CHAPTER ONE

INTRODUCTION

1.1General Background

Phytogeography of Nepal shows wide range of species diversity due to wide range of climatic and altitudinal variation. Nepal is ranked on 10^{th} highest position in flowering plants diversity in Asia and 27^{th} position in the world biodiversity richness. The estimated number of flowering plants in Nepal is 6500 species of which about 4 % species are endemic to the country and 30 % species are endemic to the Himalayas the non flowering plants comprise over 400 species. Gymnosperms alone add 24 species and a wide land area in the country (Shrestha 1974, 1985-86), hence they form important component of the forest flora of Nepal.

Plants have their vital role in maintaining environment. Every plants has its own value. For environmental safety, 43 % of jungle to remain safe in Nepal; many plants have their important values such as medicinal, fibre, wood (fuel, fire and turpentine wood) and their products are used in the huge amount. Due to their importance and values, the overgrowing population highly exploited the plant products and caused depletion of important plants and nature's beauty. To maintain the safe environment and to fulfill the needs of overgrowing population, it is necessary to conserve the germ plasma of such valuable plants by different means of propagation. For the conservation of species propagation and cultivation is important. Plants propagate by sexual, asexual and vegetative.

The propagation of plants and conservation of germplasm through seeds is not always successful due to some biological constraints like short viable period of seeds and nonviable seed shed. These plants are multiplied by artificial methods from cuttings and also *in-vitro*. Some

plants possess very long time to complete their lifecycle, propagation of such plants by cuttings help to promote their fast multiplication. The propagation of endemic and threatened species of plants contribute a lot for biodiversity conservation. For clonal propagation, propagation by cuttings is a very common and popular method of multiplication of similar clones. This method is widely used for the propagation of herbaceous and woody or nonmetal plants. The cuttings are used widely in commercial green house propagation of many ornamentals and fruit species. Many new plants can be started in a limited space from a few stock plants. It is inexpensive, rapid, and simple and does not require sophisticated apparatus (Hartmann and Dale 1972 and Nanda and Kochhar 1995).

The four main types of stem cutting are herbaceous, soft wood, semi-hardwood, and hardwood. These terms reflect the growth stage of the stock plant, which is one of the most important factors influencing whether or not cutting will root (Dirr and Heuser 1987).

- ⇨ Herbaceous cuttings are made from non-woody, herbaceous plants such as *Coleus, Chrysanthemums*, and *Dahlia*.

- ⇨ Softwood cuttings are prepared from soft, succulent, new growth of woody plants, just as it begins to harden (mature). Shoots are suitable for making softwood cutting when they can be snapped easily when bent and when they still have a gradation of left size (oldest leaves are mature while newest leaves are still small). For most woody plants, this stage occurs in may, June or July.

- ⇨ Semi hardwood cuttings are usually prepared form partially mature wood of the current season's growth, just after a flush of growth. These types of cutting normally are made form mid-July to early

fall. Many broadleaf evergreen shrubs and some conifers are propagated by this method.

- ⇨ Hardwood cutting are taken from dormant, mature stems in late fall, winter or early spring, no signs of active growth. The wood is form and does not bend easily. Hardwood cutting are used most often for deciduous shrubs and many evergreens.

1.2. *In-vivo* Rooting

The emergence of root form plant parts is generally two types *in-vitro* rooting (controlled condition) and *in-vivo* rooting (natural, condition). From this process a detached plant parts regenerate into a whole plants. The process of rooting of cutting on natural environment is called *in-vivo* rooting. At first fibrous (primary) roots are developed then after tap (secondary) roots are developed. For the rooting process generally hard wood, semi-hard wood and soft wood stem cuttings are used. Hard wood cutting is easy to handle which does not perish in short time and may be taken safely over long distance if necessary. Cutting of this types are often slow to root taking several months to a year. Some species root much more readily (fast) than other, but some do not root at all. The basic reason for this has not been understood (Hartmann *et al.* 1981). However, evidences are available to show that rooting of cutting is influenced by a number of external factors (light, temperature, humidity, aeration etc),and internal factors include tissue characteristics of mother plants, use of root promoters, root inhibitors and synthetic growth substances (auxins, gibberellins and cytokinns.

The formation of roots on cuttings initiates form the pericycle cells and form callusing. Callusing is the groups of undifferentiated meristematic cells. They are formed by the activity of the cambial cells near the wound. From the mass callus by the differentiation root primordia are develop. From the root primordia roots are developed (Hartmann and Dale 1972, Nanda and Kochhar 1995).

Rooting time varies with the types of cutting the species being rooted, and environmental conditions. Conifer requires more time than broad leaf plants i.e. Angiosperms. Late fall or early winter is a good time to root conifers. Once rooted, they may be left in the rooting structure until spring. Species difficult to root should be wounded (Dirr and Heuser 1987).

The nutrition and other substances, which needed for root formation, are supplied by leaves and buds to the cuttings. Macronutrients like potassium, calcium, and phosphorus, micronutrients like Boron and zinc and Nitrogenous substances like amino acid and nitrates promote the root formation on cuttings. A specific root forming substance is ‘Rhizocaline’, which is the compound of three factors. They are ortho diphenolic groups produced in the leaves in light, an auxins and enzyme probably of phenol-oxides type. Rhizocaline located in the pericycle, phloem, and cambium. It is manufactured in the leaves and Translocated towards stem/ cutting and accumulated at the base of cuttings is considered to be responsible for rooting. The polarity of root formation is due to polar transport of endogenous auxins, which accumulates at the base and cause rooting. (Nanda and Kochhar, 1995). There is convincing evidence that auxins, one of the natural growth hormones is essential for rooting. Other natural growth hormones such as gibberellins and cytokinns also influence adventitious root formation. It may be necessary to supplement

exogenous auxins to promote the formation of adventitious root primordial. The capacity of regeneration in a plant depends upon genetic factors.

The external factor seasons greatly influence the adventitious root formation of cuttings (Ono *et al.*, 1997; Palanisamy and Kumar 1997). Cutting of some plants can induce roots throughout the year while others are seasonal. According to some workers, cutting taken from the mother plant during the active season, growth of roots much better than at any time in the year. Similarly light affects rooting by virtue of its intensity, quality, and duration. High light intensity promotes adventitious root formation in some species but others favor low intensity. The effect of temperature also varies in different species. The effect of temperature on root initiation may be different from that of their development. In general, high humidity and aeration of the soil as well as humidity in the air promote root formation on cutting. As root initiation on cutting is stimulated in the presence of leaves, it is necessary that the atmospheric vapor pressure is maintained at the same level as that of the intercellular spaces to prevent excessive transpiration and consequently to prevent the leaves from drying (Meson *et al.*, 1997). The medium used for rooting of cutting serves to hold them in position and to provide them with moisture and aeration. Therefore, an ideal rooting medium should be porous enough to allow good aeration and should possess high water holding capacity. The type of rooting medium determines to some extent the nature of root produced (Meson *et al.*, 1997).

The cuttings taken from vigorously growing plants rooting better than those taken from weakly growing and smaller ones (Xie and Lin 1997). Although in many cases this might not be applicable. Cuttings taken from juvenile trees rooting better than those taken from old trees. The rooting

ability decreases with the age so much so that the cuttings taken from old trees sometimes do not root at all. The capacity of rooting potential of younger cutting is higher due to possession of high nutritional content. The old twigs have less nutritional content so less rooting of cutting. The twigs obtained from the lower half of the crown is suitable to get better rooting then those from the middle or apical part (Xei and Lin, 1997). But this also varies according to the plant materials. The cutting taken from the apical part do not rooting at all. The ability of cuttings for rooting varies with the parts of a plant and age of the plant also. In general cuttings are made to a length of 3.5-15 cm for rooting (Nanda and Kochhar 1995: Hartmann *et al.,* 1981). For better rooting the cuttings are planted at angle of 45^{o} (Hartmann *et al.,* 1981) on the beds.

According to some workers the initiation of roots on stem cuttings were stimulated by some substances accumulated at the base of cuttings which move from the leaves and buds. From this evident the rooting of leafy cuttings are better than that of cuttings which have no leaves. The increase in leaf number increases the root number on cuttings (Dick *et al.,* 1996). This is because the leaves serve as a source for the supply of nutrients and specific root forming substances like vitamins and hormones, which are essential for root formation on cuttings. The absence of leaves suppress root formation. The presence of actively growing buds are either inhibitory or have no effect on rooting. The vegetative buds in general promote rooting but the floral buds inhibit it. The inhibitory effect of floral buds on root formation on cuttings is attributed to utilization of growth substances for the development of reproductive parts leaving very little for rooting. This is particularly so when the area of leaves on the cutting is low. Thus, some workers have reported that the buds do not effect root formation. The number of roots

which are produced on cuttings does not differ whether they are taken form the vegetative or reproductive branches provided the leaf area remains the same (Nanda and Kochhar, 1995).

The formation of roots on cuttings also depend on anatomical character. The presence of parenchymatous tissue facilitates the emergence of roots while the presence of sclerenchymatous and fibrous tissue inhibits the emergency of root formation (Hartmann and dale 1972, Nanda and Kochhar 1995). The decreasing ability of cuttings of plant species to root with the advancement of age is thus, ascribed to the increased sclerenchymatous tissue as juvenile parts are rather free of this tissue. The sclerenchymatous tissue is either scantly distributed or is in the form of patches of fibrous tissue which allow the vascular rays to open freely in the cortex and in fact in some cases the vascular rays actually push aside the fibrous bundles and expanded out into well- marked funnel shaped dilations. In marked contrast to this in difficult to root species, there exist one or more rings of sclerenchyma surrounding the vascular tissue which are strengthened many a time, by a complete ring of collenchymatous hypodermal tissue. The vascular rays are abutted against the dead sclerenchymatous tissue. These fibrous or hypodermal rings act as, mechanical barrier to the penetration of water and to the emergence of root initials. This is evident from the fact that the roots emerge in linear manner if longitudinal slits are given to break this barrier. However failure of cutting to root may not always be due to the density and continuity of sclerenchymatous tissue, rooting does not occur even when the cortical, phloem and cambium cells proliferates to break the continuity of sclerenchyma. In fact in conifers for instance, there is no sclerenchymatous ring, yet the cuttings do not root. The failure of these cutting to root may be due to other causes such as lack of nutrition or

auxins or the accumulation of resins in the stem or some inhibitory substances (Nanda and Kochhar 1995, Hartmann and Dale 1972).

Propagation of plant by inducing adventitious roots in stem cuttings is preferred because it reduces the time taken from seedling stage until the trees are old enough to produce flowers and fruits. If grown form seeds there can be delay of several years between seed germination and the time at which the tree matures and begins to form fruits and flowers. This delay can be overcome by taking cutting from adult trees. Such cutting will continue to produce flowers and fruits within 1-2 years of planting into the field (Carter and Paudyal, 1987).

***Ginkgo biloba* L**

Ginkgo biloba L. is considered as living fossil due to the presence of primitive character. Propagation through seeds in this plant is found to be rare. According to Dallimore and Jackson (1948) this species is represented by five verieties. Arnold (1947) mentioned that "*Ginkgo biloba* is one of the oldest living plants and may indeed be the oldest living genus of the seed plants." The living species *Ginkgo biloba* originated in the Triassic period of the Mesozoic era and since then it has continued to live unchanged and undeterred survived after many environmental hazards, that counts down to about two hundred million years ago. It came into existence during the Permian and achieved world wide distribution and luxuriance during the Triassic and Jurassic periods of Mesozoic age. It gradually thinned out and started fading out of existence during the cretaceous and on ward into the Cenozoic. The name *'Ginkgo'* was first proposed by Kaempfer (1690), an European botanist, and the same name was adopted by Linnaeus (1771) due to its fan shaped bilobed leaves. It is commonly called maiden-hair tree in Europe (Sporne

1965) because its new leaves resemble very much like those of *Adiantum* (called maiden hair fern).

