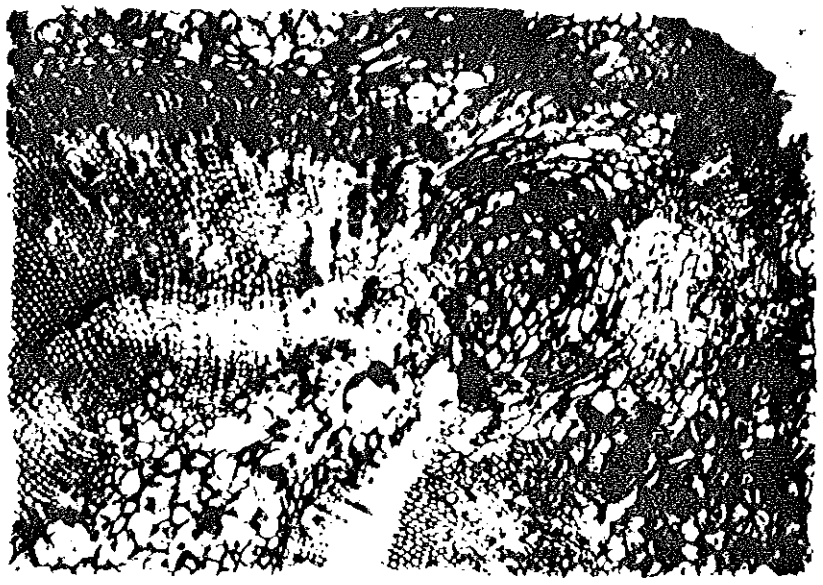


Fig. 15. Transverse section of base of branch cutting showing the dedifferentiation of meristematic cells from the pith.

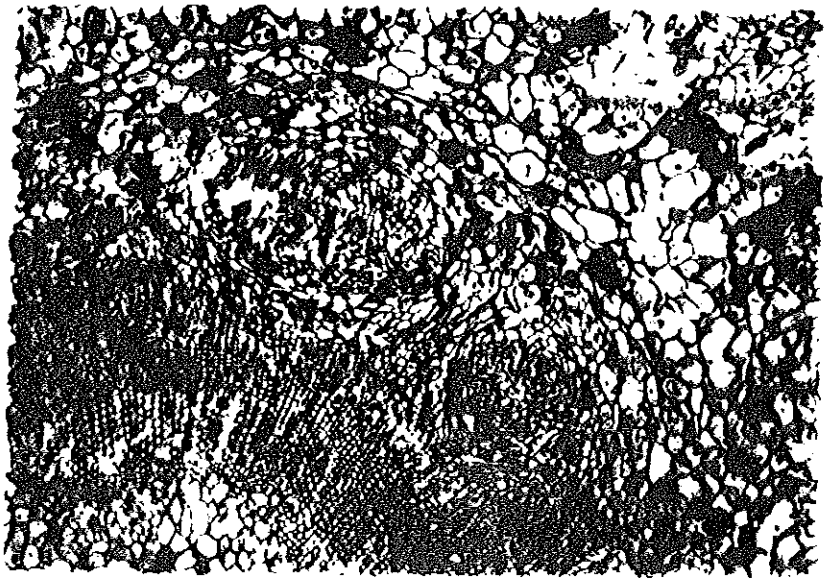


Fig. 16. Transverse section of base of branch cutting showing the dedifferentiation of meristematic cells from the phloem and cortex tissues.

Fig. 17. A) Transverse and B) Longitudinal sections of callus showing the development of root primordia.

