ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

JUNIPERUS PROCERA HOECHST. EX ENDL.: ROOTING OF BRANCH CUTTINGS UNDER A LOW-COST POLYPROPAGATION SYSTEM

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A Thesis Presented to
the School of Graduate Studies
Addis Ababa University

In Partial Fulfilment of the Requirement for
the Degree of Master of Science in
Biology (Botanical Sciences)

By

Adane Assefa

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ABSTRACT

The rooting responses of branch cuttings obtained from Class I, Class II and Class III stock plants of Juniperus procera Hoechst. ex Endl. to indolebutyric acid (IBA) were examined in sand rooting media contained in the compartments of a modified low-cost polypropagation system. This system is cheap, easy to construct and has no special and complex requirements. The results indicated that rooting of J. procera branch cuttings is promoted by applied IBA. Overall rooting percentage was obtained in cuttings derived from Class III stock plants as compared to cuttings from Class I or Class II stock plants. Hence, 38.9 % of the total cuttings collected from Class III stock plants rooted 21 weeks after inserting these into the rooting medium. Of the cuttings obtained from Class II stock plants, only 27 % were rooted 21 weeks after treatment. The maximum attainable rooting percentage was 50 % and was obtained from cuttings of Class I stock plants when these were treated with 0.2 % IBA. The optimum concentrations required for maximum root initiation and root growth varied with the age of stock plants from which cuttings were derived. Thus, better rooting percentage was observed at 0.2 % IBA for cuttings from Class I stock plants; it was 0.4 % for cuttings obtained from Class II stock plants. Consistent and relatively good rooting percentages were recorded at 0.1, 0.2 and 0.4 % IBA for cuttings collected from Class III stock plants. The largest root number (45) was recorded when cuttings collected from Class I were treated with 0.2 % IBA. Cuttings derived from Class II and treated with 0.8 % IBA yielded relatively longer mean root length (9.9 cm). Well-developed calli were recorded in most of the juvenile cuttings of Class I stock plants. In this species most roots formed directly rather than indirectly.

1. INTRODUCTION

1.1 Vegetative propagation

Vegetative propagation is the production of new plant directly from vegetative parts of existing ones, not from seed. This type of propagation is used to produce a plant identical in genotype with the parent plant (Hartmann *et al.*, 1990; Haissig *et al.*, 1992). Vegetatively propagated trees are an important research tool. Because they are genetically uniform, the effects of environment and internal changes within the tree can easily be separated from inherent effects (Longman, 1993). Vegetative propagation is possible because of two fundamental characteristics of plant cells. These are: every cell contains the genetic potential necessary to generate the entire plant, *totipotency*; and the phenomenon of *dedifferentiation* the capability of already developed and differentiated cells to return to a meristematic conditions. Since these two characteristics are more pronounced in some cells and plant parts than in others, the propagator must do some manipulation to provide the proper conditions for plant regeneration.

There are different methods for the achievement of vegetative propagation. **1**. Cutting - usually by encouraging root and/or shoot to form on a piece of stem, so that it becomes an independent plant; **2**. Grafting - joining a piece of stem or bud to another plant to induce root system; **3**. Budding - to induce root systems from detached pieces of buds or branches from the mother plant; **4**. Planting shoot or roots- when the storage organs are usually separated and replanted; **5**. Suckers - when shoots arise from adventitious roots of a root; **6**. Separating offsets - dividing up plants that form clumps or sets of buds near to the ground level; and **7**. Micropropagation - growing small pieces of tissue in sterile culture, stimulating to form many plantlets (Spurr and Barnes, 1980; Hartmann *et al*., 1990; Longman, 1993).

According to Longman (1993), there are two major merits of vegetative propagation. These are: **1**. To be free to grow a species- many trees flower and fruit rarely; and/or their seeds are not easy to store. So by rooting cutting, plenty of nursery plants can be obtained at any time, without having to rely on seeds. Vegetative propagation is particularly valuable: if pests or diseases destroy most of the seed crop; when fruits or seeds are hard to collect; and with young material, before trees reach the flowering stage. **2**. To get improved trees quickly - most tree species have not undergone selection by mankind; and breeding is much slower than with annuals. Vegetative propagation allows more rapid genetic improvement, because trees with desirable characters can be used directly to produce improved planting stock; direct selection captures more of the desired characters than is possible by seed; with each selected clone existing as many plants, it is easier to check which characters are strongly inherited; clonal trails can provide a more rigorous test than seedling progeny and results can be used at once.

1.2 Propagation by cutting

In propagation by cutting, a portion of stem, branch, root or leaf is cut from the stock plant and induced to form root and/or shoots. In most cases the resulting clones or the new independent plants produced are identical with the parent plant. Cuttings are the most important means of propagating ornamental shrubs, deciduous plant species as well as the broad- and narrow-leaved types of evergreens. It is also used widely in commercial glasshouse propagation of many florists' and foliage crops, certain fruit species, and woody plants (Hartmann *et al.*, 1990). In propagation by branch, stem and leaf-bud cuttings, only a new adventitious root system is formed but in root cutting from its adventitious bud, both a new shoot system and adventitious roots or extending the existing root pieces are initiated. Generally, cuttings can then be classified according to the part of the plant from which they are obtained (Hartmann *et al.*, 1990; Longman, 1993): These are: branch cuttings, stem cuttings, leaf cuttings, leaf-bud cuttings and root cuttings.

For large-scale afforestation program, however, stem and branch cuttings are more applicable and are quite oftenly used. Sometimes root and leaf cuttings can also be used for propagation purposes. In selecting cutting material, it is important to use stock plants that are free from diseases, moderately vigorous, and of known identity. In so doing, stock plants with the following features should be avoided Hartmann *et al.*, 1990). **a**. Plants that have been injured by frost and drought; **b**.

Defoliated by insects; **c**. Stunted by excessive flowering or fruiting; **d**. Stunted by lack of soil moisture or shortage of proper nutrition; and **e**. Plants that have made rank, overly vigorous growth.

Cuttings of softwood (narrow-leaved evergreen species such as *Juniperus* species, *Podocarpus falcatus, Thuja*, etc.), for example, must be rooted under moisture conditions that will prevent excessive drying, as they usually are slow to root. Moreover, cuttings taken from the young seedling stock plants root much more readily than those taken from the older trees because of the juvenility and certain environmental factors. Treatments of cuttings with plant growth substances (e.g., IBA, IAA, NAA) at an optimum concentration are usually beneficial in stimulating the formation of new roots, increasing the speed of rooting, the percentage of cuttings rooted, and obtaining heavier root system (Hartmann *et al.*, 1990; Longman, 1993; Kassa Semagn and Legesse Negash, 1996; Desta Berhe and Legesse Negash, 1998). Systems for the vegetative propagation of *Podocarpus falcatus* (Thunb.) Mirb. and *Juniperus procera* Hoechst. ex. Endl. had already been described (Kassa Semagn and Legesse Negash, 1996; Desta Berhe and Legesse Negash, 1998). The present work on *J. procera* is based on a different system in which, instead of a glasshouse, low-cost polypropagators are used. The design of the propagators is based on that of Leakey *et al.* (1990).

J. procera belongs to the family Cupressaceae. It is distributed in some countries of the African continent fairly well. The distribution of this species generally encompasses Zaire (Haut-Katanga), Sudan (Red Sea Hills, Didnga Mountains), Ethiopia, Djibouti, Somalia, Uganda, Kenya, Tanzania, Malawi and Zimbabwe (one locality only) (Friis, 1992). *J. procera* is common in the eastern slope of the Northwestern Highlands of Ethiopia and along the mountain ranges in Northern Somalia, but often depleted by cutting, burning and encroachment. It is widespread particularly in the Central, Northern, Southeastern parts of Ethiopia, as well as in the western and eastern plateaux of Shoa, Gojjam, Wollo, Tigry, Arsi, Hararge, and parts of Bale (Uhlig and Uhlig, 1991; Demel Teketay, 1992; Sebsebe Demissew, 1997); and rarely around Negelle Borana, Mega and Yabello (Haugen, 1992). The distribution of this species with respect to altitude and rainfall ranges from 1500-3150 m and 500-1500 mm per year, respectively (Uhlig and Uhlig, 1991; Friis, 1992).

In the South-eastern parts of the country, the species is usually dominant in very open forest with discontinuous canopy of the transitional types, associated with *Olea europaea* sub-species *cuspidata, Euclea schimperi, Teclea nobilis, Podocarpus falcatus, Pistacia aethiopica*, etc. (Friis, 1992). In the North and Northern Central Ethiopia *Juniperus procera* is common in church compounds and in small forest patches surrounding the churches (Friis, 1992; Legesse Negash, 1995). Of course in some of these places the trees appear to be planted.

Juniperus procera is a useful tree species particularly in timber production sectors and in other construction activities. It is valued for its resistance to decay and termites, and it is easy to split. The wood is used for many purposes, including for manufacturing lead-pencil, strip and parquet flooring, for out-door works (such as roofing-shingles, fence-posts), plant-trays, water flumes, telephone poles and bridges (Dale and Greenway, 1961). In Ethiopia, timber from *J. procera* had been highly valued for the construction of the Orthodox churches and houses of early nobility (Desta Berhe and Legesse Negash, 1998).

