ASEXUAL PROPAGATION OF *JUNIPERUS PROCERA* HOCHST. EX ENDL. THROUGH ROOTING OF BRANCH CUTTINGS

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ABSTRACT

The rooting responses of branch cuttings of Juniperus procera *Hochst. ex Endl., obtained from juvenile and mature source plants to four plant growth regulators (PGRs), namely, indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthaleneacetic acid (NAA), and 2,4-dichlorophenoxyacetic acid (2,4-D) at various concentrations (10^{-3} to 10^{-9} M) were examined in sand culture. Assessments on survival, callusing and rooting of the cuttings were conducted 16 and 32 weeks after the treatments. Origin of root primordia was examined microscopically, and establishment and performance of stecklings were done on sample rooted cuttings. It was found that the developmental stage of the source plants from which the cuttings were derived, and the type, as well as the concentration of the PGR, markedly affected the survival of the cuttings. About 77% of the cuttings obtained from Class III source plants (mature) died out within the first 30 days after treatment; while 34% of the cuttings obtained from Class II (mature), and 90% of the cuttings obtained from Class I source plants (juvenile) survived until the end of the investigation. Callusing and rooting of the cuttings were greatly affected by the developmental stages of the source plants. Sixteen weeks after treatment, mean percentage callusing of cuttings obtained from Class I source plants was significantly greater than that obtained from Class II source plants ($p \leq 0.05$). The highest attainable percentage of callused cuttings obtained from Class I source plants was 56, treated at 10^{-4} M IAA and 10^{-5} M NAA. The corresponding percentage for the cuttings obtained from Class II source plants was 29, treated at 10^{-4} M IBA. At this time, only 2% of the cuttings obtained from Class I source plants were rooted. Thirty two weeks after treatment, 24% of the cuttings obtained from Class I source plants were rooted. By this time, only a single cutting from Class II source plants rooted. The highest attainable percentages of rooted cuttings obtained from Class I source plants was 60 treated at 10^{-7} M IAA. The corresponding percentages were 50, 44 and 25 for NAA (treated at 10^{-3} M), IBA (treated at 10^{-5} M) and 2,4-D (treated at 10^{-6} and 10^{-7} M), respectively. The control resulted in 29% rooting. The mean maximum attainable root numbers per rooted cuttings in IAA (treated at 10^{-6} M), IBA (treated at 10^{-3} M), 2,4-D (treated at 10^{-9} M) and NAA (treated at 10^{-5} M) were 17.0 $\pm$ 4.1, 14.5 $\pm$ 12.5, 9.7 $\pm$ 6.7 and 7.0 $\pm$ 3.0, respectively. The mean root number per rooted cuttings in the control was 17.0 $\pm$ 1.7. The highest attainable mean root length was 372 $\pm$ 51.5 mm, for cuttings treated at 10^{-3} M NAA. The corresponding values for IBA (treated at 10^{-3} M), IAA (treated at 10^{-6} M) and 2,4-D (treated at 10^{-4} M) were 113.7 $\pm$ 11.7 mm, 90.7 $\pm$ 5.6 mm and 67.7 $\pm$ 8.8 mm, respectively. The mean root length of the cuttings rooted in the control was 93.9 $\pm$ 7.3 mm. Regression analysis showed that the contribution of callusing to rooting is very small ($R^2 = 0.2070$, $p \leq 0.05$). In this species, cells of callus tissue, xylem rays, tracheids, cortex and cambium resulted in the formation root primordia. Stecklings with well developed root systems were easily established and grew well, indicating the possibility of propagating the species by asexual means.*

1 INTRODUCTION

The climatic climax vegetation of Ethiopia include afroalpine, subafroalpine, forest, woodland and savannah, steppe, and, semidesert. Forest and woodland and savannah regions comprise about 54.3% of the total land mass of the country (FAO, 1984a).

Friis (1992) categorized the floristic types of natural forests of Ethiopia as: (1) lowland dry peripheral semi-deciduous Guineo-Congolian forest, (2) transitional rainforest, (3) Afromontane forest, (4) undifferentiated Afromontane forest, (5) dry single-dominant Afromontane forest (of Ethiopian highlands), (6) dry single-dominant Afromontane forest of East African evergreen and semi-evergreen bushland, and, (7) riverine forest.

Juniperus procera Hochst. ex Endl. is an important component of undifferentiated Afromontane and dry single-dominant Afromontane forests. The tree is also an important component of forests that are transitional between dry single-dominant Afromontane forest and semi-evergreen bushland and thicket. Moreover, it is also common in church compounds and small forest patches that surround churches (Friis, 1992).

Undifferentiated Afromontane forests are either *Juniperus-Podocarpus* or predominantly *Podocarpus* forests both with an element of broad-leaved species. They occur both in the NW and SE highlands, especially on the plateaux of Shoa, Wollo, Sidamo, Bale and Hararge at altitudes between 1500-ca 2700 m with average annual rainfall between 700-1100 mm with most of the rain falling in July. The forest to the east of the Rift Valley is often dominated by *Podocarpus falcatus* (Thunb.) Mirb. (at least below about 2200-2500 m). Similarly, dry single-dominant Afromontane forest

occurs in the NW and SE highlands but at higher altitudes or drier sites (especially in the Tigrean and Harar plateaux); at altitudes from (1600)-ca 3200 (3300) m, with annual average temperature ranging from 12-18^0 C, and annual rainfall between 500-1500 mm. The typical dominant species in the upper storey of this forest is *J. procera* and *Olea europaea* subsp *cuspidata* (Wall. ex DC.) Ciffieri and a number of other species in the lower storey (Friis, 1992). According to one study made by Uibrig and Abdu Abdukadir (1990) on Hubota mountain (in Alemaya catchment), as altitude increases from 2200 to 2775 m, the dominance of *J. procera* increases. Similarly, Hillier (1977) reported that the species is widely distributed in the mountains of East Africa.

J. procera and *P. falcatus* are important timber forests of Ethiopia (Westphal, 1974). *Juniperus* and *Podocarpus* forests represent the coniferous forests of Ethiopia. The potential area coverage of these forests amounted to about 170,550 km^2 or 14.1% of the total land mass (FAO, 1984a). About 76,000 km^2 of *J. procera* woodland may be added to the potential coniferous forest area (Murkland and Odenyo, 1974: quoted in Uibrig and Abdu Abdukadir, 1990). At the beginning of this decade it was reported that only about 1,600 km^2 is estimated to be stocked by coniferous forests (Uibrig and Abdu Abdukadir, 1990).

J. procera, commercially known as the African pencil cedar, belongs to the family Cupressaceae. It is evergreen dioecious timber tree with a dominant height of 45 m at ages between 100-150 years (Pohjonen and Pukkala, 1992). The species attains its maximum development at altitudes between 1830 and 2895 m (Hillier, 1977). *J. procera* has two distinct developmental stages, the juvenile and adult stages. Foliage

characteristics vary depending on the developmental stage of the plant. In the juvenile stage, leaves are prickly and needle-like while in the adult stage they are grey, glaucous, paired, scale-like, triangular, sharp pointed with conspicuous resin glands on the back. Moreover, the juvenile stage is characterized by a fairly straight trunk and pyramidal crown, while the adult stage is distinguished by a still straight trunk but a high, irregular and spreading crown. Its bark is pale-brown, thin, fibrous, cracking and peeling in long narrow strips (Dale and Greenway, 1961). The male cones of the species are small, solitary, rounded, terminal and are yellowish in colour. They occur in short branchlets. The female cones are berry-like, rounded, and, upon ripening become fleshy and soft. They produce 1 to 4 seeds (Legesse Negash, 1995).

The soft wood is characterized by tracheids which provide a system of tubes to conduct water from the root and to act as supporting skeleton that gives the wood its rigidity (Feininger, 1968; Jane, 1970). It is composed of an outer sapwood and an inner heartwood. The sapwood is white, and is limited to a narrow band in specimens that are mature. But in poles under 45 cm diameter, the sapwood may comprise nearly half the diameter, thus representing three-quarters of the timber volume (Dale and Greenway, 1961). It contains live cells (Feininger, 1968). The heartwood varies from pale yellow-brown to deep purple-red, which turns to more uniform reddish-brown on seasoning (Dale and Greenway, 1961). It is infiltrated by resin and contains dead cells (Feininger, 1968). The heartwood has distinctive scent of pencil cedars. The heartwood of cedars and junipers remains sound for many years. Practically all cedars and junipers are durable (Boyce, 1938). The heartwood of *J. procera* is not attacked by mold fungi, and is extremely resistant to termite attack (Dale and Greenway, 1961). Resin is of double factor in durability, being toxic to wood destroying fungi and has some value as a water-proofing substance (Boyce, 1938). Unfortunately, old trees are

often piped out by the heart rot fungus *Fomes juniperinus* (v. Schr.) Sacc. and Syd.. The sapwood on the other hand is perishable in the ground and is rapidly destroyed by termites (Dale and Greenway, 1961). The wood is light to medium in weight (32 to 38 lb ft^{-3} of air dry). It is straight grained, with growth zones of plainly marked. In seasoning, the wood is liable to surface checks and end splitting. It works easily, but rather brittle at the edge and splits in nailing. It takes good polish (Dale and Greenway, 1961).

The members of the genus *Juniperus* L. occupy a continuous broad belt round the northern hemisphere, with some species extending southwards. The distribution of *J. procera* begins in Arabia and extends southwards through the Sudan, Ethiopia, Djibouti, Somalia, Kenya, Uganda, Tanzania, Zaire, Malawi and Zimbabwe (Lind and Morrison, 1974; Friis, 1992).

The tree, which is the largest juniper in the world, produces timber of economic importance. The wood is used for great many purposes such as for manufacture of lead pencil and pen-holders, for construction and lining of buildings, for joinery, for strips and parquet flooring, for all sort of outdoor works such as roofing-shingles, fence-posts, plant-trays, water-flumes and telegraph poles (Dallimore and Jackson, 1954; Dale and Greenway, 1961; Lind and Morrison, 1974; Thirakul, undated). In Ethiopia, timber from *J. procera* has been highly valued for construction of large buildings such as churches (Pohjonen an Pukkala, 1992). The species can also be a source of cedarwood oil, an important product for fragrance compounding, used to scent soaps, room sprays, disinfectants, technical preparations and similar products as clearing agents for microscopic sections and for oil immersion lenses (Guenther, 1952: quoted in Adams, 1987).

Terrestrial ecosystems in Ethiopia are facing pressure of various dimensions (Abebe Demissie, 1990) because there is a competition for various types of land use. These include cultivation and construction purposes accompanied by exploitation of forests for fuel and timber, which is intensified due to increased population growth (Abebe Demissie, 1990; Bekele Tegene and Tesfaye Bekele, 1990; Becker and Abraham Desta, 1991). Consequently, *Juniperus* forests have been substantially depleted and hence only patches of relic forests remain today (FAO, 1984b), where mature trees of *J. procera* are now very rare (Legesse Negash, 1995). Uibrig and Abdu Abdukadir (1990) reported the natural regeneration of the species as low and proposed that in such cases supplemental enrichment planting is necessary.

Reforestation strategy using this indigenous conifer instead of the exotic *Cupressus lusitanica* Mill. and *Eucalyptus globulus* Labill. would be beneficial, because: (1) it is a way to diversify species selection; (2) it is also a means of supporting durable timber for high local and national preference (Pohjonen and Pukkala, 1992); and, (3) it may increase soil fertility for growth of understorey herbaceous and shrub species (Michelsen *et al.*, 1993).

Although Legesse Negash (1995) has described procedures for the propagation of the species through seeds, alternative method(s) for rapid propagation of the tree is(are) lacking.

The aim of the present work is to develop an alternative method for the rapid propagation of *J. procera*. It is proposed that the use of branch cuttings under proper treatment conditions will result in stecklings.

2 LITERATURE REVIEW

2.1 Vegetative propagation

In nature, the method of plant propagation is either asexual (by multiplication of vegetative parts) or sexual (through seeds). Sexually propagated plants show a high amount of heterogeneity, since their seed progenies are not true-to-type unless they have been derived from inbred lines. Asexual reproduction, on the other hand, gives rise to plants which are genetically identical to the parent plant and this permits perpetuation of unique characters of cultivars (Razadan, 1993).