5. D I S C U S S I O N

5.1. Effect of age of stock plants

The age of stock plants was found to be the most important factor determining the root forming capacity in *P. falcatus* branch cuttings. Significantly higher rooting percentage was obtained for cuttings collected from the youngest age class than from the intermediate and oldest age classes (Fig. 3). The rooting percentage obtained for cuttings collected from the youngest age class and six months after treatment was at least 2.6 and 5.6 times greater than those collected from the intermediate and oldest age classes, respectively. The mean number and length of roots differentiated per rooted cutting collected from the youngest age class were also significantly higher than that of the oldest age class (Fig. 3). Therefore, the rooting ability of cuttings significantly declined with increasing age of stock plants. Similar results were recorded by various workers (e.g. Grace, 1939; O'Rourke, 1952; Hartmann and Kester, 1975; Piskornik *et al.*, 1982; Kennedy and Selby, 1985; Hong *et al.*, 1986; Moon *et al.*, 1987; Williams, 1987). For example, the results obtained for the rooting ability of stika spruce cuttings collected from 2, 3, 5, 9, 20 and 32 years old trees indicated that cuttings collected from trees upto 5 years old rooted at high frequencies and produced well developed root systems whereas cuttings from older trees (9 years and above) rooted poorly

(Kennedy and Selby, 1985).

Root formation in *P. falcatus* branch cuttings was influenced more by the age of the stock plants than by the PGRs (Figs. 4, 6, and 8). For example, untreated cuttings (control) collected from the youngest age class gave 84.4 % rooted cuttings. Such cuttings differentiated on the average 14 roots which on the average measured 22.6 mm . On the other hand, only 37.8 and 2.2 % of the untreated cuttings collected from the intermediate and oldest age classes were rooted six months after treatment, respectively (Fig. 4). Also, cuttings collected from the oldest age class gave poorly developed root systems.

Kennedy and Selby (1985) have found that untreated cuttings of stika spruce collected from 3 years old trees were rooted at 100 % and formed on the average 11.6 roots per rooted cutting. On the other hand, cuttings collected from 20 and 30 years old trees rooted at approximately 50 % and produced poorly developed root systems. It has also been found by Hinesley and Blazich (1980) that exogenous application of PGRs did not affect rooting percentage but increased root number per rooted cutting in juvenile plants whereas it increased both rooting percentage and number of roots per rooted cutting in adult plant cuttings. Also, rooting in cuttings collected from juvenile stock plants was faster and more symmetrical than rooting in cuttings collected from old

trees (Hong *et al.*, 1986).

O'Rourke (1952) indicated that due to some internal physiological factors that take place in plants, cuttings from the younger plants root more easily than the same plants in a mature or senescent condition. The reason as to why the rooting potential of cuttings decreased with increasing age of stock plants may be due to: (1) the presence of rooting inhibitors in amounts high enough to mask the effect of root promoting substances (e.g. Mostaffa and Hartmann, 1967; Hartmann and Kester, 1975); (2) the decrease in the production of essential rooting co-factors (auxin synergists) (Girouard, 1969), and, (3) the decrease in the responsiveness of mature stem/branch tissues to the root promoting substances that have a capacity to change mature tissues from non-meristematic condition to a meristematic condition (Leopold, 1955).

5.2. Effect of plant growth regulators

Plant growth regulators were found to have no significant effect in increasing the rooting responses of cuttings collected from the youngest and intermediate age classes as compared to the control. However, significantly higher rooting percentage and mean root length were obtained when cuttings collected from the oldest age class were treated with IBA as compared to the control. Although there was no

significant difference among the rooting responses of the three PGRs, relatively better rooting responses were obtained when cuttings collected from the intermediate and oldest age classes were treated with IBA than IAA. Also, higher rooting percentage was obtained when cuttings collected from the youngest age class were treated with IBA than with IAA.

The effect of the various concentrations of the three PGRs on rooting percentage, mean root number and length did not follow the usual physiological response curve (supra-optimum, optimum and sub-optimum) and also the optimum concentrations were not consistent for the three rooting responses and PGRs (Figs. 5, 7 and 9). However, higher mean root number and length were recorded when cuttings collected from the youngest age class were treated with 10^{-4} and 10^{-3} M solutions. Such variations in the rooting responses may be due to the physiological differences of the cuttings which will have a direct impact on the endogenous concentration of the PGRs and rooting co-factors.