Currently, *Juniperus procera* is facing a number of serious problems including its unsustainable use for timber and furniture. As a result, mature trees are now very rare. Moreover, information concerning the physiological requirements for successful rooting of cuttings and the effect of the stock plant age on rooting of cuttings are not well studied.

2. OBJECTIVES

2.1 General objective

- To evaluate the rooting efficiency of branch cuttings derived from different age classes of *J. procera* in a low-cost propagation system, as well as to identify the optimum IBA treatment for maximum rooting response.

2.2 Specific objectives

a. To evaluate the effectiveness of low-cost polypropagators for use in the vegetative propagation of *J. procera.*

b. To investigate the effects of various levels of IBA on successful rooting of branch cuttings of different age classes of *J. procera.*

c. To study the effect of age of stock plants on rooting of branch cuttings of *J. procera.*

3. REVIEW OF LITERATURE

3.1 Plant propagation

The propagation of plants is a fundamental occupation of mankind. Civilization might have started when ancient man learned how to plant and grow kinds of plants that fulfilled the nutritional needs of him and his animals. As civilization advanced, man added to the variety of plants, thus cultivating not only additional food crops but also those which provided fiber, medicine, recreational values and beauty. From the great diversity and variation in plant life, man has been able to select kinds of plants particularly those that are useful to his welfare.

Much progress in improvement of crop plants was made long before the modern period of plant breeding started (Allard, 1960). Our cultivated plants originated from three general groups of plants. Some kinds of plants were selected directly from wild species, evolved into types that differed radically from their wild relatives (e.g. lima bean, tomato, barley, and rice); other kinds of plants arose as hybrids between species, accompanied by changes in chromosome number (e.g. maize, wheat, tobacco, pear, and prune). Further noted that other group of plants occur naturally in wide geographical areas and may be useful to man, (e.g., cabbage, broccoli, etc.).

However, the progress made in plant improvement had a little significance since there had not been methods whereby improved forms could be maintained in cultivation. Consequently there have been some processes of invention and discovery of techniques for plant propagation. As new advances in propagation techniques developed, the number of plants available for cultivation became increased.

3.2 Basic types of plant propagation

There are two basic types of plant propagation. These are known as sexual and asexual methods of propagation.

3.2.1 Sexual propagation

The sexual cycle utilizes propagation by seeds in which new individual offspring of plants are created whose characteristics reflect the genetic contribution of the parents.

3.2.2 Asexual (vegetative) propagation

The asexual cycle utilizes various vegetative methods of propagation such as cuttings, layering, apomictic embryo, runners, suckers, grafting, separation, budding, micropropagation and others (Spurr and Barnes, 1980; Hartmann *et al.*, 1990; Longman, 1993). Vegetative/asexaul propagation is important for producing plants with genetic homogeneity. It is crucial in horticulture and forestry where desirable genotypes must be produced in large quantities for commercial and agricultural purposes (Haissig, *et al.*, 1992). Vegetative method of propagation enables one to preserve the unique characteristics of any individual parent plants in the offspring plants. This means that the genotypes of the source plants can also be preserved intact (Hartmann *et al.*, 1990; Haissig, *et al.*, 1992).

In propagation by cuttings, in addition to favourable environmental conditions root and/or shoot production may also require application of root or shoot inducing hormones. Rooting stem or branch cuttings is the most important type of vegetative propagation practised for mass production of improved materials within a short period of time (Nanda and Anand, 1970).

3.3 Factors which affect regeneration of plants from stem/branch cuttings

In propagation by cutting, any vegetative part is cut from the stock plant and is induced to form roots and/or shoots by chemical, mechanical, and/or environmental manipulation. It is well recognised that this process of rooting of cuttings is determined by a complex interplay of both external and internal factors (e.g., Molnar and Cumming, 1968; Smith and Thorpe, 1975; Eliasson, 1980, 1981; James, 1983; White and Lovell, 1984a).

Important external factors that control rooting are light, temperature, aeration, moisture, the physical and chemical properties and types of the rooting medium (e.g., Hansen *et al.*, 1978; Rajagopal and Anderson, 1980; Strömquist and Hansen, 1980; Economou and Paul, 1986; Maynard and Bassuk, 1987; Norcini and Heuser, 1988; Hartmann *et al.*, 1990; Davis *et al.*, 1991; Longman, 1993; Shiembo *et al.*, 1996b).

Among the internal factors are plant growth substances, physiological conditions and nutrition of the stock plants, position of cuttings, the age of stock plants, type and size of wood selected, season, and inherent root forming capacity and premature leaf abscission (e.g., Sykey and Williams, 1959; Eliasson and Areblad, 1984; Hong *et al.*, 1986; Thompson, 1986; Greenwood, 1987; Moon *et al.*, 1987; Collet, 1988; Sunil, 1990; Klambt *et al.*, 1992; Longman, 1993).

3.3.1 External factors

3.3.1.1 Moisture

Moisture is one of the factors in plant vegetative propagation program and is the most serious problem confronting the propagators. Loss of water from leaves may reduce the water content of the cuttings to such a low level that they do not survive, although the presence of leaves on cuttings is strong stimulus to adventitious root and bud initiations. Hence, the propagation of plants from leafy cuttings requires that the cuttings retain their turgidity until new roots have developed (Hartmann *et al.*, 1990).

Numerous writers have stressed the maintenance of turgor in the cuttings. It has been remarked "even a slight water deficit which may be insufficient to cause any visual symptoms of distress results in considerable delay or reduction in the rooting response" (Evans, 1952; Loach 1977; Hartmann *et al.*, 1990). Darbyshire (1971) found that IAA oxidase activity was increased and endogenous auxin therefore reduced by increasing water stress (from -0.2 to -1.4.5 MP). This means that root initiation may be decreased by low water potentials or increased water stress. In a review of plant response to water stress, Hsiao (1973) also investigated that cell growth and wall synthesis are severely curtailed by stresses of -0.5 MP or less.

Various methods have been used to ensure an environment that will reduce wilting (O'Rourke, 1949; Hartmann *et al.*, 1990). These are: **(1)** taking cuttings early in the morning when the plant material is in a turgid condition; **(2)** use of closed cases (enclosed frame covered with glass or plastic materials); **(3)** use of bell-jars or large inverted glass jars; or **(4)** covering the rooting container with polyethylene sheets so as to maintain a sufficient increase in relative humidity to give good rooting.

The use of heavy shading in propagation frames, for example, reduces leaf temperatures and decreases the vapour pressure within the leaf, although shading depresses photosynthetic process to provide the cuttings with carbohydrates for the root formation processes. Thus, propagation under polyethylene sheet just below a wooden bed covered with certain leaves ensures that the atmosphere surrounding the cuttings is almost permanently saturated, so that water loss is determined mainly by leaf temperature through its influence on leaf vapour pressure (Loach, 1977).

Water loss from a cutting is determined by the vapour pressure difference between its leaves and the surrounding air as well as by resistance offered by the leaf surfaces (stomata, epidermis, and cuticle). Improvements in propagation techniques have thus been achieved either by decreasing the leaf air-pressure gradient or increasing the leaf resistance by using a variety of anti-transpirant coatings (Loach, 1977).

3.3.1.2 Light

Light is one of the most important physical factors that influences stock plant condition and subsequent rooting of cuttings (Dykeman, 1976; Hansen *et al.*, 1978; Delargy and Wright, 1979; Strömquist and Hansen, 1980; Eliasson, 1980, 1981; White and Lovell, 1984a; Hartmann *et al.*, 1990). However, photosynthesis by cuttings is not an absolute requirement for root initiation processes, because the rate of apparent photosynthesis during this process is too low; but it is probably more important after root initiation has occurred for root development and rapid growth of a rooted liner (Davis and Potter, 1981; Hosoi and Ooishi, 1987; Svensen and Davis, 1989).

It was reported by Davis and Sankhla (1989) that even under normal conditions light intensity has not always promoted rooting, and the required photosynthesis of unrooted cutting is saturated at relatively low PAR levels; hence, high PAR will not increase photosynthesis but could lead to desiccation of cuttings. The inhibitory effect of light on adventitious root formation or reduction of root number in stem cuttings has been observed in a number of plant species (e.g., pine hypocotyl cuttings, pea stem cuttings, and *Pinus radiata* seedling cuttings) both when the irradiation was applied to the intact stock plant before the cuttings are taken or to the base of the cuttings during the rooting period (Kawase, 1965; Eliasson, 1978; Hansen *et al.*, 1978; Strömquist and Eliasson, 1979; Strömquist and Hansen, 1980). This is because light suppresses the action of certain plant growth hormones, including indole-3-acetic acid, IAA (Eliasson, 1980). However, the negative effect of light could be counteracted by the application of a very low concentration of indole-3-butyric acid (IBA). This indicates that light lowered the level of endogenous auxins or it prevented the root-forming action of these auxins (Hansen *et al.*, 1978; Eliasson, 1980).