Ginkgo biloba has also been reported native plant in eastern part of China and Japan. It can grow on within subtropical to temperate climate. In China and Japan it is grown as a sacred tree in the temple garden and also cultivated for edible seeds. In America it is cultivated as good shade tree. It has high medicinal value. Its extract is used in different disease like psychiatric, antiulcer, antiallergic, cardiovascular disease, analgesic drug, ophthalmic drug, dermatology, antibacterial drug, immunological drug, digestive and endocrine drug. *Ginkgo biloba* plant also might be applied in urban air quality assessment for detecting the existing effect of air pollutants and micro environmental stress in an urban ecosystem and so also called pollution bioindicator. From the *Ginkgo* extracts different types of ginkgolides obtained which is rich of terpenoid lactones.

IUCN Category: Globally IUCN categorized *Ginkgo biloba* highly threatened and rare (R) category.

1.3 Hormones

The word hormone derived from the Greek Word ‘hormao’, Which means to urge on or to stimulate. These are organic substances and at the beginning they are used as herbicides on crop field throughout the world. Later extensively used in horticulture. They are also called plant growth substances, plant growth regulators, or phytohormones, the concept that plant growth and development are regulated by substance produced in minute quantities in one organ that elicits a response in another was first suggested by Julius Von Sachs, the father of plant physiology, in the latter half of the nineteenth century.

Thimann (1948) suggested to use the term phytohormone for hormones of plants. According to Pincus and Thimann (1948) hormones are organic substances produced naturally in the higher plants, controlling the growth or other physiological functions at a site remote from its place of production and active in minute amount. Similarly Phillips (1971) defined growth hormone “as substance which are synthesized in particular cells and which are transferred to other cells where in extremely small quantities influence developmental process” In many plants physiological functions are controlled by hormones which are produced on one tissue in minute amount and migrates to the another tissue of the plant where they can effects the growth promote inhibit or modify the physiological processes. The natural hormones and other materials are essentially “Chemical messengers” influencing the many patterns of plant development.

The PGRs can be broadly classified into growth promoting and growth retarding or naturally occurring growth substances and synthetic (artificial) growth substances. There are five recognized group of natural plant hormones auxins, gibberellins, cytokinns, ethylene and abscisic acid, in this work, study on effect of auxins on rooting is carried out.

1.3.1 Auxins

The term “auxin” was first used by F.W. Went in 1926. They are widely distributed throughout the plant body, greatest amount found in growing apices least amount found in non growing regions. They are the first group of plant hormones discovered 1930’s and thereafter studied intensely throughout the world. These are weak organic acid capable of cell elongation, cell enlargement and root initiation. The natural auxins originate in shoot-tips, meristems and enlarging tissues, such as actively

growing terminal and lateral buds, lengthening internodes and developing embryos in the seeds. Auxins are readily transported through the plant, principally in the apex to base direction through the vascular tissue. The movement of auxins is slow process with the speed of 0.5 cm/hour to 1.5 cm/hour. (Rao and Gupta).

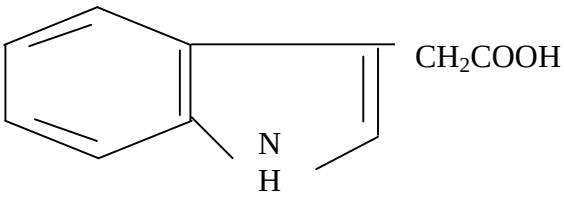
One of the first responses attributed to auxins was the stimulation of root initiation in stem cuttings. The initiation of roots and shoots in small pieces of plant tissue culture under aseptic condition using auxins has become of standard method of micro propagation of some plant species. The subsequent study of auxins has been dominated by interest in its control over growth. Went (1928) proposed that “Ohne wuchsstoff, Kein Wachstum” (without auxins no growth). The term growth hormone becomes synonymous with auxins (Leopold 1975). Plant hormones and plant growth regulators have slight difference. A plant hormone is a natural substance produced by the plant itself that acts to control plant activities. Plant hormones that are synthesized chemically can initiate reactions in the plant similar to those caused by the natural hormones known as PGRs. PGRs on the other hand, include plant hormones natural and synthetic but also other non-nutrient chemicals not found naturally in plants but that when applied to plants influence their growth and development. (Hartmann 1981, Salisbury and Ross, 1986). Of these Naphthalene acetic acid, Indole-3 butyric acid, Indole-3 acetic acid, 2-4 Dichlorophenoxy acetic acid etc are well known.

Some important auxins used in this study discussed separately.

1.3.1 (a) IAA

Indole-3 acetic acid occurs in plant is the most wide spread auxin. It’s bioassay was discovered by Went in 1928 (Holland) and chemically

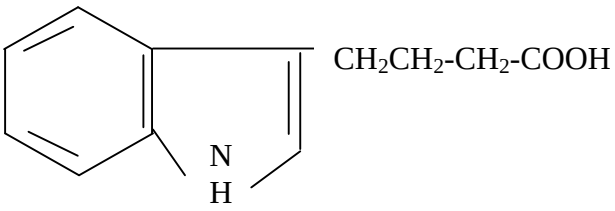
calibrated by Kogl *et al.,* in 1934 as an auxin (Lepold 1975). It has been experimentally proved to be active in promoting root initiation in cuttings by several investigations. It is effective as the natural material.



IAA (Indole-3 acetic acid)

1.3.1 (b) IBA

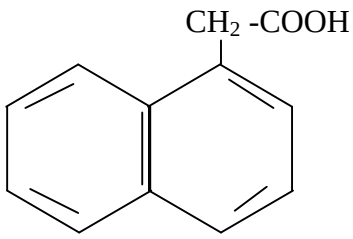
Indole-3 butyric acid acts as root inducer and fruit set enhancer with trade name 'Hormodin'. It has also experimentally proved in 1934 that induces root initiation in stem cuttings. It is best and commonly used auxins, which is decomposed relatively slowly by the auxin destroying enzyme in plants (Auds 1965).



IBA (Indole-3 butyric acid)

1.3.1 (c) NAA

Naphthalene acetic acid is also one of the most important auxin. Since NAA is not synthesized by plants, it is not considered as a hormone, is classified as plant growth regulators. It has been proved by many workers in 1934 that it is more effective in rooting. Activity of the NAA was observed by Zimmermann *et al*., in 1936 (Lepold 1975).



NAA (Naphthalene acetic acid)

1.4 Importance of this Study

Conservation of germ plasm through seeds is not always successful due to some biological constraints like short viable period of seed and non-viable seed-shed; this study helps to minimize such constraints and help in multiplication of such plants in short time. The plantlets obtained from this process are genetically identical to the parent plants. Many trees possesses very long time to complete their lifecycle, propagation of such plants by cuttings helps to promote their fast multiplication and support the sustainable use. Some terrestrial plants need waters for their fertilization, due to lack of water during fertilization propagation of such plants by seeds is rare, by applying cutting methods these plants can be propagated easily in mass and helps to conserve germplasm. *Ginkgo biloba* tree is rare and threatened, propagation by their seeds is difficult. It can be easily propagated through cutting methods. Propagation of endemic and threatened species of plants by this process contribute a lot in biodiversity conservation.

1.5 Objectives of the Study

The main objectives of this study are:

1. To find out the effect of different plant growth regulators in rooting from stem cuttings.

2. To assess the hormone and right environmental condition for propagation in stem cuttings of *Ginkgo biloba*.

3. To support ex-situ conservation through *in-vivo* propagation.

1.6 Limitation of this Study

The rooting ability of *Ginkgo biloba* depend upon the age of the tree used for propagation. So old tree's cuttings do not root at all. The cuttings from apical part of the tree have low ability of rooting in comparison to the cuttings from basal part. The presence of sclerenchymatous and fibrous tissues inhibits rooting while abundance of parenrenchymatous tissue facilitates the emergence of roots. Lack of proper green house is one of the major limitations of the work. The cuttings planted in pot should be provide with controlled climatic condition for proper rooting, so lack of well facilated green house is the major problem to carry out the work. Phytohormones used in this study are too costly, so lack of finance is also another limitation of the study.

CHAPTER TWO

LITERATURE REVIEWS

Dabadi (1975), studied the seasonal changes in the rooting behaviour of stem cuttings of *Salix tefrasperma* Roxb. and *Populus nigra* Linn in response to Indole-3 butyric acid, Naphthalene acetic acid and 2,4-Dichlorophenoxy acetic acid. The cuttings treated and planted between at May-June, November and December months showed 100 % rooting but those cuttings treated and planted at between late December to early January and April showed minimum rooting. Thicker cuttings showed maximum rooting than the thinner cuttings.

Gupta *et al.*, (1989), studied the rooting response of branch cuttings of *Melia azedarach* L. The cuttings treated with 50 ppm IBA and planted has been found to increase rooting and callus formation in the February where as May planted cuttings, the IBA 50 ppm has been found more effective inducing rooting.

Lin and Cin (1990), studied the rooting of semi hardwood and hard wood cuttings of oriental pear (*Pyrus serotina* Rehd.). Hardwood cutting obtained from early to mid-January, pre-treated with 25000 ppm IBA, and using vermiculite as the rooting medium showed 62 % rooting. Semihard wood cuttings showed highest percentage of rooting i.e. 60 % when it was sampled in June and treated with IBA and rooting medium was a 1:1 mixture of vermiculite and perlite.

Lee and Bilderback (1990), propagated. *Heptacodium jasminoides* Airy-shaw by softwood and semihard wood cuttings. They observed basal and middle softwood cuttings exhibited greater rooting (65 % and 55 % respectively) than the terminal cuttings (42 %). The cuttings treated with

K-IBA rooted in higher percentage and produced more roots than untreated cuttings.

Shamet and Dhiman (1991), studied the effects of auxins (IAA, IBA, NAA) on rooting behaviour of *Grewia optiva* Burrett. Stem Cuttings under intermittent mist. Soaking the cuttings base for 20 hours in 100 mg/litre IAA has given the maximum rooting i.e. 77.55 % in June.

Nautiyal *et al.*, (1992), studied the rooting response of branch cuttings of teak *Tectona grandis* as influenced by growh hormones and position of the cuttings on the crown. They found that the cuttings treated with IAA and IBA showed good rooting response in all cases than NAA.

Siagian and Hutan (1992), studied the effect of IBA on the survival rate of stem-cuttings of *Gmelina arborea* Lin. It is recommended that for vegetative propagation of *Gmelina arborea* Lin. by stem cuttings is better to use 400 mg/lit dosage.

Rashid *et al.*, (1993), propagated kiwifruit (*Actidinia chinensis*) by stem cuttings. The cuttings obtained from 'Hayward', (pistillate) and 'Matua' (staminate) treated with IBA at different concentration and grown in course sound at 20-22^{0}C temperature. Hayward (pistillate) hard wood cuttings showed maximum rooting (62 %) at IBA 4 gm/litre where as Matura (staminate) hard wood cuttings showed maximum rooting (58 %) at IBA 5 gm/liter. Lower the concentration i.e. 4 gm/lit showed poor rooting.

Nanda and Kochhar (1995), studied the vegetation propagation of plants and dealt with three steps required for successful rooting from stem cuttings.(i) initiation of groups of meristematic cells callusing, (ii) differentiation of meristematic cells into recognizable roots primordia and

(iii) development and emergence of new roots by rupturing other tissues of stem after the formation of vascular connection with the conducting tissue of the cuttings (connection of root vascular strand with main vascular stands.)