Comparative investigations on the effect of PGRs on rooting of various plant species were performed by several workers (e.g. Nickell, 1982; Gupta *et al.*, 1984; Thompson, 1986; Widiastoety and Soebijanto, 1988; Thimmappa *et al.*, 1990). Generally, IBA is very effective in increasing per cent rooting in cuttings than IAA (Hartmann and Kester, 1975;

Nickell, 1982; Gupta *et al.*, 1984). Good rooting of *Hibiscus rosasinensis* (Widiastoety and Soebijanto, 1988), *Epacris impressa* (Thompson, 1986) and *Pelargonium graveolens* cultivars (Thimmappa *et al.*, 1990) were obtained when cuttings were treated with IBA than with IAA or NAA. It is generally assumed that the greater ability of IBA over IAA to promote rooting of a large number of cuttings is due to: (1) its greater resistance to bacterial decomposition (Hartmann and Kester, 1975); (2) its resistance to light destruction or photo-inactivation (Yamakawa *et al.*, 1979); (3) its resistance to degradation by the enzyme IAA oxidase (Fawcett and Wightman, 1960); (4) its non-toxic effect over a wide concentration range (Nickell, 1982); (5) its better sources after conjugation reaction (Wiesman *et al.*, 1988); and (6) unlike IAA which induces the formation of rooting inhibitor, apart its stimulatory action, IBA does not induce the formation of rooting inhibitor (Eliasson, 1981) and also counteracts the inhibition caused by IAA (Eliasson and Areblad, 1984).

Although auxins (e.g. IAA, IBA, NAA) are important in the formation of adventitious roots in cuttings, the mechanism of action of auxins is not clearly understood. However, different possible mechanisms were suggested by various workers: (1) auxins determine the cells that will differentiate to root initials (Friedman *et al.*, 1979); (2)

auxins promote the subsequent development of predetermined root initial (Friedman *et al.*, 1979); (3) auxins enhance the transport of assimilates/carbohydrates to the site of root initiation (Altman and Wareing, 1975); (4) auxins stimulate cell division and they have an impact on the division of cambium leading to the formation of xylem and phloem vascular tissues (Wareing and Philips, 1970), and (5) auxins (e.g. IBA) favour the synthesis of proteins and nucleic acid necessary for the formation of root initials in cuttings (Ryugo and Breen, 1974).

5.3. Effect of nicotinic acid

The presence of 0.5 ppm nicotinic acid at the various concentrations of the PGRs showed a positive interaction with IAA and NAA on rooting percentage of cuttings collected from the youngest age class. It also decreased the rooting duration required for maximum rooting in IAA or NAA treated cuttings (Fig. 11A). For example, the rooting percentage obtained 6 months after IAA or NAA treatment was 56.2 and 85.7%, respectively. When nicotinic acid was added to IAA or NAA, 71.4% of the IAA treated cuttings and 94.3% of the NAA treated cuttings were rooted within 4 months after treatment. Therefore, the time required for root formation in IAA or NAA treated cuttings decreased by 33% in the presence of NA. This finding was in accordance with Basu and Roy (1970: cited in Hartmann and Kester, 1975). The authors found that the use

of 0.5 ppm NA in combination with NAA significantly promoted root formation in *Justicia* cuttings. Note, however, that the rooting percentage of cuttings treated with a combination of IBA and nicotinic acid decreased to some extent (Fig. 11A).

The mechanism for the positive or the negative effect of NA on rooting has so far remained unclear. Some workers (e.g. Euler, 1936 : cited in Miller, 1973) found that nicotinic acid is a constituent of two coenzymes, nicotinamide-adenine dinucleotide (NAD) and nicotinamide-adenine dinucleotide phosphate (NADP). NAD and NADP are involved in carbohydrate metabolism. Robinson (1973) further found that NAD is involved in the dehydrogenation of B-hydroxybutyric acid to acetoacetic acid. Therefore, the probable reason for the decrease in rooting response of cuttings treated with a combination of IBA and nicotinic acid may be due to the involvement of nicotinic acid in the conversion of the side chain of indole-3-butyric acid (butyric acid group of IBA) to acetoacetic acid or other compounds.