3.3.1.3 Rooting medium

The medium used for the rooting of stem or other organ cuttings include soil, sand, gravel, peat moss, perlite, compost, shredded bark, sphagnum moss, vermiculite and sawdust. The use of mixtures of some of the media, instead of any one alone, is favourable for the normal development of roots. For example, sand and/or sand mixed media can provide adequate amount of moisture and aeration that favours rooting of cuttings of a large number of plant species (Hartmann *et al.*, 1990).

Hartmann *et al.* (1990) describe four functions of the rooting medium: **(1)** to hold the cuttings in place during the rooting period; **(2)** to provide moisture for the cuttings; **(3)** to permit the penetration to and exchange of air at the base of the cuttings; and, **(4)** to create a dark or opaque environment by reducing light penetration to the cutting base. An ideal propagation medium provides sufficient porosity to allow good aeration and has a high water holding capacity, yet is well-drained and free from pathogens.

3.3.1.4 Temperature

Temperature is one of the most crucial factors that maintain maximum root inducing capacity of certain growth regulators (e.g., IAA, IBA, NAA, etc.) the activity of which can decrease radically when the temperature is below or above the optimum level. A day air temperature of 21 to 27^0 C and night temperature of 15^0 C is satisfactory for rooting of most species, although some species rooted better at low temperature (Hartmann *et al.*, 1990). In cutting beds, some types 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).

3.3.2 Internal factors

3.3.2.1 Plant growth substances

The induction of adventitious root and/or shoot in cutting is a key step in clonal propagation of plants especially for woody plants that are difficult to propagate or to root (Jones *et al.*, 1979; Striskandarajah, 1983; Welander, 1983; Samartin *et al.*, 1986; Collet and Le, 1987). The discovery that natural and synthetic auxins stimulated the production of adventitious roots in cuttings was a milestone in the history of clonal propagation. The response, however, is not universal; cuttings of some difficult-to-root species still root poorly after treatment with auxin. Hence, auxin is not always the limiting chemical component in rooting (Hartmann *et al.*, 1990).

Before the discovery of synthetic root-promoting auxins, many chemicals were tried to induce adventitious roots with limited success (Kefford, 1973). In 1933 for example, Zimmermann showed that certain unsaturated gases, such as ethylene, carbon monoxide, and acetylene, stimulate initiation of adventitious roots as well as development of latent, pre-existing root initials (Zimmermann, 1933: cited in Hartmann *et al.*, 1990). Later, various classes of growth regulators, such as auxins, cytokinins, gibberellins, and ethylene, as well as inhibitors, such as abscissic acid and phenolics, were known to influence root initiation.

Several researchers reported on some natural or synthetic growth factors that are important for root induction (e.g., Leopold, 1955; Wareing and Phillips, 1970; Haissig, 1974; Friedman *et al.*, 1979; Le, 1985; Wiesman *et al.*, 1989). 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 acids. Phenoxy compounds such as *para*-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid; naphthanol compounds for example, ∝-naphthaleneacetic acid are also known to induce rooting. These auxins are the only applied phytohormones that consistently enhance root primordia development at least in naturally responsive (i.e., easy-to-root) tissues and cause a directional movement of nutrients (Davis and Wareing, 1965), apparently having an effect on the transport system of the cuttings. The main effect of auxin was thus to form a general "root sink" at the zone of its application by facilitating the transport of assimilates and promoting rooting of cuttings through increasing the availability of sugar at the site of root formation (Altman and Wareing, 1975).

However, this universality of auxin effect is sometimes hindered by other factors that limit root initiation. Some of these are nutritional in their effect such as carbohydrates and nitrogenous substances, while others may be considered as growth co-factors or auxin synergists (Van Overbeek *et al.*, 1946; Altman and Wareing; 1975). Moreover, the type and the concentration of auxin, length of treatment time taken for the induction, as well as the types of cultivars could also greatly influence the results of the subject under investigation (Welander and

Huntrieser, 1981; Welander, 1983; Hartmann *et al.*, 1990; Zhou *et al.*, 1992).

3.3.2.2 Rooting co-factors

The rooting ability of cuttings depends on some factors called "rooting co-factors" or auxin synergists that are produced in plants, mainly in the leaves and buds (Hartmann *et al.*, 1990). Tests using ultraviolet spectrum analysis and infrared spectroscopy have indicated that this rooting co-factor is a complex structure of high molecular weight, and possibly is a condensation product between the applied auxin and a phenolic substance produced by the buds (Nanda *et al.*, 1971; Hartmann *et al.*, 1990).

The action of phenolic compounds in root promotion could be, at least partly, in protecting IAA from destruction by the enzyme, IAA oxidase (Donoho *et al.*, 1962; Gorter, 1962, 1969; Fadl *et al.*, 1979). On the other hand, the synergists are kinds of preparatory action, which make more cells able to react to the auxin (Gorter, 1969; Hartmann *et al.*, 1990); and they activate the conversion of tryptophan to IAA (Gorter, 1969) and slow the conjugation of auxins with other compounds (Hess, 1965). Ethylene has certain synergistic characters with auxin in that it is involved in the polar transport (Morgan and Gausman, 1966; Burg and Burg, 1967; Beyer, 1973), and on the rate of metabolism and complex formation of IAA (Beyer and Morgan, 1970; Epstein, 1982; Riov, *et al.*, 1982); but ethylene differs from auxin as it also has indirect negative effects (Nordström and Eliasson, 1984). Ethylene makes again strong synergistic interaction with auxin in regulating cell divisions during the formation of root primordia (Linkins *et al.*, 1973).

Furthermore, phloroglucinol (1,3,5-trihydroxybenzene), a phenolic material was observed to act synergistically with IBA in stimulating adventitious root initiation (James and Thurban, 1981; Zimmermann and Broome, 1981). It was again recognised that shoot culture developed in the presence of phloroglucinol subsequently rooted better than shoot grown in its absence (Welander and Huntrieser, 1981).

3.3.2.3 Nutrition of stock plants and cuttings during rooting

Since rooting is a developmental process, it has been difficult to quantify the effect of nutrition on root primordia initiation versus root primordia elongation (Hartmann *et al.*, 1990). However, it is obvious that growth and development processes of root primordia demand nutrients.

Therefore, the initiation of adventitious roots in cuttings is dependent on auxins, sugars, and nitrogenous substances, as well as on other growth factors and auxins synergists which are usually supplied by the leaves or from outside and may accumulate in the root-forming zone of the cuttings (Ikegami, 1969; Nanda *et al.*, 1971; Hartmann *et al.*, 1990). Although the relationship between carbohydrates and adventitious root formation remains controversial, the carbohydrate pools of free reducing sugars (soluble carbohydrates) and storage carbohydrates or starches (insoluble carbohydrates) are extremely important for rooting as well as for building blocks of complex macromolecules, structural elements, and energy sources (Van Overbeek *et al.*, 1946; Greenwood and Berlyn, 1973; Greenwood, 1987; Norcini and Heuser, 1988). That is probably why an increase in rooting success has often been observed when stock plants are maintained under optimum nutrition prior to the collection of cuttings (Hartmann *et al.*, 1990), and cuttings are given favourable conditions for photosynthesis, achieved by enriching the atmospheric carbon dioxide concentration (Molnar and Cumming, 1968). In contrast, it was observed that rooting is sometimes inhibited by environmental factors that might be expected to result in high rate of photosynthesis and high levels of carbohydrates in the cuttings prior to rooting. For example, Nanda and Anand (1970), Hansen and Eriksen (1974), and Hansen *et al.* (1978) showed that in certain plant species (e.g., *Pisum sativum* and pine cuttings), root formation was inhibited when the stock plants were kept at high irradiance over the range 7-60 W m^{-2}. These workers suggested that supra optimal carbohydrate contents were responsible for the inhibition of root formation.

With many species, carbon dioxide enrichment of the stock plant environment, on the other hand, has increased the number of cuttings that can be harvested from a given stock plant, although there is considerable variation of rooting response among species.

3.3.2.4 Age of stock plants

In difficult-to-root woody plant species (e.g., conifers), ease of adventitious root formation declines with age of parent stock, results in propagation problem, and rooting is a slow process in conifers compared to herbaceous plants, because cuttings of most conifers produce roots indirectly via callus (Satoo, 1956). However, it has relatively little effect on easy-to-root plants (Paton *et al.*, 1970; Morgan *et al.*, 1980; Kennedy and Selby, 1985; Greenwood, 1987; Kwon *et al.*, 1987; Moon *et al.*, 1989). Experiments with apple, pear, eucalypts, live oak, Douglas fir, *J. procera; P. falcatus*, and many other plant species have shown that the ability of cuttings to form adventitious roots decreases with increasing age of parent plants (Paton *et al.*, 1970; Hartmann *et al.*, 1990; Kassa Semagn and Legesse Negash, 1996; Desta Berhe and Legesse Negash, 1998).

In general, age characteristic of stock plants in relation to rooting may be explained by an increase in production of root inhibitors as the plant grows older and hence stem cuttings taken from young seedlings of a number of plant species (e.g., Eucalyptus species), root easily, but as the stock plants become older rooting decreases dramatically (Paton *et al.*, 1970).