Yoo and Kim (1996), studied the effects of PGRs and removal of floral buds on rooting ability in hard wood cuttings of white Forsythia (*Abeliophyllum distichum* Nakai). They found those cuttings treated with 500 ppm of IAA or 100ppm of IBA for 1 minute increased the rooting percentage up to 65 %.

Bista (1997), studied the effect of some plant Growth regulators on Morphology, yield and propagation of some plants of Nepal, he found in *Taxus baccata* 86.7 % of rooting when it is treated with 150 ppm concentration of ABT hormone. 100 ppm of ABT and 200 ppm of ABT showed 33.3 % and 46.7 % rooting. The cutting treated with IAA 50 ppm and 100 ppm showed higher rooting the percentage was 50 in each, 200 ppm showed 20 % and 50ppm showed 10 % rooting. The highest percentage of rooting 13.3 % obtained when the cutting treated with 200 ppm of IBA. All cuttings are treated for 4 hours with different concentration of different hormones.

Tewari and Dhar (1997), studied on the vegetative propagation of Indian butter tree [*Aisandra butyracea* (Roxb) Beatini]. They found those cuttings treated with 500 ppm of each IBA and NAA for 24 hours showed 79.7 % rooting.

Hossni (1998), studied on rooting of *Ficus benjamina* L. and *Ficus nitida* L. Vari, “Hawii” cuttings. The cuttings of both species treated with lower concentration of IBA and NAA seperately and placed on plastic house showed highest rooting percentage.

Pant (1998), propagated *Taxus baccata* L. Sub species wallichiana (Zucc) pilger and *Podocarpus nerifolius* D. Don. In case of *Taxus baccata*, he found, the stem cuttings treated with 150ppm IBA showed the highest rooting percentage i.e. 80 % for 6 hours; the highest number of rooting per cuttings i.e. 26 were given when the cuttings treated with 100 ppm IBA for 6 hours. The highest root length i.e. 3 cm was observed when the cuttings treated with 50 ppm ABT No. 1 for 6 hours. In case of *Podocarpus nerifolius*, the highest rooting percentage i.e. 80 % was given by IBA 100 ppm treated for 4 hours. The highest root number i.e. 26 were given by the cuttings treated with IAA 50 ppm for 6 hours and the highest root length i.e. 2.5 cm was given by the cuttings treated with ABT NO.1 50 ppm for 6 hours. Among the different concentrations of 4 types of hormomes the IAA showed 0 percentage rooting when the cuttings of *Taxus baccata* treated with 50 ppm for 6 hours, those cuttings treated with 300 ppm IAA for 6 hours showed 30 percentage of rooting. The NAA showed higher rooting on lower concentration and lower rooting on higher concentration when the cuttings of *Taxus baccata* treated with different concentration. The IAA showed 70 % rooting when the cutting of *Podocarpus nerifolius* treated with 50 ppm for 2 hours, those cuttings treated with 300 ppm IAA for 2 hours showed 30 % rooting. The cutting of *Podocarpus nerifolius* treated with lower concentration and lower the time of treatment of NAA showed higher rooting but higher the concentration and higher the time of treatment showed 0 percentage of rooting i.e. totally inhibited the rooting.

Bhuse *et al.,* (2003), studies the effect of time of planting type of cuttings and plant growth regulators on rooting in *Pelargonium graveolans* L. Herit. They observed that the cuttings from terminal part and treated at

IBA750 ppm, Middle with 1000 ppm IBA and lower/basal with IBA 1000 ppm maximum rooting.

Negash (2003), studied the vegetative propagation of the threatened East African yellow wood (*Podocarpus falcatus*). The cuttings harvested from 3 months and 2 years old stock plant and treated with 40 μg to 80 μg concentration of IBA showed well rooting. Old cutting treated with lower and more higher concentration of IBA inhibited the rooting.

Kelen and Ozkan (2003), studied the relationship between rooting ability and changes of endogenous IAA and ABA (Abscisic acid) during the rooting of hard wood cuttings of some grapevine rootstocks. It was found that low IAA and high ABA having low rooting rate where as high IAA and low ABA having high rooting rate.

Bhusal *et al.,* (2003), studied the effect of Juvenility on rooting of trifoliate orange (*Poncirus trifoliate* [L] Raf.) stem cuttings. They found that those cuttings treated with auxins have age one and two year showed 100 % rooting, increasing the ages the rooting ability decreases.

Bielenin (2003), studied the rooting and gas exchange of conifer *Jauniperus scopulorum* (skyrocket) and *Thuja occidentalis* (samaragd) cuttings treated with IBA. Cuttings of both species were treated with 0-09 % K-IBA. For *Juniperus scopulorum* 'skyrocket' the percentage of rooting as well as number of roots and quality of roots increased with the increasing IBA concentration while in *Thuja occidentalis* 'samaragd' 0.3-0.6 % IBA concentration are the best for rooting purpose of this species.

Haynes and William (2004), rooted the leafy and leafless cuttings of *Kosteletzkya virginica* with IBA. Leafy cuttings can be rooted more than the leafless cuttings.

Somashekar *et al.,* (2004), studied the macropropagation of *Gaudua angustifolia*. They found the leafy branch cuttings have length 10-15cm treated with IBA 2500 ppm induced maximum rooting (85 %) within 30days in sand bed medium at 30+/-5^{0}C temp and 70+/-5 % relative humidity in mist chamber.

Teklehaimanot *et al.,* (2004), studied the Influence of the origin of stem cuttings, season of collection and auxin application on the vegetative propagation of African sandal wood (*Osyris lanceolata*) "in Tanzania." The stem cuttings collected in September originating from the basal portion and treated with IBA solution (50 and 100 ppm) had best rooting than the terminal cuttings. The cause of best rooting on September is that the high level of stored food in the plant after undergoing active photosynthesis during rainy season, Nov-may. The high nutrition status and low nitrogen content of basal portion may play role enhanced rooting.

Ucler *et al.,* (2004), studied the effect on IBA and cutting dates on the rooting ability of semi hard wood kiwifruit (*Actinida deliciosa* A. Chev.) cuttings. The cuttings taken in July and treated with IBA 8 gm/L 15 secs. showed 76.6-100 % rooting. While the cuttings taken in August and treated with IBA showed less percentage of rooting i.e. 26-63.3 %.

Karam and Gebre (2004), studied the rooting of *Cercis siliquastrum* cuttings influenced by cutting position on the branch and IBA. The summer cutting were not give roots.

Handa *et al.,* (2005), studied the vegetative propagation of *Albizia Lebbek* through stem cuttings. It was observed that IBA was more effective than NAA in inducing rooting in this species and maximum rooting success achieved at IBA 400 ppm was 71.66 %.

Rosier *et al.*, (2005), studied the stumping height, crown position and age of parent tree influence rooting of stem cuttings of Fraser fir [*Abies fraseri* (pursh) Poir]. Among 3,5 and 7 year old stock cuttings, the cuttings from 3 year old stock plant and treated with IBA showed greatest root number and length.

Bona *et al.*, (2005), Studied the cutting propagation of *Baccharis articulate* (Lam) Pers, *Baccharis trimera* (Less). Ap de candolle, *Baccharis stenocephala* Baker with auxins. They found both IBA and NAA on 4000 ppm toxic for rooting. Lower the concentration less rooting and higher the concentration toxic for rooting. At the temperature below 20^0C less percentage of rooting, greaten than 32^0C also less rooting.

Krisantini *et al.*, (2006), studied the adventitious roots formation in *Grevillea* (proteacease), an Australian native species. They found that *Grevillea* sps showed seasonal difference in rooting when the cuttings treated with IBA.

Stefancic *et al.*, (2006), studied the influence of IAA and IBA on root development and quality of Prunus 'GiseIAs' leafy cuttings, the dwarfing cherry rootstock. IAA proved as the most efficient treatment for roots formation on cuttings than the IBA.

CHRPTER THREE

MATERIALS AND METHODS

3.1 Rational of the Plant Selection

The plant *Ginkgo biloba*, L. has highly medicinal value and it can also be used to indicate quality of air pollution: it is use for other different purposes like ornamental, good shade and as a sacred tree. It is one of the exotic species in Nepal from China and stands on rare IUCN category. So the propagation of this plant from this method is selected.

3.2 Collection of Materials

For this study, the newly developed different sizes of (branches) twigs were collected from Tri-Chandra campus and Tribhuvan University compound. The branches were colleted in rainy season taken to CDB on 7th Sharwan, 2062 (22nd July 2005), 25th Ashad 2063 (9th July,2006) and at 24th Chaitra, 2062 (7th April, 2006) in summer season.

The branches were taken at morning from the basal part of the plant, which have high initiation rate of rooting proved already. At the beginning, the branches had cut from tree placed on bucket with 1/5 filled water to protect from drying and taken to CDB (Photo-B, Photo Plate 2). This water checks the transpiration from these branches. The braches taken preferred at morning shown on figure, from these branches cuttings were prepared. All the hormones obtained from biotechnology unit of Central Department of Botany T.U., Kirtipur.

3.3 Preparation of Cuttings

The new of the year branches were cut into 12-15 cm length by a sharp clean cutter. The wound on cuttings were avoided. Each cutting contained

4 to 5 buds and 1 to 2 leaves (Photo-D, Photo Plate 2). Thus prepared cuttings were dipped into water so as to prevent water loss from the tissue.

3.4 Preparation of Hormones Solutions

For the preparation of hormones stock solutions 500 mg (0.5gm) of each hormone weighed and dissolved on few drops of absolute alcohol, mesh up to final volume 500 ml by adding distilled water. Thus the final concentration of the solution became1000 ppm. These stock solutions of hormones were kept in bottles with lid and kept them inside the refrigerator.

From these stock solutions different concentrations of hormone solution were prepared by the dilution process using following formula.

$$S_1 V_1 = S_2 V_2$$

Where, S_1 = Strength of Stock Solution i.e. 1000 (ppm)
V_1 = Volume of Stock solution to be taken.
S_2 = Strength of the required hormone Solution (ppm)
V_2 = Total volume required.

Hormones concentration and treatment periods.

Hormones	Concentration (ppm)	Treatment periods (Minutes)				
IAA	1000	5	10	15	20	25
IBA	1000	5	10	15	20	25
NAA	1000	5	10	15	20	25

Hormones	Concentration (ppm)				Treatment periods (Minutes)
IAA	250	500	750	1000	30
IBA	250	500	750	1000	30
NAA	250	500	750	1000	30

3.5 Bed Preparation

The 26.5 b × 21 h sized clay pots were filled with few pebbles at the bottom for good aeration and rest portion filled with sand and soil at (3:1)

ratio. The debris of plants i.e. leaves and pieces of twigs were avoided during bed preparation to keep safe from fungus (Photo-A, C and E, Photo Plate 2).

3.6 Treatment of Cuttings

Each cutting was dipped about 2-3 cm in the different concentration of the different hormone solution for different time periods. The cuttings treated from lower part not from upper parts because if it is treated from upper (growing) parts these cause inhibition to root. The experiment started from late morning to early noon. For one treatment 10 pieces of cuttings were taken (Photo-F, Photo Plate 2).

3.7 Plantation of Cuttings

Before the plantation of cuttings the beds were pierced with piece of stick or pencil. After the calculated time of treatment with particular hormone solution, the cuttings were planted one on each hole in a slanting (45^0) position (Photo-G, Photo Plate 2). After the plantation of cuttings immediately the pots were watered with water. The cuttings should not be moved. A set of cuttings were planted in control. Some of the planted pots were covered with transparent colourless plastic bags while other were kept in plastic house (Photo-H, Photo Plate 2). For the good aeration inside the plastic house different sizes of holes on plastic were made. The soil surfaces of planted earthen pots were watered with water every day till the final observation. In regular intervals of time the weeds growing around the cuttings were uprooted and thrown away. After three months of plantation the cuttings were taken out from the beds and dipped into water and measured the length, average length of roots and also counted the number of roots on each cuttings.