5.4. Growth of seedlings and stecklings

The growth of stecklings after the "adaptation" period of two months was much higher than the growth of seedlings. The reasons for the faster height increment of stecklings over seedlings may be: (1) unlike the seedlings that produced only one main root at the time of transplantation, stecklings have

a large number of adventitious roots (well developed root system) for the absorption of water as well as for the exploitation of the mineral nutrients of the soil, (2) stecklings contain larger number of expanded phyllodes which are important for maximum light gathering and hence for photosynthesis as compared to the seedlings which possessed smaller and fewer phyllodes, and (3) the mean height of the stecklings at the time of the first measurement was 34.7 % higher than that of the seedlings. Hence, this initial difference between the stecklings and seedlings may account for the faster growth of the stecklings than the seedlings and this demonstrates the importance of propagation by cutting than by seeds.

5.5. Histological origin of root primordia

Callus in *P. falcatus* branch cuttings was formed after the dedifferentiation of cells from the pith, phloem and cortex tissues. Root primordia were observed after callus formation. This finding was in agreement with Cameron and Thomson (1969), Haissig (1974a,b) and Esau (1977). Cameron and Thomson (1969) found that although callus meristems in *Pinus radiata* stem cuttings were formed mainly from the cortex and pith, cambium, phloem and xylem parenchyma can also be involved in the formation of callus meristems. Root initials of *P. radiata* were formed in or near active callus meristems and gave rise to root primordia which rapidly developed to

roots. Satoo (1956: cited in Haissig, 1974a) found out that in most species of conifer which are difficult to propagate through cuttings, root primordia originate from calli. The author stated that in coniferous cuttings basal calli developed initially from cambial and phloem cells, and latter enlarged by division of pith and cortical cells. Xylem elements then differentiated within the callus in connection with stem xylem. Phloem cells initiated root primordia before cambium developed within the callus. A somewhat different pattern of callus development and root initiation in conifer, slash pine, cuttings was described by Mergen and Simpson (1964). According to these authors, callus originated from pith and enlarged by division of stem cambium and phloem. Cambium produced within the callus cut off xylem cells centripetally, and root primordia were initiated within centrifugally developed phloem cells.

6. CONCLUSIONS

1. The root forming ability of branch cuttings of *P. falcatus* significantly decreased with increasing age of stock plants. Better results were obtained in cuttings collected from the youngest age class than from the intermediate and oldest age classes.

2. Plant growth regulators were found to have no significant effect on rooting percentage, mean number and length of roots differentiated per rooted cutting collected from the youngest and intermediate age classes but they are important for cuttings collected from the oldest age class.

3. When plant growth regulators such as IAA and NAA were used for root induction of cuttings collected from the youngest age class, better results were obtained at a shorter rooting duration when they were used in combination with nicotinic acid than when they were used alone.

4. Growth in stecklings was significantly faster than that of the seedlings.

7. RECOMMENDATIONS FOR FURTHER INVESTIGATIONS

1. The growth habit of the stecklings, whether it is orthotropic or plagiotropic, needs a continuous follow up before this method is appropriately considered for the propagation of the tree.

2. Investigations on the effect of the position of cuttings on the stock plants (terminal, intermediate or basal) should be carried out.

3. Determination of the appropriate length of the cuttings may also be important either to get better rooting or to get a readily field plantable stecklings at the end of the rooting period.

4. Selection of best rooting media may also be important to get best rooting results.

8. L I T E R A T U R E S C I T E D

Altman, A. and Wareing, P. F. (1975). The effects of IAA on sugar accumulation and basipetal transport of ^{14}C-labelled assimilates in relation to root formation in *Phaseolus vulgaris* cuttings. *Physiol. Plantarum* 33:32-38.

Argles, G.K. (1959). Root formation and development in stem cuttings : A re-examination of certain fundamental aspects. Ann. Appl.Biol. 47:626-627.

Bandzaitene, Z. Y. (1981). Biological and Biochemical Characterization of Cowberry : 9. Effects of growth stimulators on rooting of cuttings and growth of shoots. *LIET TSR MOKSLU AKAD DARB SER C BIOL MOKSLAI* 0: 5-15.

Bissaria, A. K. (1988). Influence of indole-3-butyric acid, environmental factors and posture on the regeneration of stem cuttings and leaves of *Hibiscus cannabinus*. *Adv.in Plant Sci.* 1:54-66.