3.4 Histological origin and initiation of root primordia

3.4.1 Initiation of root primordia

Rooting is often the limiting step even in whole plant regeneration *in vitro* that provides an alternative to rooted cutting for propagation of forest trees, particularly of conifers (Grönoors and von Arnold, 1985). Insufficient rooting and large variations in rooting have been reported in different experimental investigations (Smith and Thorpe, 1975). Therefore, a better understanding of the various stages before and during root formation is needed.

Although rooting is a very slow process in conifers compared to herbaceous plants, adventitious roots form naturally on various kind of plants (Hartmann *et al.*, 1990). These adventitious roots are two types: **(1)** preformed roots, and **(2)** wound roots. The preformed roots develop naturally on stems, while the wound roots develop only after the cutting is made, in response to the wounding effect in preparing the cuttings (Davis *et al.*, 1982; Hartmann *et al.*, 1990).

It has long been recognised that the process of adventitious root formation can be divided into at least two developmental stages: the first is the initiation of primordia following cutting or wounding, and the other is a stage of root emergence (Mohammed and Eriksen, 1974; Smith and Thorpe, 1975; James and Thurban, 1979).

Therefore, from a survey of the literature, one can summarize the following sequence of events that may eventually lead to root initiation: **(1)** necrosis near the cut surface; **(2)** cell expansion; **(3)** cytoplasmic movement; **(4)** tracheid differentiation; **(5)** wound tissue growth; **(6)** periderm differentiation; and **(7)** tracheid-nest differentiation (Dalgas, 1973; Smith and Thorpe, 1975; Brutcsh *et al.*, 1977; Cline and Neely, 1983; Montain *et al.*, 1983a, b; Hartmann *et al.*, 1990).

The time period preceding the formation of locus (i.e., stage 1, 2, and 3), can be considered as the pre-initiative phase. The formation of the meristematic locus with subsequent changes in the peripheral cells (stages 4, 5, and 6) constitutes the initiative phase, and the development of the meristemoids and their ultimate differentiation to form root primordia (stage 7), comprises the post-initiative phase. However, not all of these steps have been shown to occur in the same species and hence it is not certain that all species have the same developmental pattern (Grönroos and von Arnold, 1985).

There is also a direct root initiation reported from other tissues such as cambium, parenchyma formed by meristematic activity of cambium and phloem cells, parenchyma in the proximity of vascular elements, parenchyma external to differentiating resin ducts, and tissue close to differentiating resin ducts (Dalgas, 1973; Smith and Thorpe, 1975; Montain *et al.*, 1983a, b; Desta Berhe and Legesse Negash, 1998).

3.4.2 Origin of root primordia

The precise location inside the stem where adventitious roots originate has intrigued plant anatomists for decades (Hartmann *et al.*, 1990). The first study was made by Duhamel du Monceau (1958: cited in Hartmann *et al.*, 1990), a French dendrologist. Thereafter, studies of adventitious root formation of a variety of plant species suggested that initiation usually takes place in the vicinity of differentiating vascular tissues of the organ from which the root arises (Hartmann *et al.*, 1990).

The point of origin of root initial varies between species, and even within the same plant. For example, in herbaceous plants adventitious roots usually originated just outside and between the vascular bundles, but the tissue involved at the site of origin can vary widely depending upon the kind of plant. For example, in tomato, pumpkin, and mung bean adventitious roots arise in the phloem parenchyma, in coleus they originate from the pericycle, in crassula they arise in epidermis, in carnation cuttings they arise in a layer of parenchymatous cells inside a fiber sheath, and in castor bean cuttings roots arise from the vascular bundles (Blazich and Wright, 1979; Hartmann *et al.*, 1990).

In conifers, especially those that are difficult to propagate, root primordia arise usually within basal callus (Satoo, 1956). In *Juniperus procera*, for example, some of the cells from within callus mass gave rise to root primordia which, eventually, developed into roots (Desta Berhe and Legesse Negash, 1998). In other woody perennial plants adventitious roots in stem or branch cuttings usually originate from living parenchyma cells, but sometimes from vascular rays or xylem, cambium, phloem, lenticels, or pith (Ginzburg, 1967; White and Lovell, 1984b; Kassa Semagn and Legesse Negash, 1996; Desta Berhe and Legesse Negash, 1998).

3.5 Significance of plant propagation by cutting

Vegetative propagation of plants from stem or branch cuttings has been used extensively in the horticultural industry on both woody and herbaceous plant species (Allan, 1978). The production of rooted stem cuttings of conifers has become important for producing plants for experimental purposes (Kleinschmit, 1974; Longman, 1993). Vegetative propagation can also be used to speed-up the introduction of genetically superior stock (Allan, 1978).

Hence, the advantages of vegetative propagation may be summarised as follows: **(1)** many new plants can be stared in a limited space from a few stock plants; **(2)** it is inexpensive, rapid, and simple, and does not require the special techniques necessary in grafting, budding or micropropagation; **(3)** it has no problem of incompatibility; **(4)** a greater uniformity is obtained by absence of the variation which sometimes appears as a result of the variable seedling; **(5)** the parent plant is usually reproduced exactly with no genetic change; **(6)** selection and maintenance of clones is relatively easy; **(7)** shortens the time of reproductive maturity; and, **(8)** clonal variation is possible (Hartmann *et al.*, 1990).

Genetic uniformity within monoclonal plantations can, on the other hand, be a disadvantage because members of a clone have uniform susceptibility to insects, disease, or other environmental hazards (Duvick, 1978; Zobel *et al.*, 1987).

4. MATERIALS AND METHODS

4.1 Development of stock plants

Stock plants were raised from rooted cuttings obtained from the previous study conducted in the campus of Science Faculty, Addis Ababa University. The plants were maintained in polythene bags (diameter, 23 cm; depth, 12 cm) filled with 2.3 kg of a mixture of red soil (collected from Gullalle area) and horse dung in a ratio of 3:2. The stecklings were categorised into Class I (9-month-old), Class II (11-month-old), and Class III (12-month-old) and were let to develop in the glasshouse (Fig. 1). The mean minimum and maximum temperatures of the glasshouse were 21.3^0 C and 24.4^0 C, respectively; and the relative humidity (RH) was 62 to 85 % (measured with an automatic testo 452 ARMATHE RM GUNTHEL GMBH, Germany). This RH range of the glasshouse was maintained throughout the experimental period by spraying the glasshouse with tap water.

Fig. 1. The three age class of stock plants in the glasshouse

4.2 Construction of low-cost polypropagators

Two polypropagators were constructed following the modified model of Leakey *et al.* (1990). The propagators are a wooden frame that was internally divided into six compartments for allowing the independent use of the rooting media with various treatments (Fig. 2). The bases and sides of the compartments were covered with thick polythene sheets so as to make them watertight. The external parts of propagators were covered tightly with transparent polythene sheets.

The polythene sheets at the base of the compartment were covered with a thin layer of sand so as to prevent the sheets from being punctured by the large stones that were placed on them. Successive layers of small stones and gravel then covered the stones. The gravel provided support for the rooting medium that constituted the uppermost layer. Hollow plastic tube (diameter, 5 cm; length, 52.5 cm) provides an open system down to the base of each compartment. The tube was used for observing the water level and for adding water when necessary during the rooting period.