3.8 Anatomical Study

After 40 days of plantation some cuttings were taken for anatomical study. Whitish brown callus were observed at the base of cuttings (Photo-B, Photo plate 1), later from that callus roots developed. Thin section at the region of rooting were made (Photo- C and D, Photo Plate 2).

3.9 Data Collection

Primary data were collected, after three months of the plantation period. The cuttings were taken out from the beds and dipped in water. The number of roots on each cuttings counted, measured the average length of the roots. From the anatomical study differences of fresh cuttings and rooted cuttings were observed.

3.10 Statistical Analysis of Data

Statistical analysis was carried out with the help of 12^{th} version of SPSS program. The concentration variables and time variables were considered as factor or independent variables and the percentage of rooting of cuttings and each length of root are considered as dependent variables. Compare means, L.S.D. and Duncan homogeneity test were also performed. The different tables of test shown in Annex.

3.11 Acclimatization

Acclimatization is the process to provide suitable condition for newly developed plants for their stabilization. The direct sunlight and high temp might be dry the newly developed plants and altogether they may be killed. So after the completion of the study the rooted stem cuttings were transferred to the pots. The pots were placed on shade and watered time to time (Photo-F, Photo Plate 4).

CHAPTER FOUR

OBSERVATION AND RESULTS

4.1. Morphology of the Plant

Ginkgo Biloba L. is exotic in Nepal and found in an altitude of about 1300 m in the central parts of Nepal. The plant branched, dioecious, slow growing and pycnoxylic wood. There are two types of branches long shoots (growing rapidly) bearing foliage, single, spirally arranged, and deeply lobed leaves. Dwarf shoots (growing slowly) bearing groups of leaves and not deeply lobed. Bierhorst (1971) opined that differences in the growth pattern of long and dwarf shoots may be due to the quantities of auxins produced in the apical meristem..

Male and female plants, however difficult to be differentiated when young. This distinction is only possible when they bear male and female fructification and by sex chromosome. Both sex organs i.e. male and female strobilus develop terminally on the dwarf shoots of male and female plant separately. The leaves surrounding the both strobilus do not show their bilobed character. Both strobilus are arise in the axil of scale leaves or foliage green leaves.

Male strobilus resembles the catkin like inflorescence and is arranged loose structure of several microsporophylls. Each microsporophyll usually contain two micro-sporangia, they are tubular structure, from that haploid microspores formed due to reduction division of sporogenous mother cells. The development of microsporangium is eusporangiate type i.e. from single archesporial cells. Sporangium dehisces by means of longitudinal slit. Terminal part of sporangium are abortive type. Pollen (rounded microspores) are shed by wind in 4 celled stage. The development of male gametophyte *in situ* i.e. within sporangium.

Female strobili having a long stalk or peduncle, which bifurcates apically and each bearing one sessile ovule, one generally aborts earlier each bear two or three or rarely more ovules at the tip out of four megaspores only the lowermost is functional develop female gametophyte, the female gametophyte possesses abundant chlorophyll (Sporne, 1965).Pollination is anemophilous; the germination of the seed is of hypogeal type.

The Planted cuttings of *Ginkgo biloba* L. were taken out from the beds for observation after three months of plantation period. The cuttings under control in rainy season only showed rooting but summer season did not show any rooting.

Table: 1

Effect of IBA on Roots Formation on Cuttings under; Varying Concentrations on Constant Time of Treatment at Rainy Season

S.N.	Concentration of IBA (ppm)	Number rooted cuttings	Callus formation	Successfully rooted cuttings (%)	Total number of roots emerged	Number of roots per cuttings	Average length of roots (cm)
1	250	3	+	30	17	5.66	2.21
2	500	4	+	40	18	4.5	3.16
3	750	8	+	80	87	10.87	1.91
4	1000	4	+	40	40	10	1.97
5	Control	1	+	10	9	9	1.5

Number of Cuttings planted: 10
Time of Treatments (Minutes): 30
Season of Cuttings planted: Rainy

Table: 2
Effect of IBA on Root Formation on Cuttings under; Varying Time of Treatments on Constant Concentration at Summer Season

S.N	Time of treatment (minutes)	Number rooted cuttings	Callus formation	Successfully rooted cuttings (%)	Total number of roots emerged	Number of roots per cuttings	Average length of roots (cm)
1	5	5	+	50	29	5.8	1.44
2	10	2	+	20	4	2.0	1.37
3	15	3	+	30	13	4.33	2.73
4	20	2	+	20	11	5.55	2.62
5	25	1	+	10	6	6	1.86
6	Control	-	-	-	-	-	-

Number of cutting planted: 10
Concentration of IBA (ppm): 1000
Season of cuttings planted: Summer
(-) Absence of root formation

From table 1 the highest number of roots and percentage of rooting i.e. 80 % was observed on the cuttings treated with IBA 750 ppm for 30 minutes at rainy season (Photo-E, Photo plate-3). The roots developed on the cuttings treated with IBA 500 ppm for 30 minutes showed healthy and longest root i.e. 10 cm length and their average length 3.16 cm. The cuttings treated with IBA 250 ppm for 30 minutes showed lower the rooting (Photo-c, Photo plate-3). It showed that more higher the concentration lower the root formation. Cuttings under control showed 10 % rooting (Photo-A, Photo plate-3).

From table 2 the highest percentage of rooting i.e. 50 percent was found on the cuttings treated with IBA 1000 ppm for 5 minutes at summer season. The cutting treated with IBA 1000 ppm for 5 minutes showed the healthy and longest root i. e. 7.5 cm length and their average length 1.44 cm. The cuttings treated with IBA 1000 ppm for 25 mins. showed lower rooting percentage (Photo-E, Photo plate-4). It showed that the higher the concentration and higher the time of treatments lower the roots formation. Cuttings under control did not show any rooting.

From table 1 and 2 was found that the percentage of roots formation at rainy season from cuttings higher than the summer cuttings. Thick cuttings showed more rooting than the thinner cuttings. Higher the concentration i.e. 1000 ppm showed the lower the root formation. Among both seasons the highest percentage of rooting i.e. 80 percent was found on the cuttings treated with IBA 750 ppm for 30 minutes at rainy season. The cuttings under control in rainy season only showed rooting.

Table: 3

Effect of NAA on Roots Formation on Cuttings under; Varying Concentration on Constant Time of Treatments at Rainy Season

S.N	Concentration of NAA (ppm)	Number rooted cuttings	Callus formation	Successfully rooted cuttings (%)	Total number of roots emerged	Number of roots per cuttings	Average length of roots (cm)
1	250	1	+	10	2	2.0	2.0
2	500	2	+	20	72	36.0	2.33
3	750	2	+	20	7	3.5	1.64
4	1000	-	+	-	-	-	-
5	Control	1	+	10	9	9	1.5

Number of cuttings planted: 10
Time of treatments (minutes): 30
Season of cuttings planted: Rainy
(-) Absence of root formation.

Table: 4

Effect of NAA on Root Formation on Cuttings under; Varying Time of Treatments on constant concentration at summer season:

S.N	Time of treatment (minutes)	Number rooted cuttings	Callus formation	Successfully rooted cuttings (%)	Total number of roots emerged	Number of roots per cuttings	Average length of roots (cm)
1	5	4	+	40	30	7.5	1.05
2	10	4	+	40	32	8.0	2.06
3	15	2	+	20	11	5.5	2.59
4	20	1	+	10	3	3.0	2.59
5	25	-	+	-	-	-	-
6	Control	-	-	-	-	-	-

Number of cutting planted: 10
Concentration of IBA (ppm): 1000
Season of cuttings planted: Summer
(-) Absence of root formation.

From table 3, the higher percentage of rooting i.e. 20 % was observed on the cuttings treated with NAA 500 ppm for 30 minutes in rainy season. The longer roots were found on the cuttings treated with NAA 500 ppm, which have average length 2.33cm. The higher the concentration i.e. 1000 ppm for 30 minutes could not give any roots (Photo-D, Photo plate-3). From that the NAA showed lower rooting when concentration increased.

From the table 4 the cuttings treated with NAA 1000 ppm for 10 minutes showed higher rooting i.e. 40 % in summer season. Those cuttings treated with NAA 1000 ppm for 15 minutes showed long roots, which have 2.59 cm average length. The cuttings treated with NAA 1000 ppm for 25 minutes could not give any roots (Photo-E, Photo plate-4). From that NAA showed lower rooting when the concentration and time of treatments increased.

From the table 3 and 4 it was found that those cuttings treated and planted in rainy season showed higher rooting in comparison to summer season. Thicker cuttings showed more rooting in comparison to thinner cuttings. Higher the concentration i. e. 750 ppm showed lower the roots formation, at 1000 ppm totally inhibited the rooting. Among both seasons the cuttings treated with NAA 500 ppm showed higher numbers of roots i.e. 36 per cuttings in rainy season (Photo-D, Photo plate-3).

Table: 5

Effect of IAA on Roots Formation on Cuttings under; Varying Concentration on Constant Time of Treatment at Rainy Season

S.N	Concentration of IAA (ppm)	Number rooted cuttings	Callus formation	Successfully rooted cuttings (%)	Total number of roots emerged	Number of roots per cuttings	Average length of roots (cm)
1	250	4	+	40	25	6.25	1.54
2	500	4	+	40	78	19.5	3.84
3	750	8	+	80	108	13.5	3.0
4	1000	9	+	90	140	15.55	4.66
5	Control	1	+	10	9	9	1.5

Number of cuttings planted: 10
Time of treatments (minutes): 30
Season of cuttings planted: Rainy

Table: 6

Effect of IAA on Roots Formation on Cuttings under; Varying Time of Treatment on Constant Concentration at Summer Season

S.N	Time of treatment (Minutes)	Number rooted cuttings	Callus formation	Successfully rooted cuttings (%)	Total number of roots emerged	Number of roots per cuttings	Average length of roots (cm)
1	5	2	+	20	12	6.0	1.94
2	10	2	+	20	11	5.5	2.07
3	15	4	+	40	23	5.75	1.89
4	20	3	+	30	17	5.66	2.65
5	25	3	+	30	16	5.33	2.47
6	Control	-	-	-	-	-	-

Number of cutting planted: 10
Concentration of IAA (ppm): 1000
Season of cuttings planted: Summer
(-) Absence of root formation

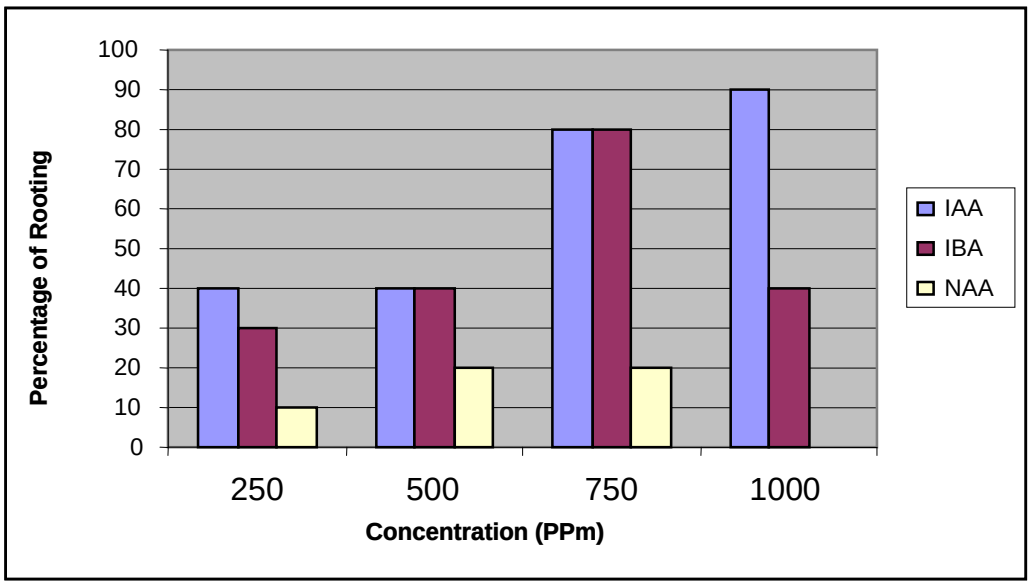
From the table 5, it was found that the highest percentage of rooting i.e. 90 % on those cuttings which were treated with IAA 1000 ppm for 30 minutes time of treatment at rainy season. These cuttings also showed the highest number of rooting per cuttings and higher average length i.e. 4.66 cm. the IAA showed that the higher the concentration, higher the root formation. The lower concentration 250 ppm showed less number of root formation (Photo-B, Photo plate-3).