Vegetative reproduction includes propagation by cutting, layering, grafting and budding; and using specialized structures such as runners, suckers, bulbs and corms (Hartmann and Kester, 1975; Richardson and Moore, 1980; Hartmann *et al.*, 1990). When plants are to be propagated by cuttings, there are three basic parts - leaves, stems and roots - from which new individuals are to be produced after they are severed from the parent plant (Edmond *et al.*, 1964; Richardson and Moore, 1980).

Nearly all plant organs such as stems, leaves, roots, and even flowers and fruits are capable of rooting (Leopold, 1963). Stem cuttings are the most commonly used ones (Edmond *et al.*, 1964; Richardson and Moore, 1980) and are the ideal rooting materials because they have sufficient undifferentiated tissues to permit easy differentiation of root primordia (Leopold, 1963). The stem cuttings could be hardwood cuttings; those obtained from narrow leaved evergreen species which are slow to root; semi-hardwood cuttings, those obtained from woody, broad-leaved evergreen species; softwood cuttings, those obtained from soft, succulent, new growth of deciduous or evergreen species; and herbaceous cuttings, obtained from succulent

herbaceous plants (Hartmann and Kester, 1975; Hartmann *et al.*, 1990).

The important advantages of vegetative propagation over sexual reproduction are:

1. Faster multiplication of plants (Denisen, 1958; Ahuja, 1991; Razadan, 1993).
2. Bypassing undesirable juvenile phases (Razadan, 1993).
3. Maintaining of plants of selected genotype for the production of standardized high quality product (Coffman and Gentner, 1977; Ahuja, 1991).
4. Easy propagation of plants which produce little or no seeds, and plants whose seeds are difficult to germinate (Denisen, 1958; Edmond *et al.*, 1964).
5. Vegetative propagation is sometimes more economical (Denisen, 1958; Edmond *et al.*, 1964).

2.2 Factors affecting rooting of cuttings

2.2.1 External factors

Light: Rooting of branch or stem cuttings could be promoted in the presence or absence of light (Denisen, 1958; Leopold, 1963; Kawase, 1965; Hess, 1969; Yeoman and Davidson, 1971; Delargy and Wright, 1979; White and Lovell, 1984a; MacCarthy and Staba, 1985). Exposing the part of the cutting above the level of the rooting medium favours root initiation due to the accumulation of carbohydrates by photosynthesis and/or due to the synthesis of phytohormones in the shoot and their subsequent transport to the root region (Edmond, 1963; Hess, 1969). Exclusion of light by placing the cuttings in the dark or by localized etiolation of the basal end promotes rooting (Kawase, 1965; Delargy and Wright, 1979; MacCarthy and Staba,

1985). Delargy and Wright (1979) reported that, greater formation and elongation of lateral roots occurred in cuttings placed in the dark. In this case, it has been shown that light has inhibitory effect on cell division in cuttings of some plants. For example, explants removed from Jerusalem artichoke tuber and exposed to white light in the presence of 2,4-D, when cultured in liquid medium exhibited much smaller dividing populations than similar tissue not exposed to light (Yeoman and Davidson, 1971). Previously Hess (1969) reported that, light inhibited root formation on parts of cuttings where root primordia are formed. The mechanism whereby etiolation facilitates rooting is believed to be through enhancing the cuttings' sensitivity to auxin (Hartmann and Kester, 1975; Hartmann *et al.*, 1990).

Temperature: Temperature is an important external factor that affects rooting of cuttings because it is important for the occurrence of normal plant processes, such as cell division (Denisen, 1958; Hess, 1969). Higher temperature enhances both shoot and root initiation and growth. However, since in rooting of cuttings, good root growth is of the foremost importance, there should be a way of increasing the temperature of the rooting medium (Jex-Blake, 1950; Leopold, 1963). As a rule, the air temperature should be below that of the medium in which the cuttings are inserted (Wright, 1955). In this respect, relatively lower air temperature retards shoot growth, hence economizing carbohydrate reserves, while high temperature of the rooting medium promotes rapid oxidation of fatty acids thus facilitating root formation and root growth (Edmond *et al.*, 1964). On the other hand, Gautheret (1969) reported alternating temperatures of 26^0 C and 15^0 C) as best for the rooting of *Helianthus tuberosus* L. var `Violet de Rennes' tuber, where high temperature initiated cambial formation followed by differentiation of the cambial cells into root primordia by lower temperature.

Moisture: Moisture is vital as it is needed in transpiration, photosynthesis and cell enlargement (Denisen, 1958). It is important for rooting of stem cuttings because rapidly dividing meristems require abundant water for root initiation and production (Edmond *et al.*, 1964; Hess, 1969). Also, high humidity is helpful for rooting because the cuttings are poorly equipped to obtain water for transpiration and so are quite susceptible to damage by low humidity (Leopold, 1963). High humidity, along with low temperature, reduces transpiration rate and keeps the guard cells turgid and the stomata open thus permitting for the diffusion of CO_2 for carbohydrate and hormone manufacture (Edmond *et al.*, 1964). Mist propagation (constant or intermittent mist) increases rooting percentage of cuttings (Denisen, 1958). But, to provide low rates of transpiration combined with high rate of photosynthesis, the intermittent mist system is preferred (Edmond *et al.*, 1964).

Rooting media: Characteristics of the rooting media, such as compaction, porosity, retentiveness of nutrients and moisture, influence the environmental conditions, such as temperature, moisture and aeration, for vegetative propagation (Denisen, 1958). Therefore, for rapid development and healthy growth of roots the media should be easily drained, porous, well aerated and should hold sufficient moisture (Denisen, 1958; Edmond *et al.*, 1964; Hewitt, 1966). Cuttings root more freely when inserted in porous media (Jex-Blake, 1950). For this purpose, sand is a most widely used material as rooting medium in propagating plants by cuttings because: (1) it is well drained, thus providing O_2 for extensive growth; (2) it is inexpensive and is usually available in adequate quantities; and, (3) it contains little or no organic matter and so does not harbour most disease causing organisms. But due to its low water holding capacity, it must be watered frequently. The sand should be washed to remove the silt and clay. For shallow beds (20 to 30 cm deep) coarser grading (0.25 to 1.00 mm in diameter) of

sand is recommended (Hewitt, 1966).

Season: In the temperate region, the time of the year when woody cuttings are taken affects their rooting response (Gardner *et al.*, 1952; Leopold, 1963). In most cases, there is high rooting response in cuttings collected during spring. Such increased rooting response in spring collected cuttings is either due to resumption of cambial activity as a result of increased levels of endogenous auxins from the sprouting buds (Digby and Wareing, 1966a) or due to increased sensitivity of the cambial cells to the auxins (Zajczkowski, 1973) or both. Unfortunately, data on the effect of season (which, admittedly, is less marked in the tropics) on rooting response of cuttings is lacking for tropical regions.

Aeration: Rooting of cuttings requires sufficient amount of O_2 (Hess, 1969). Where there is high meristematic activity (high rate of cell division), there is high rate of respiration. Rooting of woody cuttings is the result of the divisions of the cambial cells which demand higher O_2 supply (Denisen, 1958).

2.2.2 Internal factors

2.2.2.1 Plant growth regulators and their synergism

Auxins: It is widely accepted that treatment of stem cuttings with auxins promotes the development of adventitious roots. Treatment of such cuttings with these group of synthetic plant growth regulators (PGRs) alters the chemical environment thus resulting in the induction of root primordia. This is followed by tissue differentiation into root proper (Leopold and Kriedemann, 1975; Mackenzie *et al.*, 1986). Some of the PGRs include indoleacetic acid (IAA), indolebutyric acid (IBA), naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), chlorophenoxyacetic acid,

phenolpropionic acids, methyl indole propionate and various salts and esters of indole derivatives (Swingle, 1940). IAA, IBA, NAA and 2,4-D are the most widely employed ones (Edmond *et al.*, 1964; Heide, 1972; Murashige, 1974; Leopold and Kriedemann, 1975). Not only are they employed most commonly, but are also more effective in inducing rooting (Edmond *et al.*, 1964).

Gibberellins: Gibberellins are a group of closely related naturally occurring compounds. They are known in promoting stem elongation (e.g. Hartmann and Kester, 1975) and germination of seeds (e.g. Hsiao *et al.*, 1988). However, at relatively higher concentration (up to 10^{-3} M), they consistently inhibit adventitious root formation. This inhibition is believed to be a direct local effect which prevents the early cell divisions involved in the transformation of mature stem tissues to meristematic condition. Gibberellins have a function in regulating nucleic acid and protein synthesis, and may be suppressing root initiation by interfering with these processes. In *Begonia* L. leaf cuttings, gibberellic acid inhibited both adventitious bud and root formation, probably by blocking organized cell divisions which initiate formation of bud and root primordia (Hartmann *et al.*, 1990). In brittle willow, exogenous gibberellic acid markedly reduced the number of cells per primordium by blocking the action of IAA so that root development was reduced (Haissig, 1972). But the effect of gibberellic acid on cambial cell division could be promotive (Digby and Wareing, 1966b) or with no effect (Haissig, 1972). In this case, though gibberellic acid induces cambial cell divisions, it plays no role in the differentiation of the derivatives (Digby and Wareing, 1966b).

At lower concentrations (10^{-11} to 10^{-7} M), however, gibberellins are known to promote root initiation (Hartmann and Kester, 1975; Hartmann *et al.*, 1990). The effect is

believed to be mediated by increasing auxin levels in the cuttings. Gibberellins increase the conversion of tryptophan (an important auxin precursor) to auxin, hence increasing diffusible auxin level (Valdovinos *et al.*, 1967). In this case, gibberellin treatment of dwarf pea plants, normal pea plants and sunflower yielded 3, 2, and 10 times, respectively, more auxin than untreated plants (Kuraishi and Muri, 1962). This shows that, lowering of the natural levels of gibberellins in the tissue stimulates adventitious root formation in cuttings. In fact, promotion of rooting has been done experimentally by various chemical substances that interfere with gibberellin activity (Hartmann and Kester, 1975; Hartmann *et al.*, 1990).

Cytokinins: Cytokinins have been implicated in many aspects of plant growth and development such as cell division, cell enlargement, differentiation and morphogenesis, delaying senescence (Vlitos and Most, 1973), and in affecting germination of seeds (Mayer and Poljakoff-Mayber, 1975). They are also found to be part of the critical organic components of plant tissue culture media (Murashige, 1974). At relatively low concentration, when applied to decapitated pea cuttings at an early developmental stage, cytokinins promoted root initiation, while higher concentrations inhibited root initiation. Cytokinins interact with auxins. In *Begonia* leaf cuttings, cytokinins at relatively high concentration (13 ppm) promoted bud formation and inhibited root formation. Auxins, when applied at higher concentrations, gave the opposite effect (Hartmann and Kester, 1975; Hartmann *et al.*, 1990).

Ethylene: Ethylene is produced by plants and has a number of hormonal effects. With respect to rooting of stem cuttings, the effect of ethylene is controversial. Some workers reported root initiation by ethylene while others reported otherwise. The effect of ethylene as an inhibitor of rooting is due to its effect on auxin. Treatment of

epicotyl of etiolated pea seedlings with ethylene diminished the IAA level by 50% within five to six days (Lieberman and Knegt, 1977). The effect could be by affecting auxin transport or metabolism. Ethylene inhibited polar transport of auxin (IAA) in stem tissue of *Coleus blumei* Benth. (Ernest and Valdovinos, 1971). Moreover, the capacity of polar auxin transport system is markedly reduced in sections cut from etiolated pea plants grown in ethylene (Burg and Burg, 1967). It has also been observed that, ethylene decreases the conversion rate of auxin precursor (tryptophan) (Valdovinos *et al.*, 1967). It also enhances the catabolism of IAA to indole-3-carboxylic acid which is supposed to be one of the mechanisms by which ethylene reduces IAA level (Sagee *et al.*, 1990).

Ethylene induced root initiation on stem and leaf tissues and growth of pre-existing root primordia in 27 species of plants (Zimmerman and Hitchcock, 1933: quoted in Swingle, 1940). It is suggested that auxin application can regulate ethylene production, whereby auxin-induced ethylene may account for the ability of auxin to cause root initiation (Hartmann *et al.*, 1990).