_____ and Rao, P. V.(1988). Influence of indole-3-butyric acid and environmental factors on the regeneration of stem cuttings of ramie, *Boehmeria nivea* Gaurd. *Trop. Agri.* 65: 67-72.

Bonga, J. M. and Durzan, D. J. (1987). *Cell and tissue culture in forestry*. Volume 3. Martinus Nishoff Publishers, Boston, pp. 5.

Cameron, R. J. and Thomson, G. V. (1969). The vegetative propagation of *Pinus radiata*: Root initiation in cuttings. *Bot. Gaz.* 130: 242-251.

Cid, L. B., Fialho, J. D. F. and Neves, M. A. C. (1982). Influence of concentration of a mixture of 3-IAA and Boron, and that of stem carbohydrates and nitrogen contents on rooting of *Pueraria phaseoloides* cuttings. *PESQUI AGROPECU BRAS* 16: 623-626.

Dale, I. R. and Greenway, P. J. (1961). *Kenya trees and Shrubs*. Buchanan's Kenya Estates Limited, Nairobi, pp. 5-6.

Davidson, J. (1988). *Preparatory assistance to research for afforestation and conservation*. DP/ETH/85/012, Field Document 1, Volume 1, Main report. 184 pp.

Davis, T. D., Goerge, S. W., Upadhyaya, A. and Parson, J. (1991). Propagation of firebush (*Hamelia patens*) by stem cuttings. *J. Environmental Hortic.* 9: 57-61

Economou, A. S. and Paul, E. R. (1986). Influence of pH and medium composition on rooting of hardy deciduous azalea [*Rhododendron* sp.] microcuttings. *J. Am. Soc. Hortic. Sci.* 111: 181-184.

Eliasson, L. (1980). Interactions of light and auxin in regulation of rooting in Pea stem cuttings. *Physiol. Plantarum* 48: 78-82.

_____(1981). Factors affecting the inhibitory effect of indolylacetic acid on root formation in pea cuttings.

Physiol. Plantarum 51: 23-26.

_____and Areblad, K. (1984). Auxin effects on rooting in pea cuttings. *Physiol. Plantarum* 61: 293-297.

Esau, K. (1977). *Anatomy of seed plants*. 2nd ed. John Wiley and Sons, New York, pp. 251-255.

FAO (1984). Vegetation and natural regions and their significance for land use planning. ETH/78/003. Technical Report 4. 75 pp.

Fawcett, H. and Wightman, F. (1960). The metabolism of 3-indolylalkanecarboxylic acids, and their amides, nitriles and methyl esters in plant tissues. *Proc. R. Soc.* London (ser. B) 152: 231-255.

Friedman, R., Altman, A. and Zamski, E. (1979). Adventitious root formation in hypocotyl cuttings in relation to IAA translocation and hypocotyl anatomy. *J. Exp. Bot.* 30: 769-777.

Friis, I. (1992). *Forest and forest trees of Northeast Tropical Africa*. Kew Bulletin Additional Series XV, Royal Botanical Gardens, Kew, London, pp. 90-91.

Grace, N. H. (1939). Vegetative propagation of conifers I. Rooting of cuttings taken from the upper and lower regions of Norway Spruce tree. *Can. J. Res.* 17: 178-180.

Girouard, R. M. (1969). Physiological and Biochemical studies of adventitious root formation. Extractable rooting co-factors from *Hedera helix*. *Can. J. Bot.*

47: 687-699.

Gislerod, H. R. (1984). Physical conditions of propagation media and their influence on rooting of cuttings: 3. The effects of air content and temperature in different propagation media on the rooting of cuttings. *Plant Soil* 75:1-4.

Gorter, C. J. (1962). Further experiments on auxin-synergists. *Physiol. Plantarum* 15: 88-95.

_____(1969). Auxin-synergists in the rooting of cuttings. *Physiol. Plantarum* 22: 497-502.

Gupta, S., Kumar, A. and Sobti, S. N. (1984). A comparative study on the effects of auxin, IAA, IBA and NAA, on rooting of *Tylophora indica* (Burm. F.) Mor stem cuttings. *Indian Drugs* 22: 118-120.