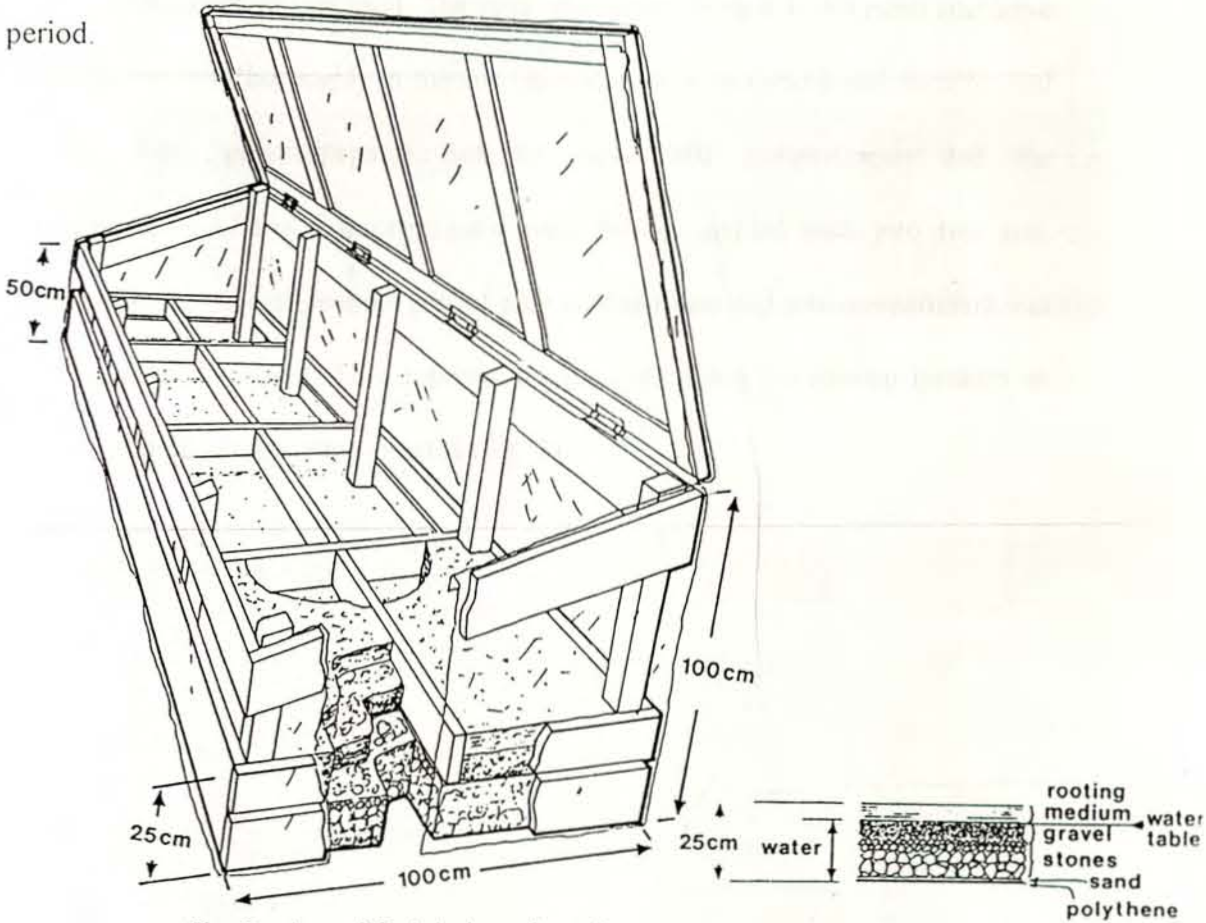

Fig. 2. A modified design of a polypropagator

(After Leakey *et al.*, 1990)

The mean minimum and maximum temperatures of the polypropagators during the whole study period were 19.5 ± 1.4 ^{0}C and 23.1 ± 1.6 ^{0} C, respectively; and the RH ranged from 70 to 85 %. Both polypropagators were kept under partial shading by making a wooden bed with a height of 1.5 m and a length of 3.6 m. The bed was then covered with leaves of *Phoenix reclinata* Jacq.

4.3 Rooting medium

The rooting medium was sand. The sand was sieved using a 1 mm mesh size sieve and was washed thoroughly in running tap water so as to remove soil particles and debris. Each polypropagator had six equal-sized compartments and the compartments of the first propagator were further divided each into two sub-compartments. The uppermost part of each compartment and sub-compartment was then layered with sand. This uppermost layer constituted the rooting medium on which branch cuttings were inserted (Fig. 3).

Fig. 3 Preparation of rooting medium in the compartments of a polypropagator

4.4 Preparation of indole-3-butyric acid

Stock solution of 0.80 % w/v of IBA (Sigma Chemical Company St. Louis, MO, USA) was prepared by dissolving the required weight of the chemical in a 1:1 mixture of absolute methanol and ethanol alcohol. The various concentrations of IBA solutions namely, 0.05 %, 0.10%, 0.20 %, and 0.40 % were prepared by serial dilution from the 0.80 % stock solution. All solutions were prepared a day before they were used and were kept in a refrigerator at 4^{0} C until use.

4.5 Preparation and treatment of cuttings

Firm branch cuttings, measuring 8 to 17 cm in length, and 0.2 to 0.6 cm in diameter were separately collected from Class I, Class II and Class III stock plants. The number of branch cutting obtained from a single stock plant ranged from 4 to 8. Angular cuts were made at the base of the branches with sharp scalpels. All branchlets near or at the base of the cuttings were removed so that insertion of the cuttings into the rooting medium was facilitated. The bases of 240 branch cuttings of Class I stock plants (40 branch cuttings X 6 treatments), 300 branch cuttings of Class II stock plants (50 branch cuttings X 6 treatments), and 180 branch cuttings of Class III stock plants (30 branch cuttings X 6 treatments) (Table 1 and 2) were treated with 10 μl of 0.00, 0.05, 0.10, 0.20, 0.40, and 0.80 % of indolebutyric acid (IBA), per cutting using a micropipette. The control treatment was to use mixture of methanol and ethanol alcohol only.

4.6 Experimental layout

Table 1. A chart showing number of Class I and Class III branch cuttings versus IBA concentrations.

IBA (%)	First polypropagator		Total
	Number of Class I branch cutting	Number of Class III branch cutting	
0.00	40	30	70
0.05	40	30	70
0.10	40	30	70
0.20	40	30	70
0.40	40	30	70
0.80	40	30	70
Total	240	180	420

Table 2. A chart showing number of Class II branch cuttings versus IBA concentrations.

IBA (%)	Second polypropagator	Total
	Number of Class II branch cutting	
0.00	50	50
0.05	50	50
0.10	50	50
0.20	50	50
0.40	50	50
0.80	50	50
Total	300	300

The IBA concentration has been randomly varied among the compartments but has not been varied between the sub-compartments of a compartment. The alcohol from the treated cuttings was evaporated by using an air pump device (Capex 2D-C, Charles Austen Pumps Ltd. England). Cuttings were then inserted into the rooting medium contained within the compartments of the polypropagators (Fig. 4), and each inserted cutting was labelled with a serial number for ease of data collection (Fig. 5). The total number of branch cuttings used for this investigation was 720.

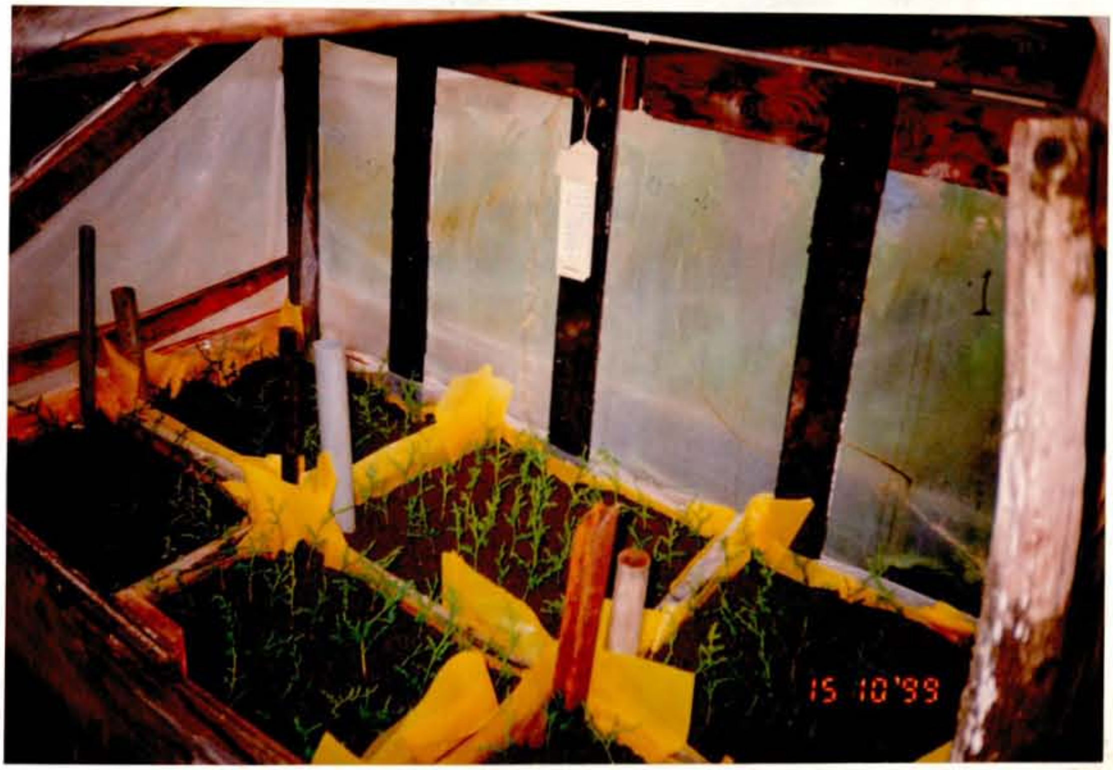

Fig. 4 Fresh cuttings of *J. procera* inserted into the rooting medium

To maintain the optimum temperature and RH of the propagators constant, the rooting media were regularly provided with tap water at least once in three days. The branch cuttings were also sprayed with a fine spray of water twice each day by using a hand-held sprayer. To avoid drastic reduction in RH, the polypropagators were closed at all times. Throughout the study period cuttings were not supplemented with mineral nutrients.

Fig. 5 Labelled branch cuttings of *J. procera* in the rooting medium

4.7 Data collection and statistical analyses

The first assessments for callus development, callus color, number of survived cuttings, number of rooted cuttings, rooting intensity and root length were made by removing the cuttings from the medium five weeks after treatment. Thereafter, assessments were made every fortnight. After data collection, all the cuttings were replaced in their respective compartments and sub-compartments. Results were then subjected to analysis of variance followed Duncan's Multiple Range Test using STATISTICA (StatSoft Tulsa, Oklahoma, USA).