Synergism: Auxins, cytokinins, gibberellins and a variety other growth substances, singly or synergistically, have been shown to regulate cambial divisions and the expansion and wall thickening of cambial derivatives (Wareing *et al.*, 1964: quoted in Denne, 1972). For example, sublethal levels of chlorogenic acid, caffeic acid, catechol, hydroquinone, ferulic acid, tryptophan, indole, α-naphthol, β-naphthol, H_2SO_4, KOH and $HgCl_2$ are synergists of IAA (Gorter, 1969). Moreover, indole is a synergist of α-NAA and 2,4-D (Gorter, 1962). The proposed mechanisms by which synergists promote the effect of auxins are several. They include:

1. Synergists frequently have structural features closely similar to the

compounds with which they work, so that synergism is believed to be due to competition for site of loss (Fawcett, 1961).

2. Synergists antagonize the effects of unspecific oxidizing (destructing) enzymes of endogenous auxins, e.g., IAA oxidase (Gorter, 1962). Rooting of *Phaseolus* L. cuttings by IAA was increased synergistically by the addition of indole; and the rate of IAA oxidation by enzyme preparation from etiolated pea seedlings decreased in the presence of this chemical (Fawcett, 1961).
3. Synergists effect preparatory actions which make more cells responsive to auxin (especially to IAA), but may not inhibit IAA oxidase or stimulate the conversion of tryptophan to IAA (Gorter, 1969). For example, phenylacetic acid is a synergist of IAA but does not reduce oxidase activity (Fawcett, 1961).

2.2.2.2 Nutritional status of source plants

Nutrient materials such as carbohydrates, nitrogenous substances and other inorganic nutrient elements and vitamins are important for rooting of branch cuttings. Carbohydrate supply affects *in vitro* organogenesis (MacCarthy and Staba, 1985) because it is high energy (ATP)- and reducing power (NADPH)-requiring process (Thrope, 1978: quoted in MacCarthy and Staba, 1985). Sucrose was found to be absolutely essential for root regeneration of embryo cuttings of *Pinus lambertiana* Dougl. (Greenwood and Berlyn, 1973). In general, when carbohydrate reserves are abundant, rooting of stem cuttings is facilitated (Leopold, 1963). This was confirmed by removing leaves and girdling. The ability of cuttings to form root primordia can be dramatically reduced by removing leaves or by girdling stems (Hess, 1969). In *Griselinia lucida* Forst. f. Prodr., the removal of leaves prevented rooting, though

some cuttings produced basal calli and survived for eight months. Therefore, leaves are beneficial for rooting because they provide photosynthates. Younger leaves are also believed to be important sources of auxins (White and Lovell, 1984a). Therefore, when cuttings are prepared for vegetative propagation purposes, the total leaf area of each cutting should be reduced such that transpiration is minimized and the provision of carbohydrates is unhampered (Edmond *et al.*, 1964).

Optimum amount of soluble nitrogen increases the rooting ability of cuttings but large amounts are inhibitory (Leopold, 1964; Hess, 1969). Under conditions of nitrogen deficiency, the rooting ability of cuttings is decreased because the production of auxins is reduced (Went, 1945). The relative level of soluble nitrogen compounds in cuttings also has a bearing on the ease of rooting. Optimum rooting is attained at a high ratio of carbohydrate to soluble nitrogen compounds (Leopold, 1963).

Among the inorganic nutrient elements, boron is an important one in affecting rooting. Since it is irreplaceable element for plant growth, lack of boron entirely eliminates rooting (Leopold, 1963). It was reported that, irrespective of auxin pre-treatment no roots were developed in cuttings from seedlings of mung bean (*Phaseolus aureus* Roxb.) without exogenous boron (Middleton *et al.*, 1978). Lack of phosphorus, potassium, calcium and magnesium is found to lower rooting but with less marked effects as compared to nitrogen and boron (Leopold, 1963).

2.2.2.3 Age of source plants

Age of source plants from which the cuttings are collected has an important effect on their rooting ability. Cuttings taken from mature trees of many forest species such as beech, eucalyptus and most conifers are often very difficult to root (Ahuja, 1991). It is

well known that the rooting ability is high in shoots during juvenile growth phase and declines after transition to adult growth phase in many woody species (Welander and Snygg, 1987). Adventitious roots and root formation is subsequently reduced, or is often absent, in plants or cuttings obtained from flowering age trees. This diminished competence for organogenesis and plant regeneration in explants or cuttings obtained from mature plants is significant for tissue culturalists and plant propagators (Haung *et al.*, 1992).

2.2.2.4 Mechanical manipulations

Mechanical manipulations during cuttings, including wounding, splitting, slanting the cuts, etc., are found to be important in improving the rooting ability of cuttings. The pattern of differentiation can be modified by physical confinement of the cells and tissues. The act of wounding a cutting (including incision wound) alters the physical environment in which the tissue differentiation occurs (Mackenzie *et al.*, 1986). It is well known that cell division and proliferation are wounding responses (White and Lovell, 1984b). Moreover, wounding by the removal of buds can result in improved rooting response through the release of a wound-induced stimulus, postulated to be specific wound hormone (Howard, 1968). Sometimes, cuttings which are difficult to root may root if splitted upwards from 1.25 to 2.5 cm according to size (Jex-Blake, 1950). Splitting the bases of cuttings in apple rootstock gave a 16-fold improvement in rooting over the control, while twin incisions (2 deep incisions) gave a 4-fold improvement. Such splitting of the bases of the cuttings results in cambial partitioning which is suitable for callus formation (Mackenzie *et al.*, 1986).

In preparing cuttings, the cuts are generally made slanting at the lower bud. Another method is to leave a small piece of old wood or a whorl of buds at the butt end

(Shoemaker, 1950). This is because, as a rule roots arise readily from a nod or bud. Mallet or heel cuttings are also used for preparing some varieties of grape which do not root readily from straight cuttings (Knapp and Auchter, 1947). It has been known for a long time that the mallet and heel have active cambia which produce more callus or wound tissue than the new wood (Denisen, 1958).

Although not commonly used for stimulating the formation of adventitious roots, ringing of shoots intended for propagation purposes can promote rooting in cuttings of *Pyrus* L. (Higdson and Westwood, 1963: quoted in Yeoman and Davidson, 1971). In this case, Delargy and Wright (1978) reported that, ring barking proximal to etiolated segments facilitated root formation in stem cuttings of apple.

2.3 Histological origin of root initials

In some plants, adventitious root initials form during early stages of intact stem development and are already present at the time when the cuttings are made. These are called **latent root initials** (Hartmann and Kester, 1975; Hartmann *et al.*, 1990). Cuttings of such plants are easy to root. Many species, however, do not contain latent root initials in their stem tissues, and the formation of roots in branch cuttings of such species involves the following stages of development.

1. Division and differentiation of one or more cells (root initials) to become root primordium(a).
2. The formation of vascular connection between the new root and existing vascular system in the stem.
3. Growth of the root through the stem tissue and its emergence (Hartmann and Kester, 1975; White and Lovell, 1984c,d; Hartmann *et al.*, 1990).

Root initials form from some active, morphologically and physiologically differentiated tissues of the cuttings. This is because differentiated plant cells are capable of dedifferentiation (where mature and specialized cells lose their differentiated characters and rejuvenate) and redifferentiation (physiological and morphological differentiation of dedifferentiated cells) (Bloch, 1941).

2.4 Mechanism of action of plant growth regulators

The response of many types of stem and hypocotyl cells to auxin is biphasic. The initial phase of the response is similar to growth that can be induced by low pH. It is associated with cell wall loosening that is likely to be induced by an acidification mechanism which, in turn, is followed by the deposition of new cell material (Breviario *et al.*, 1992). Nooden (1968) suggested that auxins act by inducing RNA synthesis that results in the synthesis of new enzymes. These enzymes modify the cell wall thus allowing for cell expansion. High concentration of auxins (particularly 2,4-D) induce the synthesis of cellulase which is capable of hydrolyzing xyloglucan *in vitro* into nanosaccharide and heptasaccharide (McDougall and Fry, 1988). The concentration of cellulases (β-1,4-glucanase and glucanohydrase) increased in the growth region of *Pisum sativum* L. epicotyl after a 2,4-D treatment (Byrne *et al.*, 1975). Xyloglucan is a hemicellulosic polysaccharide found in cell walls of monocots, dicots and gymnosperms. Xyloglucan metabolism increases by auxin treatment in a similar way as auxin promotes cell elongation. Changes in xyloglucan reflect the means by which auxin modifies the cell wall to cause elongation (Labavitch and Ray, 1974). This is supported by the increased levels of xylose and glucose (monomers of xyloglucan) in auxin treated, ethanol-insoluble polysaccharides (Terry *et al.*, 1981). A macromelecular complex composed of xyloglucan and cellulose (components of primary cell walls of higher plants) was obtained in elongated regions of etiolated pea

stems. Xyloglucan could be solubilized by extended treatment with *end*-1,4,β-glucanase (Hayashi *et al.*, 1984), an enzyme induced by auxin treatment (Hayashi and Maclachlan, 1984).

The second phase has properties similar to other long term auxin responses such as cell divisions, differentiation and morphogenesis (Breviario *et al.*, 1992). Inspection of how PGRs at very low concentration levels can exert very large physiological effect leads one to the deduction that any mechanism of action must involve a large amplification effect. In bringing about their actions, the PGRs can be expected to exert an influence on some process that alters a large number of other molecules. These functions through which large amplification effect could be obtained would be alteration of the nucleic acid-directed protein synthesis, regulation of pace-setter enzyme, or regulation of nucleic acid synthesis and thus protein synthesis (Leopold and Kreidemann, 1975). Earlier, Evans (1974) reported that sustained growth responses to auxin of a variety of tissues depend on continual protein synthesis. An auxin regulation of DNA-directed RNA synthesis is believed to take place via an increase in template activity of DNA or alternatively, via an increase in effectiveness of RNA polymarase (Leopold and Kriedemann, 1975). In this case, Key (1969) reported that auxins markedly affect both the RNA and DNA content of tobacco pith tissue cultured on a sucrose agar medium. The auxin regulated increase in nucleic acid occurred prior to the increase in tissue fresh weight, having proportional increase in RNA and fresh weight with time. Application of IAA increases RNA content and leads to maximum growth. With increased IAA concentration and time, pentose nucleic acid, DNA and fresh weight increased (Silberger and Skoog, 1953). The fact that PGRs affect genetic expression is also supported by Scott (1972) where gibberellin and cytokinin, when applied alone or together with auxin, cause inhibition of growth in roots. The effect of

cytokinin is associated with RNA metabolism which occurs at the level of cell division and events closely related to genetic expression.

3 MATERIALS AND METHODS

3.1 The glasshouse and its environmental conditions

The investigation was conducted in a glasshouse (area 6.6 m^2 and height 2.9 m) located within the Addis Ababa University, Science Faculty campus from mid-March to mid-November, 1996. Mean minimum and maximum temperatures of the glasshouse during the investigation were 13.4 $\pm$ 0.1^0 C and 31.7 $\pm$ 0.3^0 C, respectively; and relative humidity (RH) was 70 to 80% (measured with Automatic Porometer Mk 3, Delta-T Device). The RH was kept within this range by watering the surface of the glasshouse at intervals.

3.2 Rooting medium

The rooting medium used for the investigation was sand. It was sieved using 1 mm mesh size sieve and was washed in running tap water to remove most of the colloidal particles and debris. About 4.5 kg of washed sand was filled in each of the synthetic flower pots (upper diameter 20 cm, depth 19 cm) before use and was kept wet until used for inserting the branch cuttings. At the base of each of the pots, there were four holes pierced with a needle (diameter ca 2 mm) for drainage purposes.

3.3 Source plants

Cuttings from three classes of source plants that are at two developmental stages were used in this investigation.

A) Class I: These were 1.5 to 2 years old seedlings. These seedlings were raised from seeds germinated in nursery beds composed of a mixture of forest soil, animal dung and compost. The germinants were transplanted into plastic bags (diameter 5.5 cm,

length 10 cm) filled with a mixture of sand, forest soil and animal dung in a ratio of 1:4:3, respectively (Legesse Negash, 1995). The seedlings were then transplanted into larger plastic bags (diameter, 20 cm; length, 35 cm) filled with the same mixture used for germinating the seeds.

B) Class II: These were trees estimated at 25 to 30 years old, located near the Phytochemical Laboratory of the Science Faculty, Addis Ababa University.

C) Class III: Tree estimated at 30 to 35 years old, located near the Geological Observatory in the Science Faculty of Adds Ababa University.