Haissig, B. E. (1972). Meristematic activity during adventitious root primordium development. Influences of endogenous auxin and applied gibberellic acid. *Plant physio*. 49: 886-892.

_____(1974a). Origins of adventitious roots. *New Zealand J. of Forestry Sci*. 4: 299-310.

_____(1974b). Influences of auxins and auxin synergists on adventitious root primordium initiation and development. *New Zealand J. of Forestry Sci*. 4: 311-323.

Hansen, O. B. (1989). Propagating apple rootstocks by semi-hardwood cuttings. *Norwegian J. of Agri.Sci*.

3: 351-365.

Hartmann, H. T. and Kester, D. E. (1975). *Plant Propagation: Principles and Practices*. Prentice-Hall, Englewood Cliffs, New Jersey, pp. 211-270.

Hess, C. E. (1965). Phenolic compounds as stimulators of root initiation. *Plant Physiol.*(Supplement) 40: XLV

Hinesley, L. E. and Blazich, F. A. (1980). Vegetative propagation of *Abies fraseri* by stem cuttings. *Hortic. Sci.* 15: 96-97.

Hong,S. H., Park, H. S., Hwang, M. S. and Kim, C. S. (1986). Rooting of juvenile cuttings of *Picea koraiensis*. *Res. Rep. Inst. For. Genet.* 0:53-58.

Hosoi, T. and Ooishi, A. (1987). The involvement of current photosynthesis in rooting of hardwood cuttings of cultivar Delaware [grape] vines. *J. Jpn. Soc. Hortic. Sci.* 55: 429-433.

Ivanova, Z. (1981). Rapid vegetative propagation of conifers. *Sci. Hortic.* (AMST) 14: 347-356.

James, D. (1983). Adventitious root formation *in vitro* in apple rootstocks (*Malus pumila*) I. Factors affecting the length of the auxin sensitive phase in M 9. *Physiol. Plantarum* 57: 149-153.

Jensen, W. A. (1962). *Botanical Histochemistry*. W. H. Freeman and Company, pp. 55-99.

Kaundal, G. S. and Bindra, A. S. (1986). Influence of

various growth regulators on the adventitious rooting of almond x peach hybrib. *J. Res. Punjab. Agric. Univ.* 22: 53-56.

Kawase, M. (1965). Etiolation and rooting in cuttings. *Physiol. Plantarum* 18: 1066-1076.

Kennedy, S. J. and Selby, C. (1985). Propagation of Stika Spruce by stem cuttings. *Rec. Agric. Res.* (Belfast) 32: 61-70.

Klahr, M. D. and Still, S. M. (1980). Effects of IBA and sampling dates on the rooting of 4 *Tilia* taxa. *Sci. Hortic.* 11: 391-398.

Krul, W. R. (1968). Increased root initiation in Pinto bean hypocotyls with 2,4-dinitrophenol. Plant Physiol. 43: 439-441.

Kukuchi, H., Ogata, R. and Hori, Y. (1983). Rooting ability of willow cuttings. *J. Jpn. Soc. Hortic. Sci.* 51: 435-442.

Kwon, O. W., Song, W. S., Park, H. S. and Park, Y. K. (1987). Study on vegetative propagation by juvenile cuttings in four economic tree species. *Res. Rep. Inst. For. Genet.* 0: 30-33.

Leakey, R. R. B. and Coutts, M. P. (1989). The dynamics of rooting in *Triplochiton scleroxylon* cuttings: their relation to leaf area, node position, dry weight accumulation, leaf water potential and carbohydrate composition. *Tree Physiol.* 5: 135-146.

Legesse Negash (1992). *In vitro* methods for the rapid germination of seeds of *Podocarpus falcatus* (Thunb.) Mirb. SINET: *Ethiop. J. Sci.* 15: (In press).

Leopold, A. C. (1955). *Auxins and plant growth*. University of California press, Berkeley and Los Angles, pp. 202-214.

Malik, M. N. and Harnard, H. E. (1984). Rooting of sour orange cuttings with IBA on sand and peat moss. *Pak. J. Agric. Res.* 4: 174-179.

Mergen, F. and Simpson, B. A. (1964). Asexual propagation of *Pinus* by rooting needle fascicles. *Silvae Genet.* 13: 133-139.