From the table 6, those cuttings which were treated with 1000 ppm IAA for 15 minutes time of treatment showed higher percentage rooting i.e. 40 % at summer season. The cutting treated with IAA 1000 ppm for 5 minutes time of treatment showed lower percentage i.e. 20 % of rooting. The healthy and longer roots were formed from those cuttings which were treated with IAA 1000 ppm for 20 minutes time of treatment. Higher the concentration and higher the time of treatments showed lower the roots formation at summer season.

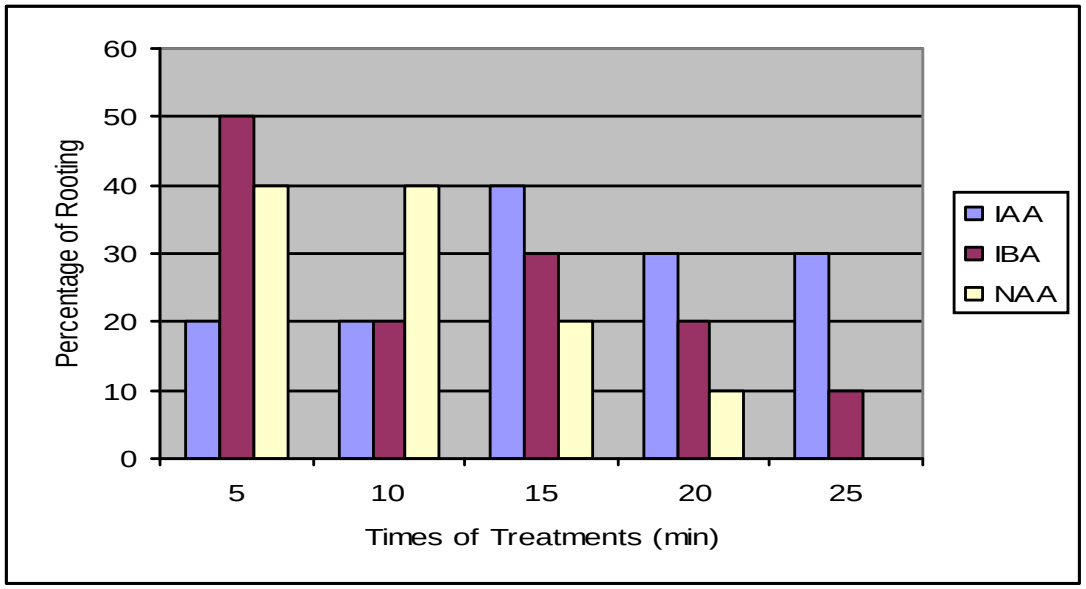
From the table 5 and 6, it was found that those cuttings treated and planted at rainy season showed higher the rooting percentage than those treated and planted at summer season. Thicker cuttings showed more higher rooting in comparison to the thinner cuttings. Here on this hormone higher the concentration showed higher the root formation. Among both seasons the cuttings treated with IAA 1000 ppm for 30 minutes time of treatment and planted at rainy season showed the highest percentage of roots formation.

4.2 Presentation of Data

4.2.1 (a) Pictorial form (Group Bar Diagram)Rainy season

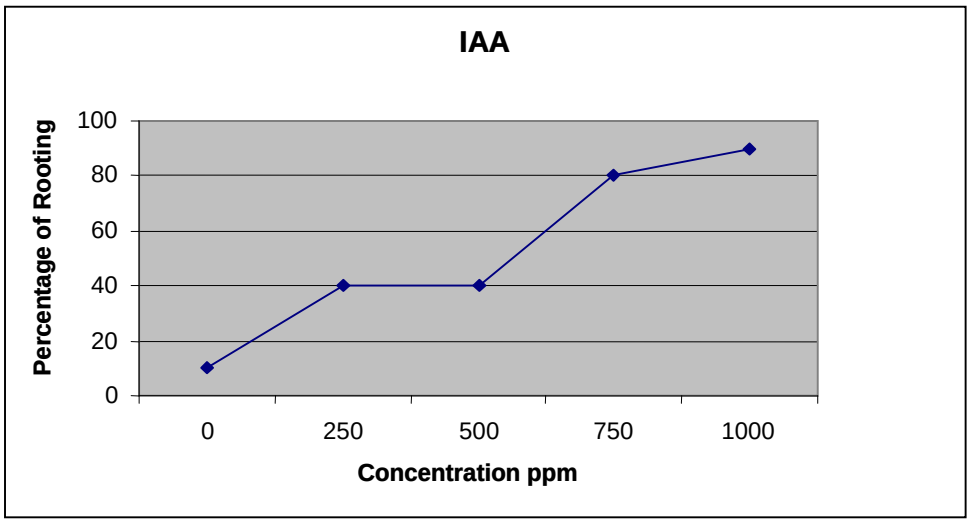


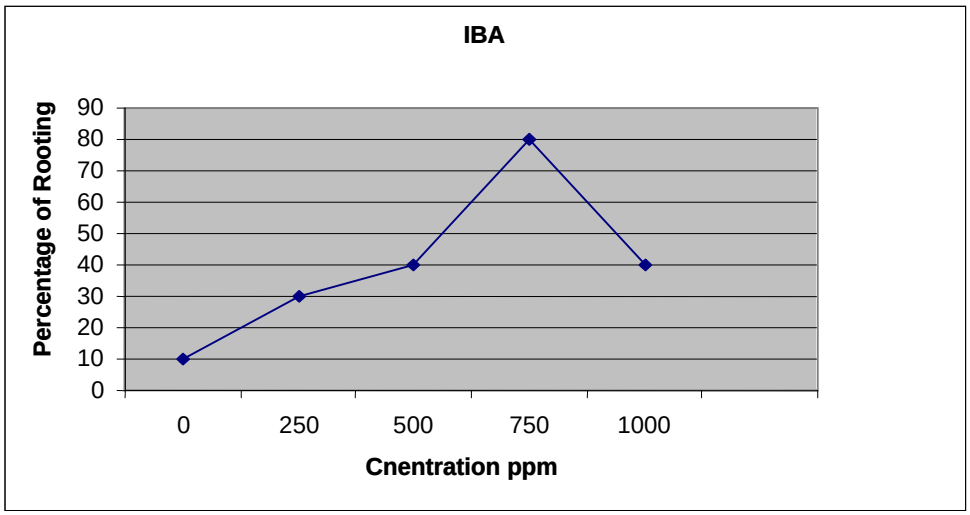
4.2.1 (b) Pictorial form (Group Bar Diagram) summer season

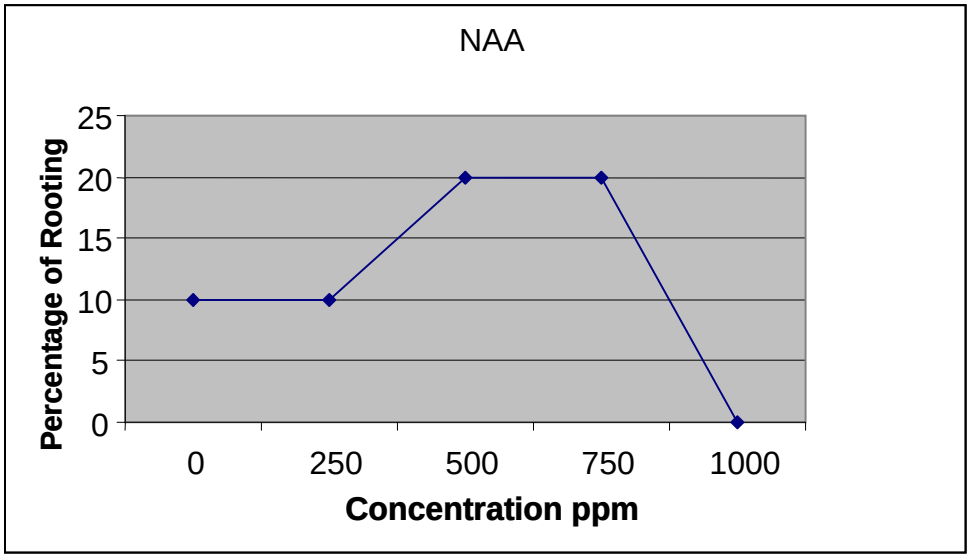


From both bar diagram, the IAA showed the highest percentage of rooting, higher the concentration of IAA showed higher the roots formation. The most appropriate concentration for rooting of *Ginkgo biloba* cuttings was found IAA 1000 ppm for 30 minutes time of treatment at rainy season. The other hormones showed higher rooting at lower concentration. The NAA at 1000 ppm concentration totally inhibited the roots formation. The IBA showed that the its higher the concentration lowered the roots formation. Among both bar diagram those cuttings which were treated with different concentration of hormone solution and planted at rainy season showed better rooting than at summer season.

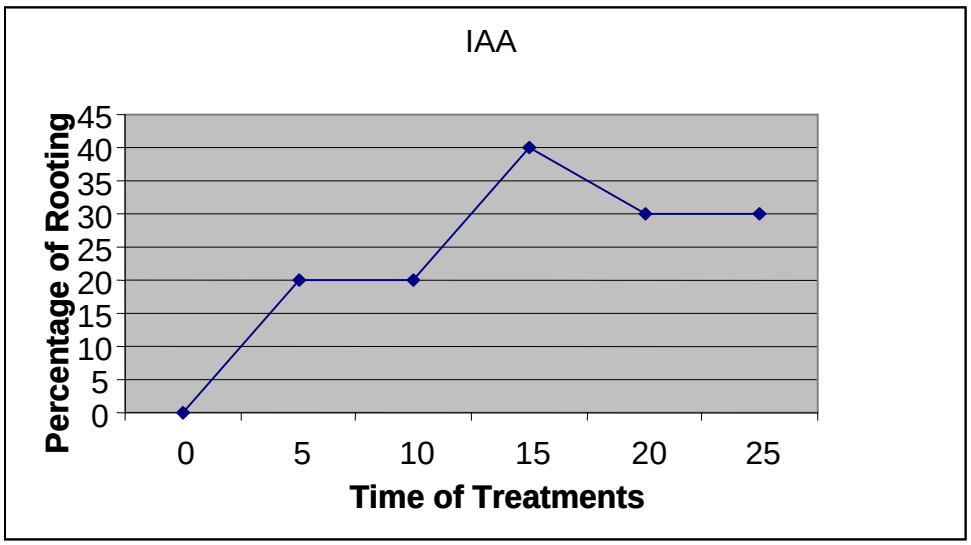
4.2.2 (a) Graphical Form (Rainy Season)