3.4 Preparation of cuttings

Four hundred sixty four cuttings from primary branches (15 to 25 cm in length, and 2 to 4 mm in diameter) were collected from Class I source plants. Cuts were made using a scalpel at the bases of the branches. Six to 20 cuttings were collected from a single seedling. The same number of cuttings of similar length and diameter were collected from each of Class II and Class III source plants. Cuttings obtained from Class II source plants were younger while the cuttings obtained from Class III source plants were older. The leaves and/or branches at the bases of the cuttings were removed immediately after the cuts were made. The bases of the cuttings were then soaked in distilled water to keep the continuity of xylem transport (transpiration column).

3.5 Preparation of PGRs

Four PGRs, namely indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthaleneacetic acid (NAA) and 2,4-dichlorophenexyacetic acid (2,4-D), were used to examine their effects on rooting of branch cuttings of *J. procera*. 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 10^{-3} M of each PGR

was prepared by dissolving the required amount in 2 ml of 95% ethanol. The volume was adjusted to 200 ml by adding distilled water. Stock solutions of IBA, NAA and 2,4-D were prepared a day before they were used, but that of IAA was prepared during the day of treatment, in dim light, to reduce the risk of loosing its strength through photo-oxidation and bacterial decomposition. Concentrations ranging from 10^{-4} to 10^{-9} M were then prepared by serial dilution from the respective stock solutions.

3.6 Treatment of the cuttings with PGRs

There were 29 treatments for each category of cuttings (seven treatments in each of the four PGRs, ranging from 10^{-3} to 10^{-9} M, and one treatment using distilled water to serve as a control). In each treatment there were 16 cuttings. The bases (4 to 5 cm long) of each group of cuttings were soaked in 90 to 100 ml of the corresponding PGRs at a given concentration for 24 hours. To avoid the entrance of light, the vessels containing cuttings treated with IAA were covered with cardboard.

3.7 Planting of the cuttings

After 24 hours of treatment, the bases (4 to 5 cm) of each of the 16 cuttings in each treatment were inserted in two pots (eight cuttings per pot). The pots were randomly arranged in the glasshouse. About 40 ml of Hoagland mineral solution (as modified by Johnson *et al.*, 1957: cited in Epstein, 1972) was added to each pot once a week throughout the experimental period. The cuttings were watered three to four times a day between 9 and 15 hours.

3.8 Data collection

Assessments on the number of survived, callused and rooted cuttings, the number of roots per rooted cuttings and the length of roots were done two times (16 and 32 weeks

after the start of the experiment) for all the treatments. During the first assessment, data collection was done by splashing the rooting medium with running water and by gently removing the cuttings from the rooting medium. After the required data were collected, all the cuttings (rooted and non-rooted) were replaced in their respective pots to be assessed after another 16 weeks. In the second assessment, data collection was done by washing the sand out by tilting the pots.

3.9 Transplantation of rooted cuttings

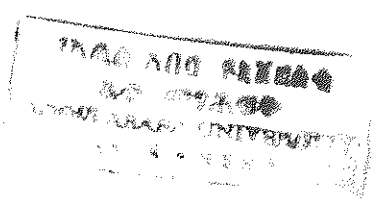
Forty rooted cuttings were transplanted into plastic bags containing a mixture of sand, soil and animal dung in a ratio of 1:4:3, respectively. The rooted cuttings were maintained in a glasshouse and were watered once a day. Data on establishment, and growth of stecklings were collected from mid-November, 1996 to mid-April, 1997.

3.10 Statistical analyses

Analysis of variance (ANOVA) followed by post-hoc comparison using LSD (Least Significant Difference) and regression analysis were done to compare the data.

3.11 Histological investigation of root initiation

Histological study on initiation of root primordia was made using cuttings obtained from Class I source plants. The tissue for sectioning was prepared by cutting and trimming 10-20 mm of the tip of the cut end. This was fixed in formalin (40%), acetic acid and ethyl alcohol (50%) in the ratio of 1:1:18 in volume for three to 20 days. It was then washed in 50% alcohol (five changes), dehydrated in alcohol series and chloroform-absolute alcohol series, and infiltrated and embedded in paraffin wax (melting point 49° C). Microtome sections were made at 12 micrometre thickness. The sections were affixed using Haupt's adhesive and were stained using safranine-fast



green (Jensen, 1962; Purvis *et al.*, 1966). The preparations were then microphotographed at 40X magnification.

4 RESULTS

4.1 Survival of cuttings under the glasshouse conditions

Survival of cuttings under the glasshouse conditions varied markedly depending on the developmental stage of the source plants. Seventy seven percent of the cuttings obtained from Class III source plants died within the first 30 days. Cuttings collected from Class II source plants were also relatively difficult to handle as compared to those obtained from Class I source plants. Only 44% of the cuttings obtained from Class II source plants survived until the end of the first 16 weeks. After 32 weeks, this value dropped to 34%. On the other hand, 93% the cuttings obtained from Class I source plants survived until the end of the first 16 weeks; and 90% survived until the end of the investigation (32 weeks) (Table 1).

Table 1. Effect of the age of the source plants on survival of cuttings. Assessments were made 16 weeks (16w) and 32 weeks (32w) after treatment.

A) Cuttings obtained from Class I source plants

[PGRs] (M)	Percentage survival							
	IAA		IBA		NAA		2,4-D	
	16w	32w	16w	32w	16w	32w	16w	32w
10^{-3}	100	81	75	50	38	25	0	0
10^{-4}	100	100	100	100	100	100	94	81
10^{-5}	100	100	100	100	100	100	100	100
10^{-6}	100	100	100	100	100	100	100	88
10^{-7}	100	94	100	100	100	94	100	100
10^{-8}	100	100	100	100	100	100	100	100
10^{-9}	100	100	100	100	100	100	100	100
H_2O	94	88						

B) Cuttings of Class II source plants

[PGRs] (M)	Cuttings survived (%)							
	IAA		IBA		NAA		2,4-D	
	16w	32w	16w	32w	16w	32w	16w	32w
10^{-3}	50	31	0	0	0	0	0	0
10^{-4}	88	63	44	44	31	31	88	44
10^{-5}	6	6	44	38	94	69	13	6
10^{-6}	25	6	56	31	64	19	44	19
10^{-7}	50	44	44	44	25	6	50	13
10^{-8}	69	38	94	63	31	31	25	19
10^{-9}	63	31	50	19	44	44	75	75
H_2O	25	25						

4.2 Callus formation

Calli at/around the base of cuttings obtained from Class I source plants were observed six weeks after treatment. Calli occurred: (1) at the cut end as a result of wound healing; (2) on scars of branches and leaves; and, (3) few millimetres above the base where there were no scars of branches and leaves.

Callus formation was markedly affected by the age of the source plants from which the cuttings were obtained. After 16 weeks, the mean percentage of cuttings callused obtained from Class I source plants (21.1 $\pm$ 3.2%) was significantly greater than that of the cuttings obtained from Class II source plants (8.4 $\pm$ 2.0%) ($p \leq 0.05$). The highest attainable percentage of callused cuttings obtained from Class I source plants was 56 for the IAA (applied at 10^{-4} M) and NAA (applied at 10^{-5} M). The corresponding percentages were 31 and 25 for IBA (applied at 10^{-5} M) and 2,4-D (applied at 10^{-6} M and 10^{-7} M), respectively. The control resulted in 40% callused cuttings. Similarly, the highest attainable percentages of callused cuttings obtained

from Class II source plants were 29, 27 and 25 for IBA (applied at 10^{-4} M), IAA (applied at 10^{-8} M) and NAA (applied at 10^{-7} M), respectively. No callused cuttings were observed in any of the treatments of 2,4-D, and the control (Fig. 1).

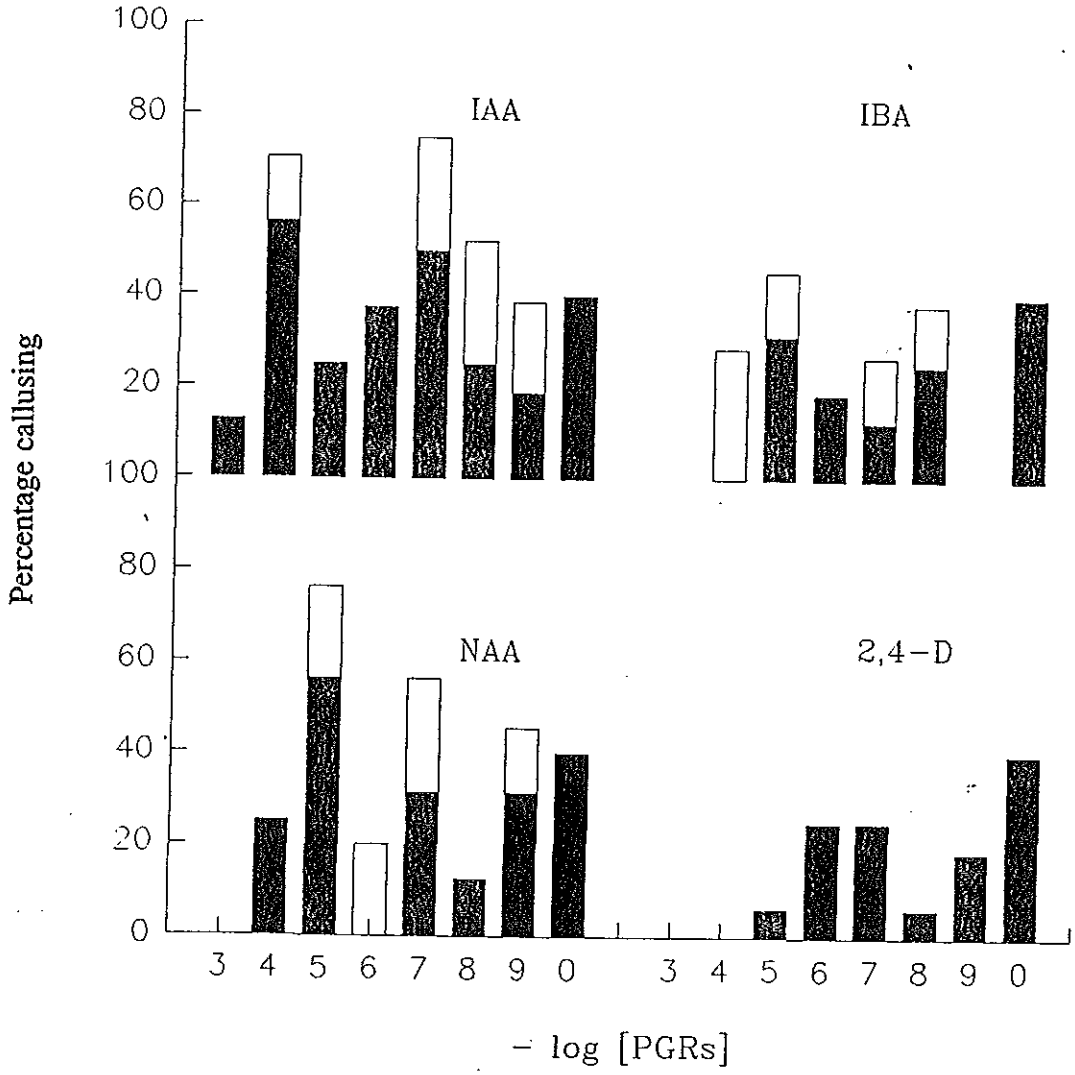


Figure 1. Effect of the age of the source plants on callusing of cuttings. Assessments were made 16 weeks after treatment; and percentages are given based on the number of survived cuttings. (▩ = Class I; □ = Class II). The zero bar on the abscissa is for the control.

4.3 Rooting response of the cuttings

The number of cuttings rooted within the first 16 weeks was very limited. Moreover, it was only in the cuttings obtained from Class I source plants that rooting was observed. In general, only 2% of the cuttings obtained from Class I source plants rooted. This was simply nine out of the 432 of the cuttings that survived. Only a single rooted cutting was obtained in each of the 10^{-7} M and 10^{-8} M of IAA, 10^{-3} M of IBA and 10^{-6} M of 2,4-D treatments. The control and 10^{-3} M of IAA treated ones resulted in three and two rooted cuttings, respectively. After 32 weeks, 24% of the cuttings obtained from Class I source plants were rooted. Some of these cuttings resulted in high degree of rooting with respect to root number and root length (Fig. 2) while most of them had fewer and shorter adventitious roots. Only a single cutting rooted among those obtained from Class II source plants.