Miller, L. P. (1973). *Phytochemistry*. Van Nostrand Reinhold Company, New York, 3: 195-220.

Miller, N. F., Hinesley, L. E. and Blazich, F. A. (1982). Propagation of Fraser fir by stem cuttings: Effects of type of cutting, length of cutting, and genotype. *Hortic. Sci.* 17: 827-829.

Moon, H. K., Park, Y. H., Lee, K. Y. and Kim, W. W. (1987). Rooted cuttings of *Quercus acutissima* by rooting substances and rooting media. *Res. Rep. Inst. For. Genet.* 0: 38-46.

Mostaffa, F. S. and Hartmann, H. T. (1967). Isolation, purification and characterization of an endogenous root promoting factor obtained from basal sections of pear hardwood cuttings. *Plant Physiol.* 42: 541-548.

Nanda, K. K. and Anand, V. K. (1970). Seasonal changes in

auxin effects on rooting of stem cuttings of *Populus nigra* and its relationship with mobilization of starch. *Physiol. Plantarum* 23:99-107.

Nickell, L. G. (1982). *Plant growth regulators: Agricultural uses*. Springler Verlag. New York, pp. 4-5.

Nordstrom, A. C. and Eliasson, L. (1984). Regulation of root formation by auxin-ethylene interaction in pea stem cuttings. Physiol. Plantarum 61: 298-302.

Ooishi, A., Machida, H., Hosoi, T. and Shiobara, Y. (1981). Studies on photosynthesis in cuttings during propagation: 3. Effects of darkness and disbudding treatments to rooting of hardwood cultivar Delaware grape vines. *J. Jpn. Soc. Hortic. Sci.* 49: 326-330.

Ooishi, A., Machida, H., Hosoi, T. and Shiobara, Y. (1982). Role of light in rooting of softwood cuttings of *Euonymus japonicus*. *J. Jpn. Soc. Hortic. Sci.* 50: 511-515.

O'Rourke, F. L. (1949). Mist humidification and the rooting of cuttings. *Mich. Agr. Exp. Sta. Quart. Bul.* 32: 245-249.

_____ (1952). The effects of juvenility on plant propagation. *Nat. Hort. Mag.* 31: 278-282.

Pearse, H. L. (1943). The effects of nutrition and phytohormones on the rooting of vine cuttings. *Ann. Bot.* 7:123-132.

Piskornik, Z., Piskornik, M. and Goc, F. (1982). The

Standardisation of propagation of scented from stem cuttings. *Indian Perfumer* 34: 56-60.

Thompson, W. K. (1986). Effects of origin, time of collection and planting media on rooting of cuttings of *Epacris impressa* Labill. Sci. Hortic. (AMST) 30: 127-134.

Wally, Y. A., EL-Hamady, M. M., Boulos, S. T. and Abu-Amara, N. M. (1981). Rooting experiments on guava using hardwood stem cuttings. *Egypt J. Hortic.* 8: 77-88.

Wareing, P. E. and Phillips, I. D. J. (1970). *The control of growth and differentiation in plants.* Pergamon press, Newyork, 303 pp.

Widiastoety, D. and Soebijanto,(1988). Rooting of stem cuttings of *Hibiscus rosa-sinensis*. *Buletin-Penelitian-Hortikultura* 16: 79-83.

Wiesman, Z., Riov, J. and Epstein, E. (1988). Comparisons of movement and metabolism of IAA and IBA in mung bean cuttings. *Physiol. Plantarum* 74: 556-560.

Williams, F. (1987). Propagation of mature western white pine (*Pinus monticola* Dougl.) by cuttings. *Can. J. For. Res.* 17: 349-352.

World Conservation Monitoring Centre (WCMC) (1991). *Biodiversity guide to Ethiopia*. Cambridge, UK. pp. 20

Yamakawa, T., Kurahashi, O., Ishida, K., Kato, S., Kodama. T. and Minoda, Y. (1979). Stability of indole-3-acetic acid to autoclaving, aeration and light illumination. *Agric. Biol. Chem.* 43: 879-880.