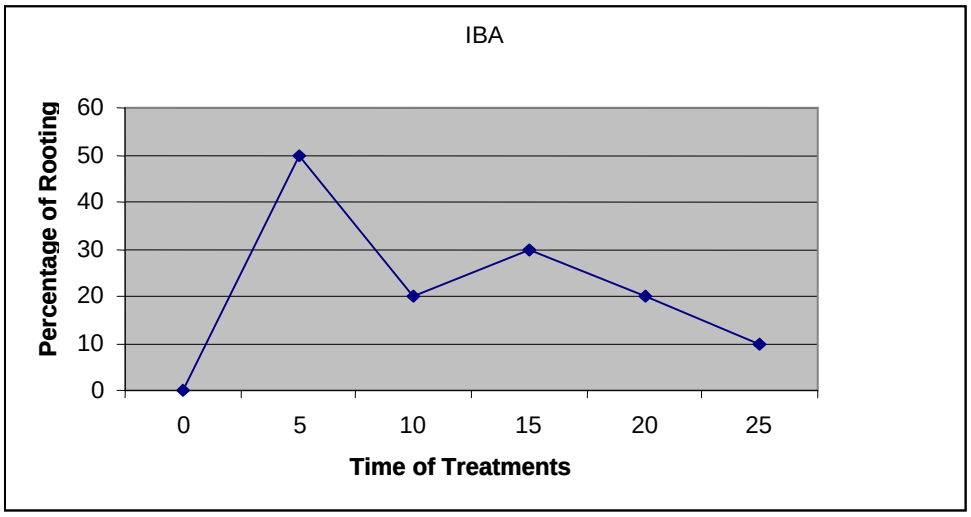


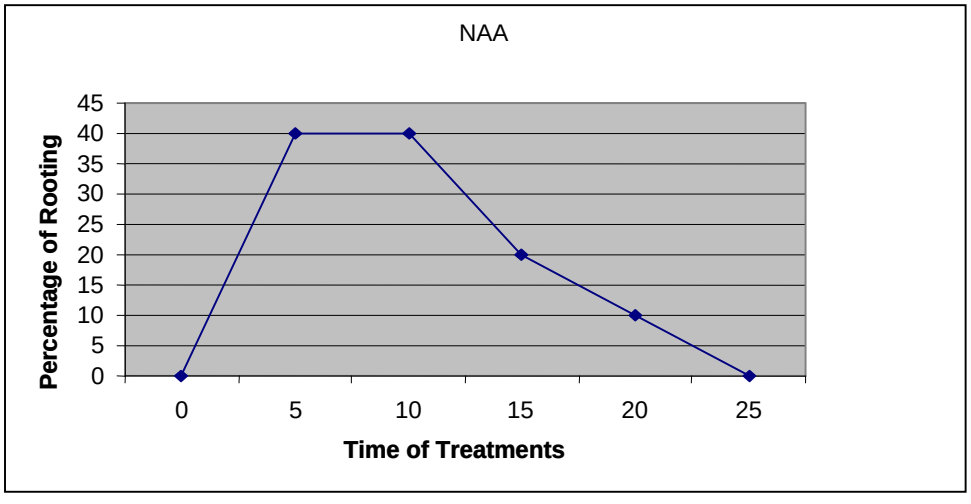




4.2.2 (b) Graphical Form (Summer Season)







From both types of graphs i.e. summer and rainy seasons it was found that those cutting treated with hormone solution and planted at rainy season showed higher rooting than at summer season. IAA is the best hormone for better rooting of this species. Higher the concentration of IAA showed higher rooting but other hormones i.e. IBA and NAA showed lower rooting when concentration and time of treatments increased. On higher concentration and longer the time of treatments of NAA i.e. 25 mins. showed the totally inhibition of roots formation. On IAA showed increasing order and others showed decreasing order of roots formation on graph. The graphs of IBA and NAA were found curve from.

4.3 Studies on Anatomical Features of Stem of *Ginkgo biloba* L.

a) **Anatomy of normal stem**: T.S. of *Ginkgo biloba* young stem (long shoot) is more or less circular in outline and remains surrounded by single-layered, thickly cuticularized epidermis made of brick-shaped cells while in older stems epidermis is replaced by periderm. Inner to the epidermis a well-marked region of parenchymatous cortex. It contained mucilaginous canals, sphaeraphides and many tannin-filled cells. Cortex was comparatively less extensive in long shoots than dwarf shoots. Endodermis and pericycle was not well-marked in long shoot. The vascular bundles are conjoint, collateral; open and endarch are arranged in ring. The phloem constituted the sieve tubes and phloem parenchyma. The xylem constituted the tracheas and bordered pits. Uniseriate medullary rays present on T.S. of stem. The pith is parenchymatous containing mucilage canals, sphaeraphides and narrow in log shoots while in dwarf shoots it is more extensive. The cambium gets activated at the early stage and give rise to secondary growth. The T.S. of normal stem of *Ginkgo biloba* shown in Photo-A, Photo Plate 1.

b) Anatomy of rooted stem: The basal cut end of *Ginkgo biloba* cuttings first gave out whitish brown mass of callus. The vascular cambium, adjacent secondary phloem (in hard wood cuttings) cells of cortical region and pericycle cells took part in callus formation. Anatomy of the rooted stem revealed that the bulk of tissues (callus) were scattered around the vascular strand. These bulks (adventitious root primordial) pushed the cortical as well as epidermal region giving out small protuberances all around, which grow out continuously giving out long roots. Finally the vascular strands of the roots get connected with the main strands. The anatomy of rooted stem of *Ginkgo biloba* cuttings shown in Photo-B and C, Photo Plate 1.

4.4 Statistical Analysis

From statistical analyzed tables which were shown in Annex. Significant values of IAA hormone found to be small but the values of NAA found to be greater as compare to the values of other hormones at the 0.05 level of significance. So, IAA is also found to be significantly best but NAA is also found to be significantly less effective for rooting of cuttings of that species. From the Duncan and Post Hoc Test there is found highly difference between the significant value of control and 1000 ppm of IAA and found separate subsets but in NAA there is less difference between the significant value of control and other concentrations and found within same subsets. So, it is found that the 1000 ppm of IAA at rainy season significantly best for rooting of cuttings of that species.

The significant values of all hormones at summer season found to be greater but the values of rainy season found to be small as compare to the 0.05. So, rainy season found to be the best season for rooting of cuttings of *Ginkgo biloba* species.

CHAPTER FIVE

DISCUSSION

Ginkgo biloba showed selective response to different hormone concentration in rooting. The different PGRs have varied effect at the different levels of concentration and at various time of treatment on rooting. Temperature, light, humidity, cutting type played the important role in rooting of the cuttings.

In the present work IAA was found to be the most appropriate hormone for rooting. It is significantly better than others for rooting for this species. The higher the concentration i.e.1000 ppm of IAA for 30 minutes showed higher roots number and rooting percentage i.e. 90 % but the other hormones showed less rooting. The higher the concentration of NAA (1000 ppm) totally inhibited the rooting of cuttings which is similar to the work of Pant (1998) on *Taxus baccata* and *Podocarpus nerifolius*. Among three hormones the IAA is the best and the IBA is better for rooting of *Ginkgo biloba* cuttings but the NAA showed very less effective and less significant than others. The control cuttings at rainy season showed 10 % rooting but at summer season did not show any roots.

The work of Yoo *et al.*, (1996), showed that treatment of PGRs and removal of floral buds affect on rooting ability in hardwood cuttings of white forsythia (*Abeliophyllum distichum* Nakai). They found that those cuttings treated with higher concentration i.e. 500 ppm IAA for 1 minute or with lower concentration i.e. 100 ppm IBA for 1 minute increased the rooting percentage upto 65 % which showed similar to the present work.

Tewari and Dhar (1997), studied on the vegetative propagation of the Indian butter tree (*Aisandra butyracea* (Roxb) Beahni). They found those

cuttings treated with 500 ppm of each IBA and NAA for 24 hours showed 79.7 % rooting which is similar to the result of present work.

In the result of present work higher concentration of IAA treatments showed higher rooting percentage, which seems to be similar to the work of Kelen and Ozkan (2003). They studied the relationship between rooting ability and changes of endogenous IAA and ABA during the rooting of hardwood cuttings of some grapevine root stocks and found that high concentration of IAA and low concentration of ABA having high rooting rate.

In the result of present work the high concentration of IBA showed less rooting parentage which is similar to the works of Negash (2003). He studied the vegetative propagation of the threatened East African Yellow wood (*Podocarpus falcatus*) found that those cuttings treated with lower and more higher concentration of IBA inhibited the rooting.

Handa *et al*., (2005) studied the vegetative propagation of *Albizia lebbek* through stem cuttings. It was observed that IBA was more effective than NAA in inducing rooting in this species and maximum rooting success achieved at IBA 400 ppm was 71.66 percentage this which is similar to the result of present work.

In the present work the rooting hormones IAA and IBA showed better rooting response than NAA which is similar to the work of Nautiyal *et al*., (1992).They studied the rooting response of branch cuttings of teak (*Tectona grandis*) as influenced by the growth hormones and position of the cuttings on the crown. They found that the cuttings treated with IAA and IBA showed the good rooting response than NAA.

IAA is the best hormones for rooting response in *Ginkgo biloba* which is similar to the work of the Shamet and Dhimam (1991) who studied the effect of auxins (IAA, IBA, NAA) on rooting behaviour of *Grewia optiva* Burett. They found that the IAA has given the maximum rooting i.e. 77.5 % in June than others auxins.

Stefancic *et al.,* (2006) studied the influence of IAA and IBA on root development and quality of *Prunus* leafy cuttings. IAA proved as the most efficient for root formation on cuttings than the IBA Which as present work.

In present study the roots formation from cuttings during the rainy season (June, July) were higher in comparison to experiment started in April. The thicker cuttings showed greater number of roots than the thinner cuttings which is similar to the work of Dabadi (1976), rooting behaviour of stem cuttings of *Salix tetrasperma* Roxb. and *Populus nigra* Linn. in response to IBA, NAA and 2,4-D. She found that those cuttings treated and planted at may-June, November and February showed 100 % rooting but those cuttings treated and planted at December, January and April very less percentage of rooting. Thicker cuttings showed maximum rooting percentage than the thinner cuttings.

The result of present work is similar to the works of Ucler *et al.,* (2004) who studied the effect of IBA on cuttings of semi hard wood kiwifruit (*Aeinida deliciosa* A. Chev.). They found that those cuttings taken at July and treated with IBA showed higher rooting percentage i.e. 76.6-100 %, than those treated with IBA at other seasons.

Krisantini *et al.,* (2006) studied the adventitious roots formation in *Grevillea*. They found that *Grevillea* species showed seasonal difference

in rooting when the cuttings were treated with IBA which is similar to the result of present work.

The work of Haynes *et al.*, (2004) showed that the leafy cuttings of *Kosteletzkya virginica* treated with IBA rooted better than the leafless cuttings as shown by present work.

Somashekar *et al.*, (2004) studying on *Guadua angustifolia* found that the leafy cuttings measuring 10-15 cm in length and treated with 2500 ppm IBA and planted on sand bed induced maximum(85 %) rooting similar result was found in *Ginkgo biloba*.

The ability of rooting depend on the presence of foliage leaves as shown by Nanda and Kochhar (1995); Hartmann *et. al*., (1981). Presence of many leaves on cuttings gave negative effect because of higher transpiration rate and water deficiency which caused leaf shedding in *Shorea leprosa* stem cuttings (Amirath *et.al*., 1997) which resemble with present investigation. The growth of vegetative parts of the stem cuttings in present hindered the proper growth and development of adventitious roots. Removal of such parts enhanced the growth of roots properly.

In present work it was also found that the soft wood and semi hard wood stem cuttings gave callus and roots easily even with low concentration of hormone solution but the hard wood stem cuttings proved difficulty in rooting. Except IAA other hormones showed negative effect on root formation treated with high concentration and long period of soaking.