5. RESULTS

5.1 Callusing and root initiation

The first calli appeared in cuttings obtained from Class I, Class II and Class III stock plants during the first 5 weeks, although the number of callused cuttings was very limited. Well-developed calli were observed in most cuttings at about week 17, especially in juvenile stock plant cuttings (Fig. 6).

The calli were white, red and yellow (Table 3). Of these, white calli were the most dominant ones followed by red and yellow calli. The total numbers of white calli were 65, 52 and 62 for cuttings obtained from Class I, Class II and Class III stock plants, respectively. The corresponding respective red calli were 41, 47 and 53. Only 6 and 1 yellow calli were recorded in cuttings collected from Class I and Class III stock plants, respectively. However, no yellow callus was observed in cuttings obtained from Class II stock plants.

Table 3. Color and number of calli in cuttings derived from Class I, Class II and Class II stock plants of *J. procera*.

Callus color	Age of stock plants			Total
	Class I	Class II	Class III	
White	65	52	62	179
Red	41	47	53	141
Yellow	6.0	–	1.0	7.0
Total	112	99	116	327

Root primodia were first observed in callused cuttings 5 weeks after treatment. Well-developed and measurable roots were observed in most cuttings within 15 to 19 weeks (Fig. 6).

Fig. 6. Rooted cuttings of *J. procera*. The photograph was taken 17 weeks after inserting the cuttings into the rooting medium. Left to right: rooted specimens were obtained from mature (0.20 % IBA), juvenile (0.20 % IBA), mature (0.80 % IBA) and juvenile (0.8 % IBA).

Branch cuttings collected from the three age class of stock plants produced roots directly (i.e. without callus formation) from the cut branch surface near the base, and indirectly (i.e. via callus formation) in roughly equal proportion (Table 4).

Table 4. Percentage of directly and indirectly (i.e. via callus formation) rooted cuttings collected from class I, Class II and Class III stock plants of *J. procera*.

Age class of stock plants	Origin of Root	
	Directly rooted cuttings (%)	Indirectly rooted cuttings (%)
Class I	15.0	12.9
Class II	14.7	12.3
Class III	17.8	21.1

Rooting response was more dependent on the IBA concentration than on the age of stock plants from which cuttings were derived. The overall rooting efficiency of the cuttings within the time frame of 21 weeks was generally low. Hence, the total rooted cuttings obtained from Class I stock plants was 67, giving rooting percentage of 27.9. The corresponding 81 (27 %) and 70 (38.9 %) total rooted cuttings were obtained from cuttings collected from Class II and Class III stock plants, respectively (Table 4). However, at the concentration of 0.20 % IBA maximum rooting percentage (50) was recorded from treated cuttings of Class I stock plants (Table 5). The maximum percentage of rooted cuttings obtained from Class II and Class III stock plants at 0.40 and 0.10 % respective treatments were 44 and 46.7 %, respectively. IBA at 0.80 % was supra optimal for the root formation in all treated cuttings of the three classes (Table 5).

Table 5. Effects of IBA on rooting of cuttings obtained from Class I, Class II and Class III stock plants. Observations were made 21 weeks after inserting the cuttings into the rooting medium.

	Class I		Class II		Class III	
IBA (%)	SP	RP	SP	RP	SP	RP
0.00	90.0	15.0	64.0	6.0	93.3	23.3
0.05	85.0	35.0	70.0	36	66.7	40.0
0.10	65.0	15.0	88.0	24	83.3	46.7
0.20	65.0	50.0	46.0	36	70.0	43.3
0.40	47.5	37.5	70.0	44	66.7	43.3
0.80	27.5	15.0	16.0	12	33.3	23.3

SP = Survival percentage

RP = Rooting percentage

5.2 Effects of IBA on the number and length of roots

The highest number of roots (45) was recorded at the concentration of 0.20 % in cuttings collected from Class I stock plants (Fig. 7). Although 0.80 % IBA reduced the rooting percentage of all the cuttings collected from the three classes considerably, it was found that a few of the cuttings that formed roots produced relatively large number of roots, more than 10 roots per rooted cutting, especially for cuttings derived from Class II and Class III stock plants (Figs. 8 and 9). Cuttings obtained from Class I stock plants and treated with 0.20 % IBA produced significantly larger number of roots per rooted cutting than cuttings treated with 0.1% (Fig. 7) ($P = 0.000285$, ANOVA). At the same time, the control cuttings and cuttings treated with 0.4 % produced significantly smaller number of roots per rooted cutting compared to the 0.20 % ($P = 0.000881$; $P = 0.000358$, respectively). The least number of root per rooted cutting (1) collected from Class I stock plants was recorded for IBA level applied at 0.20 % and 0.4 % (Fig. 7).

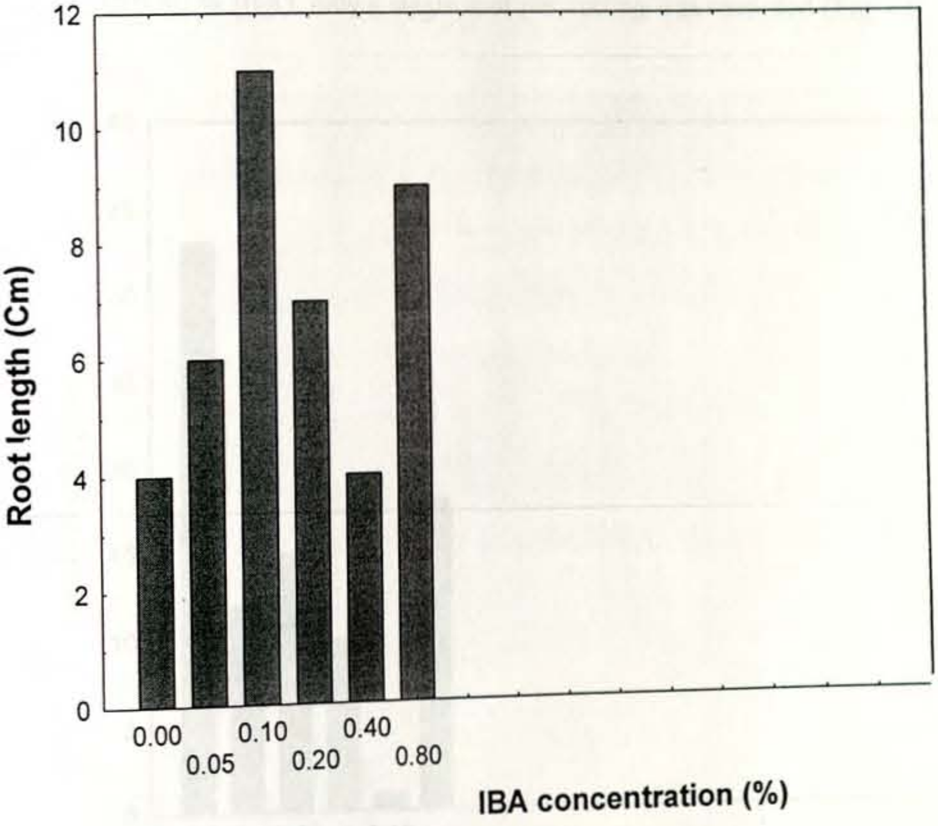

Fig 7 Effects of various IBA concentrations on the number of roots per rooted cutting of Class I stock plants of *J. procera*. Assessments were made 21 weeks after treatment.

In Class II cuttings treated with 0.05, 0.10, 0.20, 0.40 and 0.80 % IBA produced maximum number of roots (40, 35, 33, 35 and 33, respectively), compared to the maximum root number (8) that was obtained from the control (Fig. 8). Significantly large number of roots per rooted cutting was observed in cuttings treated with 0.10 and 0.80 % as compared to that of the control cuttings ($P = 0.05$; $P = 0.08$, respectively). Moreover, in eight cuttings (i.e., one cutting treated with 0.10 %, four cuttings treated with 0.20 %, two cuttings treated with 0.40 % and one cutting treated with 0.80 % IBA), only a single root per cutting was recorded (Fig. 8).