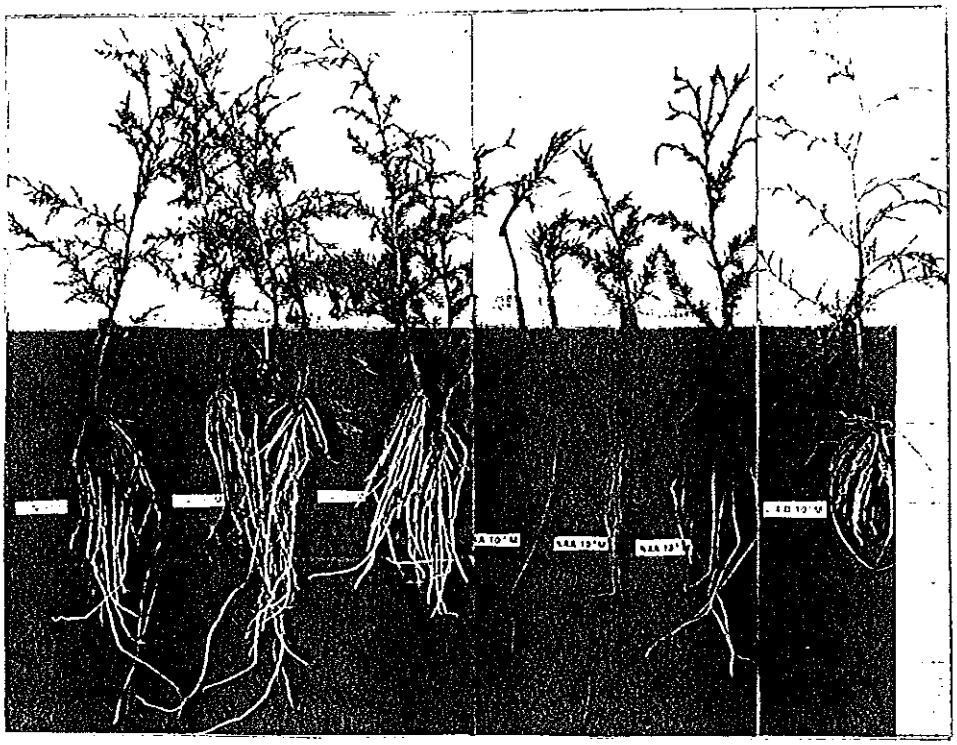


Figure 2. Rooting intensity of the cuttings obtained from Class I source plants after 32 weeks. (Left to right: control, 10^{-6} M IAA, 10^{-7} M IAA, 10^{-3} M NAA, 10^{-4} M NAA, 10^{-5} M NAA, 10^{-6} M 2,4-D).

The maximum attainable percentage of rooted cuttings obtained from Class I source plants was 60 for IAA treatment at 10^{-7} M. The corresponding percentages for NAA (applied at 10^{-3} M), IBA (applied at 10^{-5} M) and 2,4-D (applied at 10^{-7} M and 10^{-8} M) were 50, 44 and 25, respectively (Fig. 3).

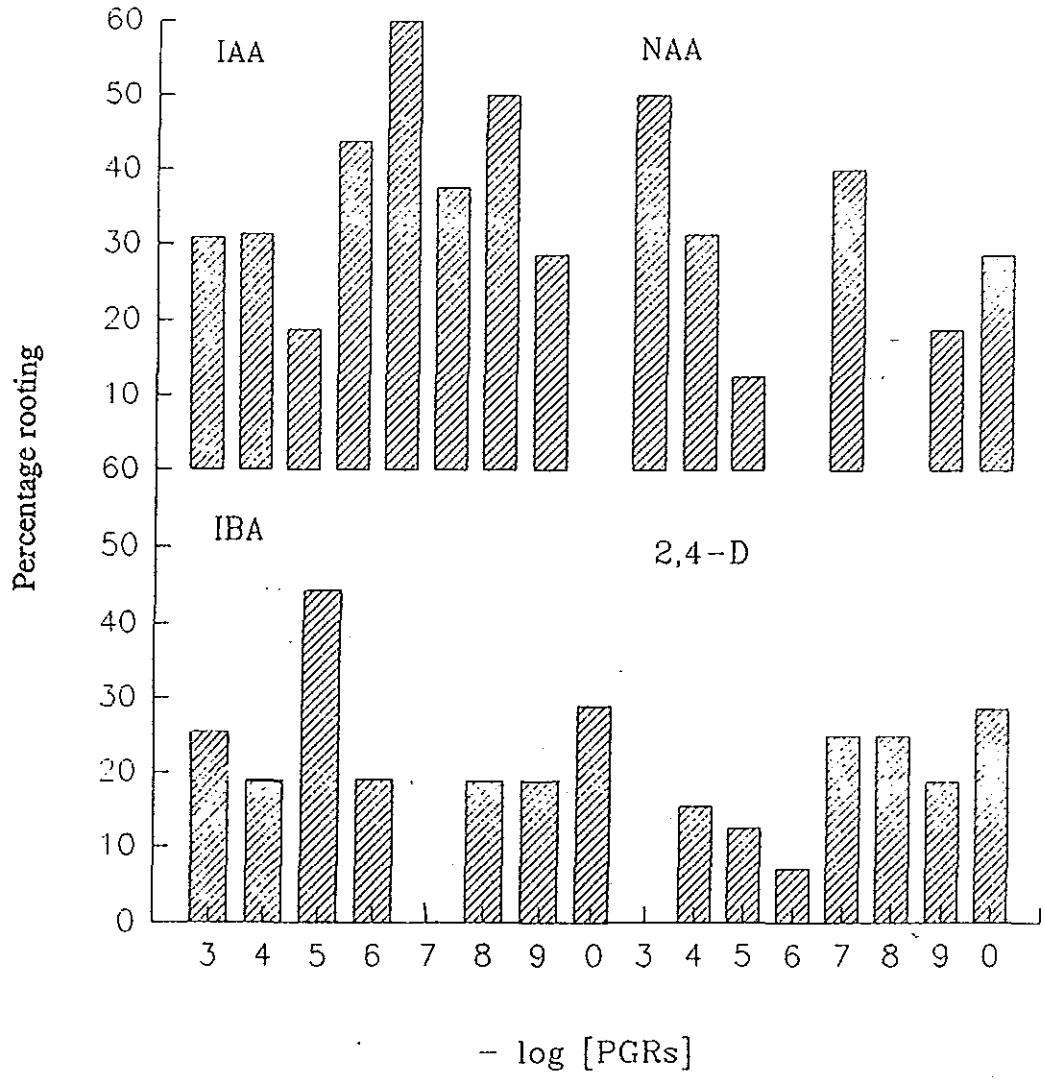


Figure 3. Effect of the PGRs on rooting of cuttings obtained from Class I source plants. Assessments were made 32 weeks after treatment; and percentages are given based on the number of survived cuttings. The zero bar on the abscissa is for the control.

Cuttings treated using lower concentrations of IAA (10^{-6} M to 10^{-9} M) gave rooting percentages of ≥ 38. But at higher concentrations of IAA (10^{-3} M to 10^{-5} M) rooting percentages of the cuttings were ≤ 31. Naphthaleneacetic acid applied at 10^{-7} M gave 40% rooting while the rest of the treatments resulted in rooting percentages of less than 40%. None of the cuttings treated with 10^{-6} and 10^{-3} M NAA produced roots. Six of the treatments in IBA resulted in rooting percentages of ≤ 25. None of the cuttings treated at 10^{-7} M IBA rooted. Rooting responses of cuttings treated with were between 7% and 25%. It is important to note that 29% of the control cuttings rooted successfully. This value is greater than the values obtained from many of the various treatments).

4.4 Effects of the PGRs on root number

The highest attainable mean root number per rooted cutting obtained from Class I source plants was 17 ± 4.1 for the IAA treatment applied at 10^{-6} M. The corresponding mean root numbers for IBA (applied at 10^{-3} M), 2,4-D (applied at 10^{-9} M) and NAA (applied at 10^{-5} M) were 14.5 ± 12.5, 9.6 ± 6.7 and 7 ± 3.0, respectively. Cuttings treated at 10^{-8} M and 10^{-7} M of IAA resulted in relatively greater root number; 16.3 ± 3.9 and 11.7 ± 4, respectively. Cuttings treated with IBA at 10^{-8} M and 10^{-9} M gave 11.7 ± 2.9 and 10.3 ± 4.2 roots, respectively. Most of the cuttings rooted in 2,4-D resulted in mean root number of ≤ 8 ± 7.0. In the NAA treatments, four of the five groups gave mean root number of ≤ 4.2 ± 1.3 (Table 2).

Table 2. Effects of the PGRs on mean root number per rooted cutting obtained from Class I source plants after 32 weeks.

[PGRs] (M)	Mean root number ± SE			
	IAA	IBA	NAA	2,4-D
10^{-3}	8.6±1.9^{ab}	14.5±12.5^{ab}	1.5±0.5^{b}	-
10^{-4}	7.6±2.9^{ab}	4.3±1.7^{b}	4.2±1.3^{ab}	8.0±7.0^{ab}
10^{-5}	3.0±1.5^{b}	4.4±1.4^{b}	7.0±3.0^{ab}	4.0±3.0^{b}
10^{-6}	17.0±4.1^{a}	4.0±1.2^{b}	-	*
10^{-7}	11.7±4.0^{ab}	-	3.8±1.4^{b}	6.8±3.3^{b}
10^{-8}	16.3±3.9^{a}	11.6±2.9^{ab}	-	6.8±3.3^{b}
10^{-9}	4.0±1.4^{b}	10.3±4.2^{ab}	1.7±0.3^{b}	9.7±6.7^{ab}
H_2O	17.0±1.7^{a}	17.0±1.7^{a}	17.0±1.7^{a}	17.0±1.7^{a}

* Single rooted cutting.

Within the same column means followed by different letters are significantly different ($p \leq 0.05$).

The mean root number of cuttings treated with IAA at 10^{-6} M and those of the control was significantly greater than 13 and 11 of the 23 treatments, respectively, in all of the PGRs ($p \leq 0.05$) (Table 3).

Table 3. Results of Post-Hoc comparison on the effects of the various treatments on root number.

Probabilities for LSD Test are presented in a matrix form (Differences are significant at $p \leq 0.05$). Highlighted values indicate significant differences. (MRN = mean root number; T = treatment)

																							MRN	T
1																							8.8	10^{-3} M IAA
2	.814																						7.6	10^{-4} M IAA
3	.304	.389																					3.0	10^{-5} M IAA
4	.074	.030	.006																				17.0	10^{-6} M IAA
5	.507	.319	.078	.150																			11.7	10^{-7} M IAA
6	.110	.051	.011	.869	.227																		16.3	10^{-8} M IAA
7	.290	.388	.839	.000	.033	.002																	4.0	10^{-9} M IAA
8	.364	.260	.081	.669	.620	.758	.072																4.5	10^{-3} M IAA
9	.429	.540	.823	.013	.134	.022	.946	.130															4.3	10^{-4} M IBA
10	.364	.459	.776	.001	.052	.004	.909	.088	.984														4.4	10^{-5} M IBA
11	.395	.500	.866	.011	.118	.019	1.00	.118	.955	.932													4.0	10^{-6} M IBA
12	.601	.446	.148	.291	1.00	.367	.123	.671	.221	.153	.201												11.7	10^{-8} M IBA
13	.776	.608	.221	.188	.784	.246	.202	.532	.316	.243	.290	.823											10.3	10^{-9} M IBA
14	.253	.319	.822	.009	.078	.014	.665	.078	.671	.617	.707	.130	.187										1.5	10^{-3} M NAA
15	.354	.462	.822	.003	.069	.007	.961	.094	.980	.957	.970	.164	.252	.658									4.2	10^{-4} M NAA
16	.782	.921	.549	.090	.418	.120	.603	.306	.689	.660	.652	.484	.617	.452	.647								7.0	10^{-5} M NAA
17	.298	.395	.871	.001	.044	.003	.966	.076	.922	.883	.974	.132	.210	.695	.933	.595							3.8	10^{-7} M NAA
18	.206	.267	.823	.003	.042	.005	.637	.057	.655	.584	.695	.096	.148	.890	.635	.424	.675						1.7	10^{-9} M NAA
19	.905	.947	.454	.127	.521	.165	.489	.374	.582	.542	.549	.582	.726	.374	.534	.891	.485	.343					8.0	10^{-4} M 2,4-D
20	.453	.556	.880	.028	.181	.041	1.00	.153	.960	.941	1.00	.252	.343	.732	.973	.681	.977	.726	.584				4.0	10^{-5} M 2,4-D
21	.698	.862	.502	.027	.264	.044	.539	.222	.665	.612	.662	.379	.521	.407	.603	.968	.536	.363	.843	.663			6.8	10^{-7} M 2,4-D
22	.698	.862	.502	.027	.264	.044	.539	.222	.665	.612	.667	.379	.521	.407	.603	.968	.536	.363	.843	.663	1.00		6.8	10^{-8} M 2,4-D
23	.869	.698	.265	.148	.681	.199	.253	.469	.372	.300	.343	.737	.911	.222	.307	.689	.260	.180	.802	.396	.601	.601	9.8	10^{-9} M 2,4-D
24	.113	.058	.013	1.00	.226	.887	.004	.692	.025	.007	.022	.340	.234	.016	.108	.116	.006	.007	.157	.042	.050	.050	.191	17.0 CONTROL
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

4.5 Effects of PGRs on root length

The maximum attainable mean length of roots in cuttings obtained from Class I source plants was 372.3 ± 51.5 mm for the NAA treatment applied at 10^{-3} M. The corresponding mean lengths were 113.7 ± 11.8 mm, 90.7 ± 5.6 mm and 67.9 ± 8.8 mm for IBA (applied at 10^{-3} M), IAA (applied at 10^{-6} M) and 2,4-D (applied at 10^{-4} M), respectively (Table 4).