During the experiment on rooting from stem cuttings, various factors enhance the rooting. The cutting taken from vigorously growing and juvenile trees showed better rooting than the weakly growing old trees. The cutting without buds and leaves did not give roots but gradually died.

The wounded cuttings difficult to initiate rooting. The growth of vegetative and reproductive parts had negative effect on rooting but the removal of such parts also enhanced the rooting. The cuttings which have 2-3 leaves and some buds rooted well but heavy foliage leaves hindered the rooting effect. Soft woodcuttings could give callus and produce roots easily when they were treated with low concentration (250 ppm) of hormone. Hard woodcuttings rooted well when they treated with high concentration (1000 ppm) of hormone. Much water logging, uncovered pots, the pots covered with thick plastic sheets did not give good result. The growth of unwanted weeds around the cuttings hindered the proper growth of roots. Too much water logging resulted death and decay of cuttings and callus. The beds which filled with coarse sand and soil did not give well rooting but the fine sand and soil beds gave well rooting. The sunlight is necessary for rooting but the cuttings beds which placed on direct sunlight did not give successful rooting. The cuttings towards the more sunlight produced long roots, high number of roots in few cutting only. The cuttings kept towards less sunlight produced high number of roots in more cuttings. Temperature also play vital role on rooting of cuttings, around 20 +/-1^0 C gave well rooting above and below of that temperature did not gave well rooting. Old, small, and thin cuttings did not give well rooting but the new thick cuttings gave well rooting. At the dry and summer season, the cuttings did not give well rooting but on rainy seasons, the cutting gave good results.

The dryness of the beds leads to the water deficiency in the cuttings which may die if this condition continues as in the case of exposed pots and its cuttings. The cuttings covered with thick plastic bags, the reverse effect or water logging condition might have caused for the less

successful for rooting percentage or another reason may be the deficiency of light required for photosynthesis during rooting period.

Light and heat showed positive response towards the shortening of time for the initiation and development of the roots hence the cuttings towards more light and heat gave high number of roots and long roots but the less number of successful cuttings. High heat and high light cause water evaporation leading to water deficiency might have played role in this case. Hence the cuttings towards less sunlight and heat gave higher rooting percentage but takes more times. According to Nanda and Kochhar (1995) light intensity, temperature and humidity play important role in rooting of cuttings. But it is difficult to say exactly what intensity of light, temperature and humidity is optimum. It is also determined by genetic make up of plant species.

In the case of anatomical observations of rooted stem cuttings the vascular cambium, secondary phloem (hard woodcutting), young phloem (soft woodcuttings), cortical cells, and pericycle cells took part in roots initiation. According to Hartmann and Dale (1972) adventitious roots in stem cuttings usually originate from young secondary phloem tissue, although such root also arise from the various other tissues such as vascular rays, cambium or pith. In *Pinus strobus* cuttings the initiation of roots took place in the association of rays and leaf traces. In *Taxus cuspidate* cuttings, adventitious roots originate from the secondary phloem, phloem ray and surrounding parenchyma.

From this experiment it has been found that the different PGRS have different effects at different levels of concentration and time of treatment. All the hormones i.e. NAA, IBA and IAA had good effect on rooting percentage, increase root length and number of roots. Among them higher

concentration and longer time of treatment of IBA and NAA inhibited rooting. The concentration (1000 ppm of IAA) for 30 minutes of treatment showed high percentage of rooting. The plant obtained from this experiment could survive well in the field. Acclimatization of such plants could survive well and successfully established.

CHAPTER SIX

CONCLUSION

From this experiment, it can be concluded that rooting in stem cuttings of *Ginkgo biloba* is determined by many factors: the presence or absence of leaves and buds, number of leaves present on cuttings and type of cuttings used i.e. hard wood, softwood and semi hard wood. Temperature, bed prepared, watering and humidity also determine rooting. The tissue system of cuttings also affect in rooting. Time of hormone treatment, type of hormone used and its concentration used affected rooting. In *Ginkgo biloba* IAA hormone has been found to be the most appropriate hormone for rooting of cuttings than other hormones like NAA and IBA.

RECOMMENDATIONS

The following recommendations are drawn from the present study.

(1) *In vivo* rooting is one of the best promising methods of propagation of *Ginkgo biloba*. Seed germination of *Ginkgo biloba* takes long time because of its long dormancy period.

(2) Propagation through stem cutting is an effective method for the support of *ex-situ* conservation of important plant species, thus concerned authorities should encourage to work on such plants.

(3) The results obtained from this study can be implemented for further researchers which help to uplift the conservation program.

CHAPTER SEVEN

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ANNEX

IBA : Rainy season, percentage of rooting considered as dependent and concentration as independent variables.

Oneway ANOVA

Percentage of rooting

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.600	4	.650	3.112	.024
Within Groups	9.400	45	.209		
Total	12.00	49			

Percentage of Rooting

	concentration	N	Subset for alpha = .05	
			1	2
Duncan(a)	.00	10	.1	
	250.00	10	.3	
	1000.00	10	.4	.4
	500.00	10	.4	.4
	750.00	10		.8
	Sig.		.188	.070

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10..

Post Hoc Tests **Multiple Comparisons**

Dependent Variable: Percentage of rooting

	(I) concentration ppm	(J) concentration ppm	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	1000.00	-.30	.20	.149	-.71	.11
		250.00	-.20	.20	.333	-.61	.21
		500.00	-.30	.20	.149	-.71	.11
		750.00	-.70(*)	.20	.001	-1.11	-.28
	1000 .00	.00	.30	.20	.149	-.11	.71
		250.00	.10	.20	.627	-.31	.51
		500.00	.00	.20	1.20	-.41	.41
		750.00	-.40	.20	.057	-.81	.01
	250.00	.00	.20	.20	.333	-.21	.61
		1000.00	-.10	.20	.627	-.51	.31
		500.00	-.10	.20	.627	-.51	.31
		750.00	-.50(*)	.20	.018	-.91	-.08
	500.00	.00	.30	.20	.149	-.11	.71
		1000.00	.00	.20	1.20	-.41	.41
		250.00	.10	.20	.627	-.31	.51
		750.00	-.40	.20	.057	-.81	.01
	750.00	.00	.70(*)	.20	.001	.2883	1.11
		1000.00	.40	.20	.057	-.01	.81
		250.00	.50(*)	.20	.018	.0883	.91
		500.00	.40	.20	.057	-.01	.81

* The mean difference is significant at the .05 level.

IAA: Rainy season, percentage of rooting considered as dependent and concentration as independent variables.

1. **Oneway** **ANOVA**

Percentage of rooting

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.280	4	1.070	5.872	.001
Within Groups	8.200	45	.182		
Total	12.480	49			

Post Hoc Tests **Multiple Comparisons**

Dependent Variable: percentage of rooting

	(I) concentration ppm	(J) concentration ppm	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	250.00	-.30	.19	.123	-.68	.08
		500.00	-.30	.19	.123	-.68	.08
		750.00	-.70(*)	.19	.001	-1.08	-.31
		1000.00	-.80(*)	.19	.	-1.18	-.41
	250.00	.00	.30	.19	.123	-.08	.68
		500.00	.00	.19	1.	-.38	.38
		750.00	-.40(*)	.19	.042	-.78	-.01
		1000.00	-.50(*)	.19	.012	-.88	-.11
	500.00	.00	.30	.19	.123	-.08	.68
		250.00	.00	.19	1.	-.38	.38
		750.00	-.40(*)	.19	.042	-.78	-.01
		1000.00	-.50(*)	.19	.012	-.88	-.11
	750.00	.00	.70(*)	.19	.001	.31	1.08
		250.00	.40(*)	.19	.042	.01	.78
		500.00	.40(*)	.19	.042	.01	.78
		1000.00	-.10	.19	.603	-.48	.28
	1000.00	.00	.80(*)	.19	.	.41	1.15
		250.00	.50(*)	.19	.012	.11	.88
		500.00	.50(*)	.19	.012	.11	.8845
		750.00	.10	.19	.603	-.28	.48

* The mean difference is significant at the .05 level.

Homogeneous Subsets

Percentage of rooting

	Concentration	N	Subset for alpha = .05		
			1	2	3
Duncan(a)	.00	10	.1		
	250.00	10	.4	.4	
	500.00	10	.4	.4	
	750.00	10		.8	.8
	1000.00	10			.9
	Sig.		.145	.052	.603

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10..

IAA: Rainy season, each length of root considered as dependent and concentration as independent.

Oneway ANOVA

Each length of root

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	351.146	4	87.787	21.998	.00
Within Groups	1416.660	355	3.991		
Total	67.806	359			

Homogeneous Subsets Each Length of root

	Concentration	N	Subset for alpha = .05		
			1	2	3
Duncan(a,b)	.00	9	1.5333		
	250.00	25	1.5840		
	750.00	108		3.0120	
	500.00	78		3.8397	3.8397
	1000.00	140			4.7157
	Sig.		.925	.124	.103

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 27.726.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Post Hoc Tests **Multiple Comparisons**

Dependent Variable: Each length of root

	(I) concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	250.00	-.0506	.776	.948	-1.57	1.47
		500.00	-2.306(*)	.703	.001	-3.69	-.92
		750.00	-1.478(*)	.69	.034	-2.84	-.11
		1ooo.00	-3.182(*)	.686	.00	-4.53	-1.83
	250.00	.00	.0506	.776	.948	-1.47	1.57
		500.00	-2.255(*)	.459	.00	-3.15	-1.35
		750.00	-1.428(*)	.443	.001	-2.3	-.55
		1ooo.00	-3.131(*)	.433	.00	-3.98	-2.27
	500.00	.00	2.306(*)	.703	.001	.92	3.68
		250.00	2.255(*)	.459	.00	1.35	3.15
		750.00	.8277(*)	.296	.006	.24	1.41
		1ooo.00	-.875(*)	.282	.002	-1.43	-.32
	750.00	.00	1.478(*)	.693	.034	.11	2.84
		250.00	1.428(*)	.443	.001	.55	2.3
		500.00	-.827(*)	.296	.006	-1.41	-.243
		1000.00	-1.703(*)	.255	.00	-2.20	-1.20
	1000.00	.00	3.182(*)	.686	.00	1.83	4.533
		250.00	3.131(*)	.433	.00	2.27	3.98
		500.00	.875(*)	.282	.002	.32	1.43
		750.00	1.703(*)	.255	.00	1.20	2.20

* The mean difference is significant at the .05 level.

IBA: Rainy Season, each root of length considered as dependent and concentration as independent variables.

Oneway **ANOVA**

Each length of root.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26.2	4	6.575	3.239	.014
Within Groups	336.9	166	2.030		
Total	363.218	170			

Homogeneous Subsets

Each length of root.

	Concentration ppm.	N	Subset for alpha = .05	
			1	2
Duncan(a,b)	.00	9	1.53	
	750.00	87	1.93	
	1000.00	40	1.97	
	250.00	17	2.21	
	500.00	18		3.16
	Sig.		.184	1.

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 19.085.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Post Hoc Tests Multiple Comparisons

Dependent Variable: Each length of root.

	(I) concentration ppm.	(J) concentration ppm.	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	1000.00	-.43917	.525	.405	-1.47	.598
		250.00	-.67843	.587	.250	-1.83	.481
		500.00	-1.627(*)	.581	.006	-2.79	-.479
		750.00	-.430	.498	.421	-1.38	.582
	1000.00	.00	.43917	.525	.405	-.59	1.476
		250.00	-.23926	.412	.563	-1.05	.575
		500.00	-1.188(*)	.404	.004	-1.98	-.390
		750.00	.03687	.272	.892	-.50	.57
	250.00	.00	.67843	.587	.250	-.48	1.83
		1000.00	.23926	.412	.563	-.57	1.053
		500.00	-.94935	.481	.050	-1.90	.001
		750.00	.27613	.377	.466	-.46	1.00
	500.00	.00	1.627(*)	.581	.006	.47	2.77
		1000.00	1.188(*)	.404	.004	.390	1.98
		250.00	.94935	.481	.050	-.001	1.90
		750.00	1.225(*)	.368	.001	.497	1.95
	750.00	.00	.430	.498	.421	-.582	1.38
		1000.00	-.03687	.272	.892	-.57	.50
		250.00	-.27613	.377	.466	-1.	.46
		500.00	-1.225(*)	.368	.001	-1.95	-.49

The mean difference is significant at the .05 level.