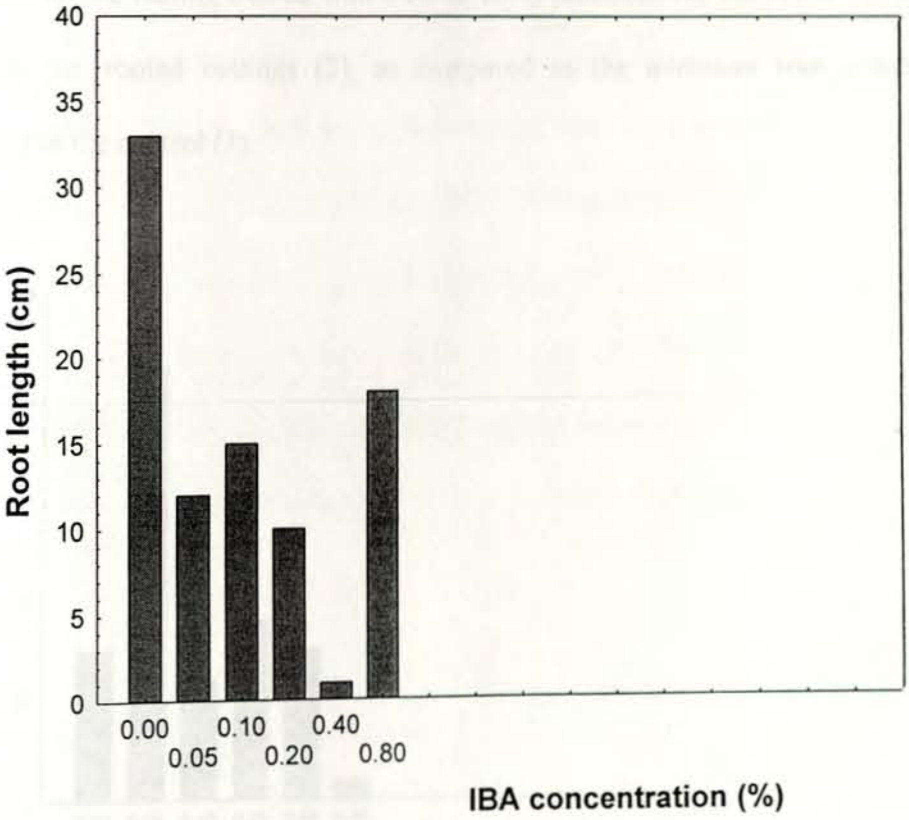

Fig. 8 Effects of various IBA concentrations on the number of roots per rooted cutting of Class II stock plants of *J. procera*. Assessments were made 21 weeks after treatment

Compared with the 0.05 % IBA-treated cuttings obtained from Class III stock plants, application of 0.40 and 0.80 % IBA induced significantly greater number of roots per rooted cutting ($P = 0.031868$; $P = 0.032166$, respectively) (Fig. 9). There were also significant differences between the 0.40 and 0.80 % IBA-treated cuttings and the control ($P = 0.002742$; $P = 0.035776$, respectively). Cuttings treated with 0.10 and 0.20 % IBA produced fewer roots per rooted cutting as compared to the 0.40 and 0.80 %. The least number of rooted cuttings were recorded in the 0.80 % treatment and the control (Fig. 9). Four cuttings (i.e. three cuttings treated with 0.05 % and one cutting treated with 0.80 % IBA) produced the minimum number of roots per rooted cuttings (2), as compared to the minimum root number observed in the control (3).

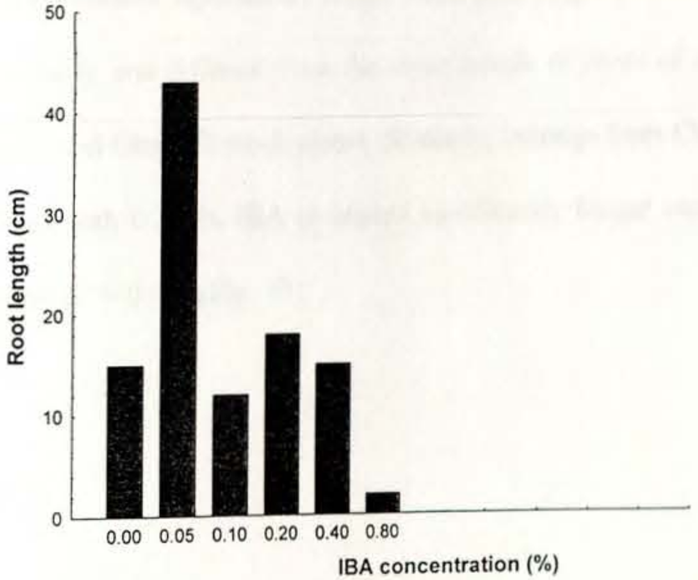

Fig. 9. Effects of various IBA concentrations on the number of roots per rooted cutting of Class III stock plants of *J. procera*. Assessments were made 21 weeks after treatment

Root length was not much affected by applied IBA, nor was it influenced by the age of the stock plants from which cuttings were derived. IBA concentration below 0.05 % for mean root length of cuttings collected from Class II stock plants was sub-optimal and concentrations between 0.05 and 0.20 % were more or less optimal for cuttings obtained from Class I, Class II and Class III stock plants. However, for cuttings obtained from Class I stock plants treatment above 0.4 % was supra optimal (Fig. 10).

Treatment 0.80 % in cuttings collected from Class I stock plants induced significantly shorter mean root length than 0.10 % and 0.20 % ($P = 0.033264$; $P = 0.025027$, respectively), which was in turn shorter than the control ($P = 0.111677$), at 10 % significant level (Fig. 10). Also, 0.80 % treated cuttings from Class II stock plants produced significantly longer mean root length (9.9 cm) than the control ($P = 0.042564$), and differed from the mean length of roots of cuttings collected from Class I and Class III stock plants. Similarly, cuttings from Class III stock plants and treated with 0.20 % IBA produced significantly longer mean root length than its control ($P = 0.09$) (Fig. 10).

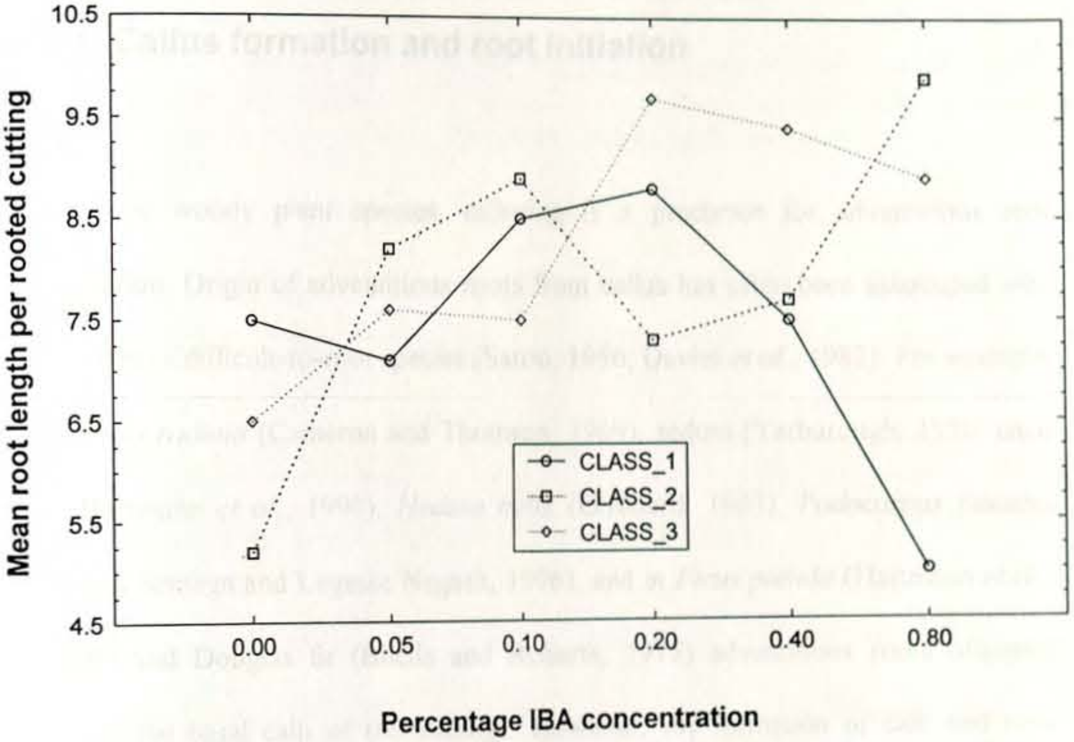

Fig. 10 Effects of various concentrations of IBA on mean root length (**cm**). Cuttings were collected from Class I (**circles**), Class II (**squares**) and Class III (**diamonds**) stock plants Measurements were made 21 weeks after treatment.

6. DISCUSSION

6.1 Callus formation and root initiation

In some woody plant species, callusing is a precursor for adventitious root formation. Origin of adventitious roots from callus has often been associated with conifers of difficult-to-root species (Satoo, 1956; Davies *et al.*, 1982). For example, in *Pinus radiata* (Cameron and Thomson, 1969), sedum (Yarburough, 1936: cited in Hartmann *et al.*, 1990), *Hedera helix* (Girouard, 1967), *Podocarpus flacatus* (Kassa Semagn and Legesse Negash, 1996), and in *Ficus pimula* (Hartmann *et al.*, 1990) and Douglas fir (Bhella and Roberts, 1975) adventitious roots originate within the basal calli of the cuttings. However, the formation of calli and root primordia in most coniferous trees is usually independent of each other (White and Lovell, 1984a, Hartmann *et al.*, 1990; Desta Berhe and Legesse Negash, 1998). Both structures involve cell division and often occur simultaneously, because they are dependent upon similar internal and external conditions (Hartmann *et al.*, 1990).

In contrast to a study made on conifers by Satoo (1956), most cuttings of *Juniperus procera* produced adventitious roots without callus formation, or there were cuttings that produced calli and root primordia at the same time (Table 4). This is consistent with the previous study that adventitious roots in most cuttings of *J. procera* arose from non-callused cuttings (Desta Berhe and Legesse Negash, 1998).