Table 4. Effects of the PGRs on mean root length (mm) per rooted cutting obtained from Class I source plants. Assessment was made 32 weeks after treatment.

[PGRs] (M)	Mean root length ± SE			
	IAA	IBA	NAA	2,4-D
10^{-3}	74.5±8.5^{b}	113.7±11.8^{a}	372.3±51.5^{a}	-
10^{-4}	51.8±6.9^{c}	25.7± 6.5^{c}	46.2±10.2^{c}	67.9±8.8^{b}
10^{-5}	8.9±1.5^{d}	32.0± 4.4^{c}	113.2±19.7^{b}	15.0±5.3^{d}
10^{-6}	90.7±5.6^{ab}	16.7± 6.5^{c}	-	52.0±2.4^{d}
10^{-7}	65.7±4.1^{c}	-	68.0±13.1^{c}	25.9±3.8^{d}
10^{-8}	44.6±3.9^{c}	72.9±12.3^{b}	-	25.6±3.6^{d}
10^{-9}	38.4±6.0^{cd}	15.2± 3.9^{c}	16.6± 8.5^{c}	39.1±5.9^{c}
H^2O	93.9±7.3^{a}	93.9± 7.3^{a}	93.9± 7.3^{b}	93.9±7.3^{a}

Within the same column means followed by different letters are significantly different ($p \leq 0.05$).

The mean root length of cuttings treated at 10^{-3} M NAA was significantly greater than the mean root lengths of cuttings in all of the other treatments which resulted in rooting ($p \leq 0.05$). Interestingly, the control had mean root length of 93.9 ± 7.3 mm. This was significantly greater than the mean root lengths recorded for the 20 of the 24 treatments which resulted in rooting ($p \leq 0.05$) (Table 5).

Table 5. Results of Post-Hoc comparison on the effects of the various treatments on mean root length.

Probabilities for LSD Test are presented in a matrix form (Differences are significant at $p \leq 0.05$). Highlighted values indicate significant differences (MRL =mean root length; T = treatment).

																									MRL	T
1																									74.5	10^{-3} M IAA
2	.038																								51.8	10^{-4} M IAA
3	.000	.013																							8.9	10^{-5} M IAA
4	.069	.000	.000																						90.7	10^{-6} M IAA
5	.333	.117	.000	.000																					65.7	10^{-7} M IAA
6	.001	.416	.027	.000	.001																				44.6	10^{-8} M IAA
7	.001	.230	.092	.000	.003	.515																			38.4	10^{-9} M IAA
8	.000	.000	.000	.014	.000	.000	.000																		113.7	10^{-3} M IBA
9	.001	.080	.405	.000	.003	.169	.405	.000																	25.7	10^{-4} M IBA
10	.000	.078	.190	.000	.000	.189	.583	.000	.681																32.0	10^{-5} M IBA
11	.000	.022	.704	.000	.000	.050	.167	.000	.628	.333															16.7	10^{-6} M IBA
12	.885	.054	.000	.046	.429	.002	.002	.000	.001	.000	.000														72.9	10^{-8} M IBA
13	.000	.001	.720	.000	.000	.002	.047	.000	.495	.155	.925	.000													15.2	10^{-9} M IBA
14	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000												372.3	10^{-3} M NAA
15	.028	.654	.044	.000	.080	.887	.553	.000	.212	.281	.088	.038	.018	.000											46.2	10^{-4} M NAA
16	.008	.000	.000	.087	.000	.000	.000	.974	.000	.000	.000	.006	.000	.000	.000										113.2	10^{-5} M NAA
17	.605	.198	.001	.032	.827	.030	.020	.000	.008	.005	.002	.697	.000	.000	.121	.004									68.0	10^{-7} M NAA
18	.008	.111	.766	.000	.021	.189	.329	.000	.710	.492	.997	.011	.950	.000	.201	.000	.025								16.6	10^{-9} M NAA
19	.643	.246	.002	.066	.855	.033	.038	.001	.015	.012	.004	.726	.000	.000	.159	.008	.996	.031							67.9	10^{-4} M 2,4-D
20	.001	.042	.787	.000	.003	.084	.203	.000	.609	.357	.937	.001	.991	.000	.167	.000	.005	.951	.008						15.0	10^{-5} M 2,4-D
21	.034	.987	.011	.000	.105	.385	.212	.000	.074	.068	.020	.049	.000	.000	.639	.000	.183	.107	.242	.039					52.0	10^{-6} M 2,4-D
22	.000	.027	.342	.000	.000	.065	.304	.000	.988	.620	.566	.000	.381	.000	.135	.000	.001	.680	.004	.560	.022				25.3	10^{-7} M 2,4-D
23	.000	.025	.350	.000	.000	.061	.293	.000	.996	.603	.579	.000	.394	.000	.129	.000	.001	.650	.004	.570	.021	.981			25.6	10^{-8} M 2,4-D
24	.002	.267	.089	.000	.006	.576	.955	.000	.388	.555	.160	.003	.047	.000	.595	.000	.026	.318	.047	.195	.249	.290	.279		39.1	10^{-9} M 2,4-D
25	.044	.000	.000	.653	.001	.000	.000	.055	.000	.000	.000	.030	.000	.000	.000	.158	.021	.000	.045	.000	.000	.000	.000	.000	93.9	CONTROL
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	

4.6 Relationship between callus formation and rooting

Regression analysis between the number of callused cuttings within the first 16 weeks after treatment and the number of rooted cuttings during the second 16 weeks showed that only 20.70% of the variation in rooting of the cuttings during the second 16 weeks could be explained by the variation in callused cuttings observed during the first 16 weeks ($R^2 = 0.2070$; $p \leq 0.05$) (Fig. 4). Most of the cuttings with well developed calli did not root.

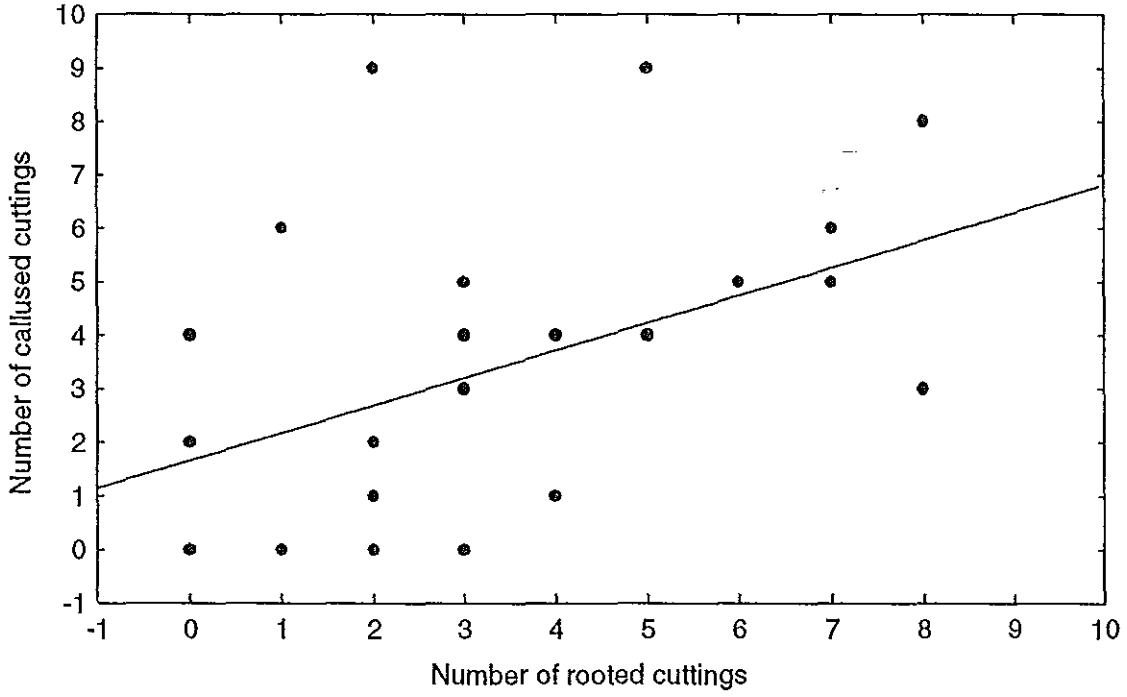


Figure 4. Relationship between callusing and rooting of callused cuttings. Cuttings were collected from Class I source plants and assessments on callusing and rooting were made 16 and 32 weeks after treatment, respectively.

4.7 Histological origin of calli and root primordia

The differentiation of adventitious roots in the cuttings arose either from callus mass or from non-callused cut ends. Calli developed from and expanded throughout the cortical layer. Some of the cells in the callus tissue gave rise to root primordia which latter developed into root proper (Fig. 5).

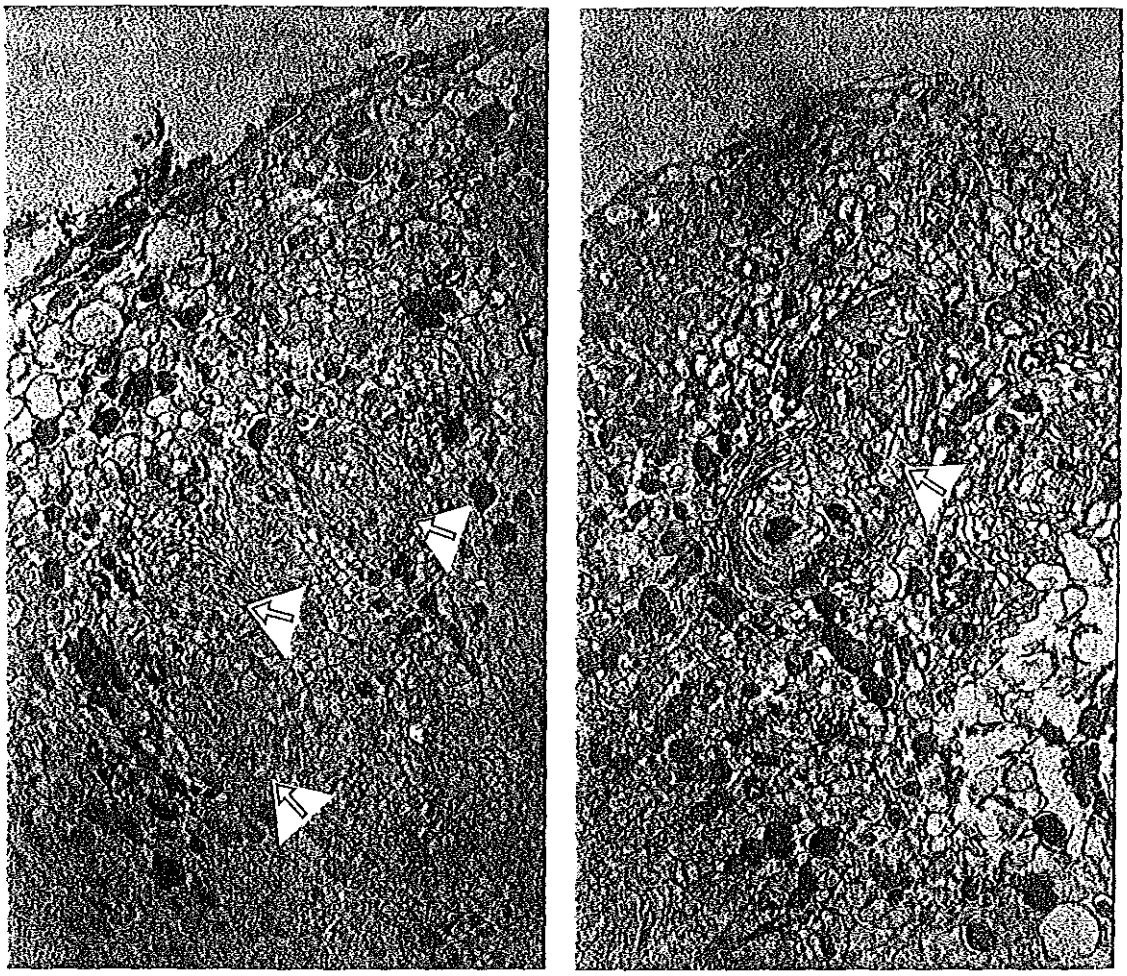


Figure 5. Transverse section of stem base showing development of callus mass from the cortex (left) and differentiation of some calli cells into root primordium (right). Arrows indicate calli and root primordium.