NAA: Rainy season ,each length of root considered as dependent and concentration considered as independent variables.

Oneway ANOVA

Each length of root.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	21.506	4	5.376	1.787	.139
Within Groups	264.806	88	3.009		
Total	286.312	92			

Post Hoc Tests Multiple Comparisons

Dependent Variable: Each length of root

	(I) Concentration ppm	(J) Concentration ppm	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	250.00	-.48	1.35	.719	-3.18	2.60
		500.00	-.81	.613	.187	-2.03	.40
		750.00	-.131	.874	.881	-1.86	1.60
		1000.00	1.51	1.15	.195	-.78	3.80
	250.00	.00	.48	1.35	.719	-2.60	3.18
		500.00	-.32	1.24	.794	-2.79	2.14
		750.00	.35	1.39	.798	-2.40	3.12
		1000.00	2.00	1.58	.210	-1.14	5.14
	500.00	.00	.815	.613	.187	-.40	2.03
		250.00	.32	1.24	.794	-2.14	2.79
		750.00	.68	.68	.322	-.68	2.04
		1000.00	2.32(*)	1.21	.5	.29	4.35
	750.00	.00	.131	.87	.881	-1.60	1.86
		250.00	-.35	1.395	.798	-3.12	2.40
		500.00	-.68	.68	.322	-2.04	.68
		1000.00	1.64	1.19	.173	-.73	4.17
	1000.00	.00	-1.51	1.15	.195	-3.80	.78
		250.00	-2.00	1.58	.210	-5.14	1.14
		500.00	-2.32(*)	1.21	.5	-4.35	-.29
		750.00	-1.64	1.19	.173	-4.17	.73

* The mean difference is significant at the .05 level.

Homogeneous subsets

Each length of root

	Concentration ppm	N	Subset for alpha = .05
			1
Duncan(a,b)	1000.00	3	.0
	.00	9	1.51
	750.00	7	1.64
	250.00	2	2.0
	500.00	72	2.32
	Sig.		.074

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 4.541.

b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

IAA: Summer season , percentage of rooting considered as dependent and Times of treatments considered as independent variables.

Oneway ANOVA

Percentage of rooting

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.933	5	.187	1.9	.410
Within Groups	9.800	54	.181		
Total	10.733	59			

Homogeneous Subsets Percentage of rooting

			Subset for alpha = .05
	Concentration ppm	N	1
Duncan(a)	.00	10	.00
	5.00	10	.20
	10.00	10	.20
	.00	10	.30
	25.00	10	.30
	15.00	10	.40
	Sig.		.070

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10

Post Hoc Tests Multiple Comparisons

Dependent Variable: Percentage of rooting.

	(I) Times of Treatments mins.	(J) Times of Treatments mins.	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	5.00	-.20	.19	.298	-.58	.18
		10.00	-.20	.19	.298	-.58	.18
		15.00	-.40(*)	.19	.040	-.78	-.0180
		20.00	-.30	.19	.121	-.68	.08
		25.00	-.30	.19	.121	-.68	.08
	5.00	.00	.20	.19	.298	-.18	.58
		10.00	.00	.19	1.20	-.38	.38
		15.00	-.20	.19	.298	-.58	.18
		20.00	-.10	.19	.60	-.48	.28
		25.00	-.10	.19	.60	-.48	.28
	10.00	.00	.20	.19	.298	-.18	.58
		5.00	.00	.19	1.20	-.38	.38
		15.00	-.20	.19	.298	-.58	.18
		20.00	-.10	.19	.60	-.48	.28
		25.00	-.10	.19	.60	-.48	.28
	15.00	.00	.40(*)	.19	.040	.0180	.78
		5.00	.20	.19	.298	-.18	.58
		10.00	.20	.19	.298	-.18	.58
		.00	.10	.19	.60	-.28	.48
		25.00	.10	.19	.60	-.28	.48
	200.00	.00	.30	.19	.121	-.08	.68
		5.00	.10	.19	.60	-.28	.48
		10.00	.10	.19	.60	-.28	.48
		15.00	-.10	.19	.60	-.48	.28
		25.00	.00	.19	1.20	-.38	.38
	25.00	.00	.30	.19	.121	-.08	.68
		5.00	.10	.19	.60	-.28	.48
		10.00	.10	.19	.60	-.28	.48
		15.00	-.10	.19	.60	-.48	.28
		20.00	.00	.19	1.20	-.38	.38

* The mean difference is significant at the .05 level.

NAA: Rainy season, percentage of rooting considered as dependent and concentration as independent variables.

Oneway ANOVA

Percentage of rooting

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.280	4	.070	.630	.644
Within Groups	5.	45	.111		
Total	5.280	49			

Homogeneous subsets Percentage of rooting

			Subset for alpha = .05
	Concentration ppm	N	1
Duncan(a)	1.00	10	.00
	.00	10	.10
	250.00	10	.10
	500.00	10	.20
	750.00	10	.20
	Sig.		.240

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10.

Post Hoc Tests Multiple Comparisons

Dependent Variable: Percentage of rooting.

	(I) Concentration ppm	(J) Concentration ppm	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	250.00	.00	.14	1.20	-.30	.30
		500.00	-.10	.14	.506	-.40	.20
		750.00	-.10	.14	.506	-.40	.20
		1000.00	.10	.14	.506	-.20	.40
	250.00	.00	.00	.14	1.20	-.30	.30
		500.00	-.10	.14	.506	-.40	.20
		750.00	-.10	.14	.506	-.40	.20
		1000.00	.10	.14	.506	-.20	.40
	500.00	.00	.10	.14	.506	-.20	.40
		250.00	.10	.14	.506	-.20	.40
		750.00	.00	.14	1.20	-.30	.30
		1000.00	.20	.14	.186	-.10	.50
	750.00	.00	.10	.14	.506	-.20	.40
		250.00	.10	.14	.506	-.20	.40
		500.00	.00	.14	1.20	-.30	.30
		1000.00	.20	.14	.186	-.10	.50
	1000.00	.00	-.10	.14	.506	-.40	.20
		250.00	-.10	.14	.506	-.40	.20
		500.00	-.20	.14	.186	-.50	.10
		750.00	-.20	.14	.186	-.50	.10

** The mean difference is significant at the .05 level.

IBA: **summer season, percentage of rooting considered as dependent and times of treatments considered as independent t variables.**

Oneway ANOVA

Percentage of rooting

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.483	5	.297	1.841	.120
Within Groups	8.700	54	.161		
Total	10.183	59			

Post Hoc Tests **Multiple Comparisons**

Dependent Variable: Percentage of rooting.

	(I) Time of treatments mins	(J) Times of treatments mins	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	5.00	-.50(*)	.17	.007	-.85	-.1401
		10.00	-.20	.17	.270	-.55	.15
		15.00	-.30	.17	.100	-.65	.05
		20.00	-.20	.17	.270	-.55	.15
		25.00	-.10	.17	.580	-.45	.25
	5.00	.00	.50(*)	.17	.007	.14	.85
		10.00	.30	.17	.100	-.05	.65
		15.00	.20	.17	.270	-.15	.55
		20.00	.30	.17	.100	-.05	.65
		25.00	.40(*)	.17	.030	.04	.75
	10.00	.00	.20	.17	.270	-.15	.55
		5.00	-.30	.17	.100	-.65	.05
		15.00	-.10	.17	.580	-.45	.25
		20.00	.00	.17	1.20	-.35	.35
		25.00	.10	.17	.580	-.25	.45
	15.00	.00	.30	.17	.100	-.05	.65
		5.00	-.20	.17	.270	-.55	.15
		10.00	.10	.17	.580	-.25	.45
		20.00	.10	.17	.580	-.25	.45
		25.00	.20	.17	.270	-.15	.55
	20.00	.00	.20	.17	.270	-.15	.55
		5.00	-.30	.17	.100	-.65	.05
		10.00	.00	.17	1.20	-.35	.35
		15.00	-.10	.17	.580	-.45	.25
		25.00	.10	.17	.580	-.25	.45
	25.00	.00	.10	.17	.580	-.25	.45
		5.00	-.40(*)	.17	.030	-.75	-.0401
		10.00	-.10	.17	.580	-.45	.25
		15.00	-.20	.17	.270	-.55	.15
		20.00	-.10	.17	.580	-.45	.25

The mean difference is significant at the .05 level.

Homogeneous Subsets

Percentage of rooting

	Times of Treatments mins	N	Subset for alpha = .05	
			1	2
Duncan(a)	.00	10	.00	
	25.00	10	.10	
	10.00	10	.20	.20
	2o.00	10	.20	.20
	15.00	10	.30	.30
	5.00	10		.50
	Sig.		.143	.133

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 10..

NAA: Summer season ,percentage of rooting considered as dependent and times of treatments considered as independent variables.

Oneway ANOVA

Percentage of rooting

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.683	5	.337	2.490	.042
Within Groups	7.300	54	.135		
Total	8.983	59			

Homogeneous Subsets Percentage of rooting

	Times of treatments	N	Subset for alpha = .05	
			1	2
Duncan(a)	.00	10	.00	
	25.00	10	.00	
	20.00	10	.10	.10
	15.00	10	.20	.20
	5.00	10		.40
	10.00	10		.40
	Sig.		.275	.101

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10..

Post Hoc Tests Multiple Comparisons

Dependent Variable: Percentage of rooting

	(I) Times of treatments mins	(J) Time if treatments mins	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.00	5.00	-.40(*)	.16	.018	-.72	-.0703
		10.00	-.40(*)	.16	.018	-.72	-.0703
		15.00	-.20	.16	.229	-.52	.12
		20.00	-.10	.16	.546	-.42	.22
		25.00	.00	.16	1.20	-.32	.32
	5.00	.00	.40(*)	.16	.018	.0703	.72
		10.00	.00	.16	1.20	-.32	.32
		15.00	.20	.16	.229	-.12	.52
		20.00	.30	.16	.074	-.02	.62
		25.00	.40(*)	.16	.018	.0703	.72
	10.00	.00	.40(*)	.16	.018	.0703	.72
		5.00	.00	.16	1.20	-.32	.32
		15.00	.20	.16	.229	-.12	.52
		20.00	.30	.16	.074	-.02	.62
		25.00	.40(*)	.16	.018	.0703	.72
	15.00	.00	.20	.16	.229	-.12	.52
		5.00	-.20	.16	.229	-.52	.12
		10.00	-.20	.16	.229	-.52	.12
		20.00	.10	.16	.546	-.22	.42
		25.00	.20	.16	.229	-.12	.52
	20.00	.00	.10	.16	.546	-.22	.42
		5.00	-.30	.16	.074	-.62	.02
		10.00	-.30	.16	.074	-.62	.02
		15.00	-.10	.16	.546	-.42	.22
		25.00	.10	.16	.546	-.22	.42
	25.00	.00	.00	.16	1.20	-.32	.32
		5.00	-.40(*)	.16	.018	-.72	-.0703
		10.00	-.40(*)	.16	.018	-.72	-.0703
		15.00	-.20	.16	.229	-.52	.12
		20.00	-.10	.16	.546	-.42	.22

* The mean difference is significant at the .05 level.