Moreover, several researchers suggested that in most woody plant species callus does not initiate rooting of cuttings. For example, in *Griselinia* species callus formation is not a prerequisite for root initiation (Bhella and Roberts, 1975). Although in both species of *Griselinia* (*G. littoralis and G. lucida*), callus production took place at about the same time as root production, more cuttings rooted than produced callus and hence callus developed only in some cuttings at the cut base whereas the root emerged higher up through splits in the epidermis (Bhella and Roberts, 1975; White and Lovell, 1984a).

6.2 Effects of age of stock plants and IBA

The rooting ability of many woody plant species is high during the juvenile growth phase and declines after transition to the adult growth phase (Welander and Huntrieser, 1981; Welander, 1983; Welander and Snygg, 1987; Haung *et al.*, 1992; Henry *et al.*, 1992; Kassa Semagn and Legesse Negash, 1996; Desta Berhe and Legesse Negash, 1998). For example, the rooting ability of eastern redcedar (*Juniperus virginiata* L.), *P. falcatus* and many other woody plant cuttings was dependent on the juvenility of their stock plants (Porlings and Therios, 1976; Henry *et al.*, 1992; Kassa Semagn and Legesse Negash, 1996). It was reported by Henry *et al.* (1992) that increased tree age of *J. virginiata* substantially reduced the rooting capacity of its stem cuttings.

Although no significantly different values of rooting percentage were recorded among the three age classes of *J. procera* in the present study, Desta Berhe and Legesse Negash (1998) found that the most important factor controlling rooting in this species was the age of the stock plants. One of the most probable reasons for the gap between the previous and the present results is perhaps the age difference among the stock plants used. That is, in the previous investigation there was a great variation in age between classes of stock plant selected for the study (e.g., juvenile or Class I and mature or Class II stock plants with 1.5 to 2-year-old and 25 to 30-year-old, respectively). However, in the present study three age classes of stock plants (e.g., young = 10-month-old, intermediate = 12-month-old and mature = 13-month-old) with closest age were used.

In a study of rooting of cuttings of a certain coniferous and deciduous species known to root with extreme difficulties, it was concluded that the age of the source plants greatly affected root initiation (Paton *et al.*, 1970; Hartmann *et al.*, 1990; Kassa Semagn and Legesse Negash, 1996; Desta Berhe and Legesse Negash, 1998). The diminished rooting ability of cuttings obtained from mature stock plants has several explanations. Some of the reasons include: **1**. Lack or reduction of endogenous rooting co-factors, (Girouard, 1967; Hartmann *et al.*, 1990); **2**. a decrease in auxin content with age (Leoplod and Kriedemann, 1975); **3**. an increase in lignified tissues (White and Lovell, 1984a); **4**. presence of a large number of resin canals, ring of schlerenchymatous tissues, and branch traces which reduce the amount of parechymatous tissues to such a low level (Nanda *et al.* 1970; Olesen, 1982; White and Lovell, 1984b); **5**. a decrease in responsiveness of mature tissues to root promoting substance (Zajczkowski, 1973; Hartmann *et al.*, 1990); and **6**. an increase in the production of rooting inhibitor as the plant grows older (Paton *et al.*, 1970; Hartmann *et al.*, 1990).

In this investigation, however, the most important factor for the rooting response was IBA treatment rather than age of the stock plants. Consequently, there were marked differences among the different IBA treatments (Table 5). For example, the rooting percentage for cuttings derived from Class I stock plants treated with 0.2 % IBA was 50 %. On the other hand, the rooting percentage for the control cuttings was only 15 %. Similarly, cuttings derived from Class II stock plants resulted in a rooting percentage of 44 % when these were treated with 0.4 % IBA. In contrast the control cuttings from the same class resulted in only 6 %.

The supra optimal effects of the 0.80 % treatment on rooting were observed more or less equally in all cuttings derived from the three age class of stock plants (Table 5). The same effect was also observed on mean root length of cuttings collected from Class I stock plants and treated with 0.8 % IBA (Fig. 10). The toxicity or deleterious effects of higher IBA concentration were also reported in cuttings derived from certain plant species which lead to either poorer rooting, a slight decline in rooting percentage, over-wintering losses or an increase in mortality (Middleton *et al.*, 1980; Harmann *et. al.*, 1990; Shiembo *et al.*, 1996b). The delayed adventitious root formation in some species (e.g., apple cultivars) was also due to supra optimal IBA concentrations (Welander and Snygg, 1987).

Although it is generally recognized that an optimum concentration of IBA promotes both the qualitative and quantitative development of adventitious roots, studies on a wide range of woody plant species have provided varying results from nil effect to highly significant effect (e.g., Smith and Thorpe, 1975; Strömquist and Hansen, 1980; Allan, 1987; Welander and Snygg, 1987; Leakey *et al.*, 1990; Ofori *et al.*, 1996; Shiembo *et al.*, 1996a and b; Tchnoudjeu and Leakey, 1996). In vegetative propagation of *Irvingia gabonensis, Milicia excelsa* and hybrid Larch, it was found that treatment of cutting with IBA had no effect on either the rate of rooting or on the level of rooting achieved (Allan, 1987; Ofori *et al.*, 1996; Shiembo *et al.*, 1996b). On the other hand, in *Pinus radiata*, for example, the number of root primordia was increased by IBA (Smith and Thorpe, 1975). Rooting also occurred faster in *Pinus slyvestris* and *Pinus contorta*, and the rooting percentage as well as the number of roots per rooted cutting were increased by IBA (Bowen *et al.*, 1975; Strömquist and Hansen, 1980).

Similarly, Leakey (1990) and Tchnoudjeu and Leakey (1996) showed that IBA application to *Nauclea diderrihii* and *Khaya ivorensis* stem cuttings greatly increased the percentages of rooted cutting and the number of roots per rooted cutting. Also, Delargy and Wright (1979) and Henry *et al.* (1992) concluded that the promotion of adventitious root formation through applied IBA is a well-established technique in vegetative propagation of apple rootstocks and eastern redcedar stem cuttings. It was further observed in *Sequoia sempervirens* that IBA treatment is a prerequisite for root induction and rooting was never obtained in the absence of IBA (Blazkova *et al.*, 1997). Thus, rooting in both young and mature clones of *S. sempervirens* depends upon the amount of IBA in the medium, that higher level of IBA was required by the mature clone to obtained equivalent percentage of rooted cuttings as in the young one. Similar relations have also been reported in a wide variety of other species, such as *Triplochiton scleroxylon* and *Gnetum africanum* (Leakey *et al.*, 1982; Shiembo *et al.*, 1996a).

In addition to hormonal and age effects, a number of other biochemical and physical factors influence rooting responses of various cuttings. These factors may include the physiological status and growth environment of the stock plants (e.g., level of irradiance during the stock plants growing period), position of cuttings (terminal, intermediate or basal cuttings), chemical (nutrients), the physical environment of propagation system (e.g. types of rooting medium, photoperiod and its quality, etc.), leaf area, node position, cutting length as well as types of cuttings (Kawase, 1965; Hansen and Eriksen, 1974; Eliasson, 1978; Hansen *et al.*, 1978; Strömquist and Eliasson, 1979; Eliasson, 1980; Leakey *et al.*, 1982; Leakey *et al.*, 1990; Shiembo *et al.*, 1996b; Tchnoudjeu and Leakey, 1996). Also, the genotype, the presence and types of buds (lateral and terminal buds), bottom heating and wounding are among the factors that greatly influence rooting of cuttings (Van Overbeek *et al.*, 1946; Heide, 1965; Biran and Halevy, 1973; Eriksen, 1973; Henry *et al.*, 1992).

7. CONCLUSION

1. *Juniperus procera* can successfully and efficiently be propagated by branch cuttings using a low-cost propagation system.

2. The application of appropriate plant growth substances (e.g., IBA), is necessary for the successful propagation of the species either in terms of root number, rooting percentages or root length. Nevertheless, it should be noted that the optimum treatment would vary with age of the stock plants.

3. For maximum rooting and root number, cuttings derived from juvenile stock plants with the corresponding optimum IBA concentration should be used.

4. A better and further understanding of this and other factors appears to be necessary to minimize the effort, maximize the success as well as to increase cost-effectiveness of vegetative propagation program of the tree.

8. RECOMMENDATIONS FOR FURTHER INVESTIGATION

1. Investigations on appropriate stock plant management, the effect of the position of cuttings on stock plants (terminal, intermediate or basal), the effect of cutting length and types of cutting etc., should be further examined.

2. An improvement that might stabilize relative humidity in a low-cost propagator would be needed. Thus, the polypropagators should be opened as less frequently as possible, and should not be opened during high heat load of the day. When opened, the cuttings should be sprayed frequently with a fine spray of water from a hand-held sprayer. This is highly desirable to prevent low relative humidity developing at mid-day.

3. Selection of rooting media may also be important to get better rooting results, because the species may display contrasting requirements with respect to various propagation media.

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DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

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