In the non-callused cuttings, the origins of root primordia were from cells of a variety of tissues. Cells of xylem rays, tracheids, cells of cortical and cambial tissues resulted in root primordia (Fig 6).

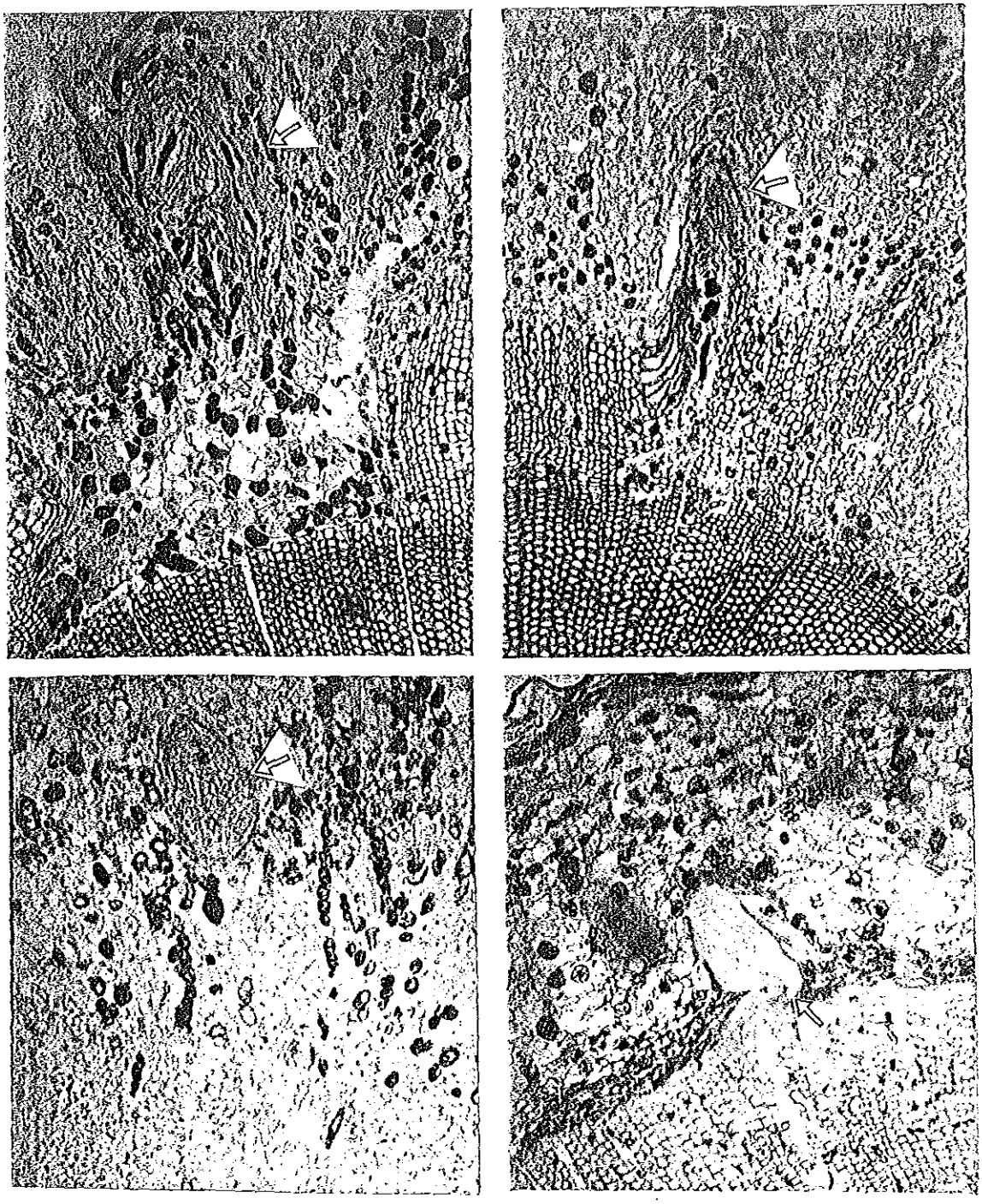


Figure 6. Transverse section of stem base showing the origin of root primordia. Origin of root primordia from xylem rays (top left), tracheids (top right), cortical tissue (bottom left), and cambial tissue (bottom right). Arrows indicate callus mass and root primordia.

4.8 Establishment and performance of the rooted cuttings

Rooted cuttings that had well developed adventitious roots established and grew faster than those with fewer adventitious roots. Eighty eight percent of the rooted cuttings were successfully established. Those which failed to establish were the ones which had fewer roots. Variations in the number of adventitious roots had significant contribution to the variations in the rate of height increment of the stecklings ($R^2 = 0.4963$; $p \leq 0.05$) (Fig. 7).

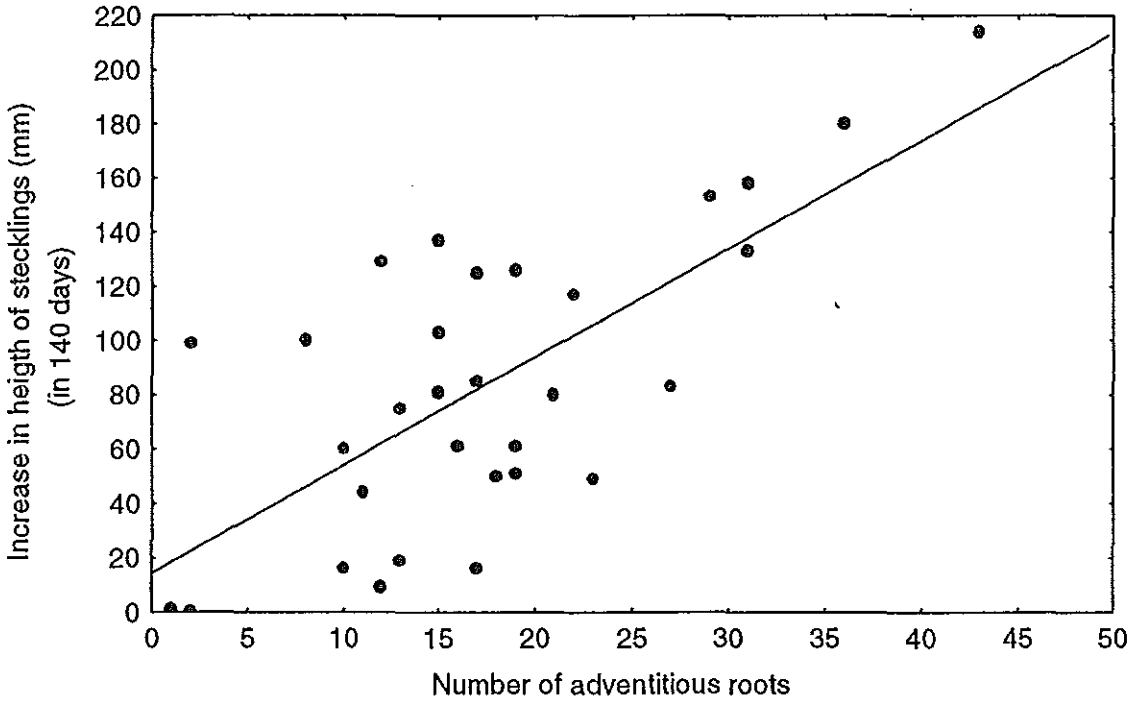


Figure 7. Regression line on the effect of adventitious root number on growth of stecklings.

5 DISCUSSION

5.1 Survival of the cuttings

Most of the cuttings obtained from Class II and Class III source plants could not survive throughout the time of the study. But, limited number of cuttings obtained from Class I source plants died.

The kinds and concentrations of the PGRs used had important contribution for the death of the cuttings. Regardless of the source of cuttings, IBA, NAA and 2,4-D at higher concentrations (10^{-3} M) killed many of the cuttings. The adverse effects of these PGRs at this concentration (manifested through bronzing the cuttings) was observed as early as 48 to 60 hours after treatment. Cuttings obtained from all the classes of source plants were totally killed by 10^{-3} M of 2,4-D. IBA and NAA at this concentration killed all the cuttings obtained from Class II and Class III source plants. On the other hand, only 50 and 25% of the cuttings obtained from Class I which were treated at 10^{-3} M IBA and NAA, respectively, survived the combined effects of internal and external factors.

5.2 Effects of age of the source plants on callusing and rooting

The most important factor that affected callusing and rooting of cuttings was the age of the source plants from which the cuttings were obtained. The cuttings obtained from Class I and Class II source plants belong to stocks of different developmental phases, juvenile and adult phases, respectively. It is indicated that, the mean percentage of cuttings callused obtained from Class I source plants is about 2.2 times more than that

of the cuttings obtained from Class II source plants. When the rooting response is considered, the difference is even far more pronounced, where only one cutting was rooted in those obtained from Class II.

It is well documented that cuttings collected from juvenile source plants are easier to root than those collected from mature ones (Edmond *et al.*, 1964; Leopold and Kriedemann, 1975; White and Lovell, 1984a,d,e; Welander and Snygg, 1987; Ahuja, 1991; Haung *et al.*, 1992; Kassa Semagn and Legesse Negash, 1996). For instance, *Agathis australis* (D. Don.) Lindl. and *G. lucida* cuttings taken from seedlings were rooted more easily and with shorter lag phase than those taken from older plants (White and Lovell, 1984a,d,e). It was also reported that the rooting ability of cuttings of *P. falcatus* is dependent on the age of the source plants (Kassa Semagn an Legesse Negash, 1996).

The superior rooting ability of cuttings obtained from juvenile stocks as compared to those obtained from older stocks is believed to be due to higher endogenous auxin content in the former than in the latter (Pilet, 1958: cited in Leopold and Kriedemann, 1975). It was also reported that application of exogenous auxin did not restore readiness to rooting of cuttings of mature ivy (*Hedera helix* L.) (Hess, 1964: cited in Leopold and Kriedemann, 1975). The results of Kassa Semagn and Legesse Negash (1996) also agree with these findings, which indicate that the decrease in rooting ability is not (only) hormonal.

Other explanations for the difficulty of rooting in cuttings from mature stocks may be due to an increase in lignified tissues so that morphological changes are slowed down or prevented are (White and Lovell, 1984a). Also, because of the presence of abundant resin canals, sclerenchyma cells and branch traces, the amount of parenchyma tissue is reduced to such a low level that the potential primordial sites are limited (White and Lovell, 1984e). It was reported that shoots of difficult-to-root species are frequently characterized by high degree of sclerification (i.e. differentiation into fibres and scleroids) of the primary phloem of the stem (Beakane, 1961). Haissig (1983) proposed that the poor rooting behaviour of cuttings from mature woody perennials is because of lack of a non-auxin endogenous root forming stimulus. This means that, the physiology of rooting would be insensitive to normally promotive endogenous and applied auxins. This stimulus is believed to be biophysical or anatomical (Haissig and Riemenschneider, 1992).

The other suggested factor is related to the decrease in the responsiveness of the mature tissues in cuttings obtained from older source plants to root promoting substances (Leopold, 1963). Zajczkowski (1973) reported that the cambium of cuttings from the apex of a stem of *Pinus sylvestris* L. to be more sensitive to auxin than those taken from the base, indicating that the difference may not be due to the variation in auxin level.

5.3 Effects of PGRs

As it has already been shown, the effects of the various treatments in each of the four PGRs and the control on rooting response, mean root number and mean length of roots

after 32 weeks were compared using cuttings obtained from Class I source plants. Cuttings treated with the different concentrations of IAA showed better rooting responses than those treated with the various concentrations of IBA, NAA and 2,4-D. Also, six of the seven treatments in IAA resulted in greater rooting response than the control. On the other hand, the effects of most of the various concentrations of IBA, NAA and 2,4-D on rooting were lower than that of the control. Out of the 20 treatments considered in this group, only four treatments gave rooting percentages greater than the control (Fig. 3). This may suggest that the effects of IBA, NAA and 2,4-D on the rooting of the cuttings of *J. procera* is not important.

PGRs differ significantly in their stability, effectiveness and influence on organogenesis (Murashige, 1974). Indoleacetic acid which is known to stimulate cell division in responsive tissue is preferred by many workers (Digby and Wareing, 1966b; Torrey and Loomis, 1967). This is because it shows minimum adversity on organ formation. But this is the weakest auxin and is readily destroyed by light and enzymatic activities (Murashige, 1974; Leopold and Kriedemann, 1975; Weisman *et al.*, 1989). However, though this hormone is metabolized very quickly, its metabolite, (indole-3-acetylaspartic acid) served as a source of IAA during most of the rooting process in mung bean cuttings (Weisman *et al.* 1989). Regardless of the fact that IAA is more popular, many workers have reported better success in rooting of cuttings treated with IBA (Swingle, 1940; Hartmann and Kester, 1975; Leopold and Kriedemann, 1975; Hartmann *et al.*, 1990; Kassa Semagn and Legesse Negash, 1996). The reason for this is believed to be due to its weak auxin activity and relatively more stability to the effect of auxin destroying enzyme systems. Like IAA, IBA has an

important intermediate metabolite called indole-3-butyrylaspartic acid, which serves as a source of IBA in the rooting process (Weisman *et al.*, 1989).

On the other hand, NAA is more resistant to the effect of auxin-destroying enzymes than IAA and IBA. However, its effect on rooting is less than the two PGRs (Leopold and Kriedemann, 1975). Similarly 2,4-D is the most potent, especially in stimulating callus formation, but strongly antagonizes organized development (Murashige, 1974).

In this investigation, the rooting responses of the cuttings subjected to various treatments of the different PGRs didn't show clear patterns. Consequently, the optimal concentration for better rooting could not be identified. In fact, when IAA and 2,4-D are considered, higher rooting percentages are observed at concentrations below 10^{-5} and 10^{-6} M, respectively. While in the case of IBA it seems that higher concentrations (10^{-3} to 10^{-5} M) are better, but the effect of NAA is more random than any of the PGR used in this work.

Different workers on various propagation studies reported better effects of IAA at lower concentrations. In this case, the stimulation of the growth of cocos (*Cocos nucifera* L. cv Mayalan Dwarf) explants at 10^{-7} M than at higher concentrations (Eeuwens, 1978); the production of greater fresh weight of callus tissue in soybean at 5.7×10^{-6} M than at 2.85×10^{-5} or 5.7×10^{-5} M (Erez, 1978); better promotive effects on the elongation of needle primordia of *Picea abies* (L.) Karst. at 10^{-6} or at 5×10^{-6} M than at higher concentrations (Anrold and Erickson, 1979); the better stimulation of root initiation in sunflower (*Helianthus annuus* L.) hypocotyl cuttings at 10^{-6} M than at

10^{-5} M and 10^{-4} M (Fabijan *et al.*, 1981); and, the presence of lower concentration of IAA in best rooted ex-adult shoots of apple rootstock (A2) (Welander and Snygg, 1987) agree with the results of the present work.

In the case of 2,4-D, it has similar effect on cocos explants at 10^{-7} M (Eeuwens, 1978). Similarly, callus initiation and growth of organ- and seedling-derived calli in rice were optimally stimulated by 2.7×10^{-8} and 9×10^{-9} M (Henke et al., 1978). The formation of adventitious roots in leaf explants from *Triticum aestivum* L. cv Kite was better at 1.6 to 6.3×10^{-7} M than at concentrations $\geq 10^{-6}$ M (Wernicke *et al.*, 1986). When the effect of IBA is considered, largest number of meristemoids and/or root primordia in cuttings of *Pinus radiata* D. Don.was formed at 1.48×10^{-5} M of IBA (Smith and Thrope, 1975a). Eliasson (1980) reported greater number of roots per cutting of pea stem at 10^{-4} and 10^{-5} M than at 10^{-6} and 10^{-7} M. Also, the optimum concentration of this auxin for root initiation of apple rootstock was 1.5×10^{-5} M (James, 1983).

When mean root numbers and mean root lengths are considered in relation to concentrations of each of the PGRs, it is not possible to reach at some generalization with decreasing or increasing concentrations. However, the mean root numbers and mean root lengths of the cuttings rooted in the various treatments of the PGRs can be compared with the mean root number and mean root length of the cuttings rooted in the control. With the exception of a single treatment, 10^{-6} M of IAA which gave equal mean number of roots with that of the control, the rest of the treatments resulted in values less than that of it. Eleven treatments resulted in significantly lower mean root number per rooted cuttings ($p \leq 0.05$). Moreover, when the mean root length is

considered, the cuttings rooted in the control treatment resulted in the fourth highest mean root length. It was significantly greater than 20 treatments. This clearly shows that the effects of the various concentrations of these PGRs on mean root number and root mean length are not important. Application of PGRs is important factor in determining the rate and the degree of regeneration phenomena, but it has little, if any, quantitative effect upon the regenerate (Swingle, 1952). Such chemicals simply speed up root production and may induce the development of large number of roots which are expected to root without treatment (Swingle, 1940; Jex-Blake, 1950; Edmond *et al.*, 1964). The result of this work agrees with these findings.

5.4 Relationship between callusing and rooting

It was believed that callusing is a precondition for rooting of the cuttings of this species. Therefore, it was expected that the number of cuttings which would root during the second 16 weeks would be proportional to the number of cuttings callused during the first 16 weeks.

After stem cuttings have been made and placed under environmental conditions favourable for rooting, calli will usually develop at the basal end of the cuttings. The appearance of roots from callus tissue leads to the belief that callus formation is essential for rooting. In most cases, callusing and rooting are independent of each other. They occur simultaneously because of their dependence upon similar external and internal conditions (Hartmann and Kester, 1975). The development of callus tissue in this species was, however, quicker than rooting and was expected to be a precondition for rooting. Despite the development of large callus tissue, most of the

callused cuttings did not root, consistent with the result of Grönroos and Arnold (1985). Regression analysis indicated that callusing cannot be taken as an important precondition for rooting, even though some cuttings developed from callus tissue. Callus formation is believed to be undesirable because:

(1) it lengthens the rhizogenesis process and

(2) the ratio of the potentially organogenic (meristemoid) cells to the total callus mass is very small (Hicks, 1980).

5.5. Histological origin of callus and root primordia

The differentiation of adventitious roots from cuttings of this species takes place from cells of a variety of active tissues. Many documented results indicate that, depending on the kinds of the cuttings, vascular rays, tracheids, cells of cortical, cambial, and callus tissue undergo dedifferentiation and redifferentiate to form root primordia. In addition to being located close or within the vascular tissue, adventitious root primordia occur at the morphological bases of the cuttings, even though they are 1-2 cm long (White and Lovell, 1984b).

In woody perennial stems with one or more layers of secondary xylem and phloem, the first evidence of root primordia is frequently found at the intersection of the vascular rays of the xylem with the phloem (Hess, 1969). In the case of this species, in some cuttings, cells of vascular rays (at the base of the younger xylem ring) underwent division accompanied by the differentiation of some cells into root primordia. On the other hand, tracheid cells of the younger xylem ring differentiated into root tip. Tracheids are known to be important cells of primordium formation. For example,

basal and sub-basal regions in cuttings of *A. australis* underwent a complex series of changes, categorized as wound responses, such as cell division in the interfascicular region. The resulting cells which differentiated into tracheids and phloem which were favourable for primordium formation (White and Lovell, 1984c). Tracheids in the vascular cylinder of hypocotyl cuttings of *P. sylvestris* developed tracheid nests from which root development commenced (Grönroos and Arnold, 1985).

Cortical cells are also known to be involved in the formation of root initials (Hartmann and Kester, 1975). It is indicated that some of the cells of the cortical tissue differentiated into root primordia. In cuttings of *P. radiata* seedlings cortical parenchymatous cells divided to give group of cells called meristemoids which differentiated into root primordia (Smith and Thrope, 1975b). Cambial cells (or their derivatives) are the most important ones where root primordia develop. Here continual cell division takes place, and soon each group of cells take the appearance of root tip (Hartmann and Kester, 1975; Zhou *et al.*, 1992). The differentiation of root primordia from the cambial tissue is also clearly indicated in the cuttings of this species. In this case root primordia originated from the cambial region making connections with the xylem tissue. In a study of adventitious rooting of apple microcuttings, root meristemoids outside the xylem developed into root primordia and make vascular connection with the xylem (Hicks, 1987).

Also, in conifers which are difficult to root, root primordia arise within the basal callus tissue (Sato, 1956: cited in John, 1978). The callus originates from the cambial and phloem cells and latter enlarge by division of the cortical cells (Hartmann and

Kester, 1975). Root primordia develop from the callus. According to Haissig (1974: cited in John, 1978) root primordia in callused conifers are initiated from cells immediately adjacent to the immature xylem present in the callus. Xylem elements differentiated within the callus in connection with the stem xylem (John, 1978). Kassa Semagn and Legesse Negash (1996) have also reported the development of root primordia from callus tissue in cuttings of *P. falcatus*. The formation of adventitious roots in conifer cuttings that involve callus formation follows the following stages (John, 1978):

1. Formation of cortical vascular cambium as a result of rapid division of some cells of the medullary ray.
2. Callus formation accompanying the development of cortical vascular cambium.
3. The differentiation of some of the callus cells near the cortical vascular system.

5.6 Establishment and performance of rooted cuttings

Better adventitious root system was reported to result in better performance of rooted cuttings when transplanted to a field (Palzkill and Feldman, 1993). In *P. falcatus* growth of stecklings was found to be greater than that of seedlings. This difference was indicated to be due to the difference in the number of roots, where the stecklings were with greater number of adventitious roots (Kassa Semagn and Legesse Negash, 1996). In *J. procera*, rooted cuttings with greater number of roots established quickly and grew well. However, another important aspect which deserves consideration is

that, the adventitious roots should be well developed. The start of growth of the rooted cuttings before they were transplanted is believed to be an important indication of functionally and structurally well developed roots. Most of the roots of such cuttings were no more brittle.

6 CONCLUSIONS

1. *J. procera* could be successfully propagated asexually through rooting of branch cuttings. But, the rooting ability of branch cuttings of this species is greatly influenced by the age of the source plants. Cuttings obtained from adult source plants failed to root. Therefore, stock plants at juvenile (seedling) stage are appropriate sources for vegetative propagation of the species through rooting of branch cuttings.
2. The limited number of cuttings rooted during the first 16 weeks shows that the duration for rooting of cuttings of this species is longer like cuttings of narrow-leaved evergreen species.
3. The effect of IAA on rooting of the cuttings of *J. procera* is more pronounced than those of IBA, NAA and 2,4-D. The effects of these PGRs (at most of the concentrations) on the rooting percentage, mean root number and mean root length were less than the control. The use of IAA, at lower concentrations, in vegetative propagation practices of the species is recommendable.
4. In this species, cells of a variety of stem tissues give rise to root primordia. The involvement of cells of such a variety of tissue types in the development of root primordia indicates the ease of the rooting ability of cuttings of the species obtained from appropriate sources.
5. The differentiation of large number of adventitious roots must have enabled the rooted cuttings to exploit water and nutrients from the soil efficiently. This is an important advantage over the limited number of tap roots of seedlings.

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