STANDARDISATION OF PROPAGATION THROUGH CUTTINGS IN *Gymnema sylvestre* R. Br.

By
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THESIS

Submitted in partial fulfilment of the
requirement for the degree

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Kerala Agricultural University

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COLLEGE OF HORTICULTURE
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DECLARATION

I hereby declare that the thesis entitled '**Standardisation of propagation through cuttings in *Gymnema sylvestre* R. Br.**' is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that the thesis entitled `**Standardisation of propagation through cuttings in *Gymnema sylvestre* R. Br.**' is a record of research work done independently by **Mr.D. Rajesh**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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We, the undersigned members of the Advisory Committee of **Mr.D.Rajesh,** a candidate for the degree of **Master of Science in Horticulture** with major in **Plantation Crops and Spices**, agree that the thesis entitled **'Standardisation of propagation through cuttings in *Gymnema sylvestre* R. Br.'** may be submitted by Mr.D. Rajesh in partial fulfilment of the requirement for the degree.

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Introduction

INTRODUCTION

Gymnema sylvestre R.Br. (Asclepiadaceae) known as 'Gurmar' in Indian folklore and locally termed 'Chakkarakolli' (Madhunashini) is a large, more or less pubescent, laticiferous woody climber found in the Deccan Peninsula extending to parts of northern and western India. Leaves of this species are opposite, usually elliptic or ovate. Flowers are small, yellow and produced in umbellate cymes.

The plant is a much sought after rare medicinal species and is valued for its stomachic, stimulant, laxative and diuretic properties. It is also used in the treatment of cough and sore eyes in the traditional system of Indian medicine. But *Gymnema* is most popular for the remarkable property of paralysing the sense of taste for sweet substances for a few hours (Chopra *et al.*, 1928) and hence it is advocated as a remedy for diabetes.

Diabetes mellitus is a common metabolic disorder of human beings and the present mode of insulin therapy imposes lots of medical complications and side effects. Though insulin is available in various forms, risk of developing insulin antibodies on long term use limits its utility. Other oral hypoglycaemic agents also possess side effects (Chaturvedi *et al.*, 1984).

In recent years, there is an increasing demand for the herbal antidiabetic preparations made from this plant such as 'Diabetea' and 'Diatea'. The efficiency of leaf powder of this plant in checking glycosuria and hyperglycaemia has already been reported (Shanmughasundaram *et al.*, 1990). Gymnema, if properly exploited can be considered as a boon to patients suffering from diabetes mellitus.

The need of the hour is to domesticate this valuable medicinal species, for which the first step should be identifying a viable procedure for large scale multiplication of superior strain. *Gymnema sylvestre*, being a member of the family Asclepiadaceae seldom sets seeds and hence seeds cannot be used as propagules for large scale multiplication. *In vitro* mass propagation methods were also attempted by Nazeem *et al.* (1991). But limited success was obtained and a viable protocol for mass multiplication is yet to be standardized. *Gymnema* is usually propagated by rooted vine cuttings, but rooting success is generally very low. It is difficult to propagate vegetatively at present. As a result, the rooted cuttings are being sold at a very high price.

Information regarding the nature of vine cuttings to be used, the effect of growth regulators and their optimum concentration for rooting and conducive environmental conditions will greatly help to standardise the propagation through cuttings in *Gymnema.* It may also help to boost up large scale multiplication of elite planting material at a cheaper cost. It is against this background and impending necessity that the present studies have been taken up in the Department of Plantation Crops and Spices with the objectives of standardising the methods to improve rooting efficiency of cuttings in *Gymnema sylvestre* and to throw light into the biochemical and anatomical changes associated with its root development.

Review of Literature

REVIEW OF LITERATURE

Gymnema sylvestre popularly known as 'gurmar' in Indian folklore is a medicinal plant with stomachic, stimulant, laxative, diuretic and other such properties. Of the many medicinal properties attributed to this plant, its hypoglycaemic property is the most valued one. The leaves of this plant, when chewed, possess the remarkable property of paralysing the sensory buds of sweet taste for a few hours (Chopra *et al.*, 1928).

Diabetes mellitus is a common metabolic disorder of human beings and the present mode of insulin therapy imposes a lot of medical complications and side effects. Though insulin is available in various forms, risk of developing insulin antibodies on long term use limits its utility. Other oral hypoglycaemic agents also possess side effects.

There are quite a few reports pertaining to the antidiabetic effect of *Gymnema sylvestre*. The hypoglycaemic property of *Gymnema sylvestre* was initially reported to be not due to any direct influence on the carbohydrate metabolism, but to indirect stimulation of insulin secretion by pancreas. No water soluble or alcohol soluble substance which destroy glucose *in vitro* has been identified in the leaves (Chopra *et al.*, 1928; Mhaskar and Caius, 1930 and Kirtikar *et al.*, 1975).

Manni and Sinsheimer (1965) could isolate various constituents from *Gymnema sylvestre* leaves. Nonacosane, hentriacontane and tritriacontane were isolated by vapour phase chromatography from a hydrocarbon fraction of *Gymnema sylvestre* leaves. The cyclic alcohol, conduritol A was also isolated from these leaves.

Srivastava *et al.* (1981) reported the experimental evidence for hypoglycaemic effect of *Gymnema sylvestre* in diabetic Charles Foster rats. Oral administration of aqueous suspension of dried leaves of the plant exhibited hypoglycaemic activity in moderately diabetic animals. This hypoglycaemia persisted even after discontinuation of the treatment. The drug also increased longevity in severe and toxic diabetic animals.

A hexahydroxy triterpene called gymnemagenin has been isolated from the leaves of *Gymnema sylvestre*. The sugar moieties obtained from the hydrolysis of the saponin were d. glucoronic acid and d. galactose (Chakrabarti and Debnath, 1981).

Another study conducted by Srivastava *et al.* (1988) revealed the importance of *Gymnema sylvestre* in diabetes mellitus. The aqueous extracts of dried leaf powder were given to patients and found improved signs and symptoms without any toxicity in liver, kidney or blood.

Three new saponins such as gymnemic acid V, VI and VII were isolated from the leaves of *Gymnema sylvestre*; of which gymnemic acid V and VI were found to have anti-sweet properties (Yoshikawa *et al.*, 1989).

The efficiency of leaf powder of 'gurmar' in checking glycosuria and hyperglycaemia has been reported by Shanmughasundaram *et al.* (1990). GS_4, a water soluble extract of the leaves, when administrated to patients with insulin dependent diabetes mellitus (IDDM) appeared to enhance endogenous insulin possibly by regeneration of the residual betacells in IDDM.

Kamei *et al.* (1992) isolated a sweet-taste-suppressing peptide called 'gurmarin' from the leaves of *Gymnema sylvestre*. They also determined the complete amino acid sequence of this peptide and observed that gurmarin consisted of 35 amino acid residues with an amino-terminal pyroglutamyl residue and had a molecular weight of 4209. Gurmarin had no significant homology with other known proteins.

Seven new dammarane type triterpene glycosides, named gymnemasides I-VII were isolated from the saponin extracts of the leaves of *Gymnema sylvestre* (Yoshikawa *et al.*, 1992). They also suggested that the antisweet activity of the saponins is increased by an increased number of acyl groups.

Chattopadhyay *et al.* (1993) reported that oral administration of the water-soluble ethnolic extract of *Gymnema sylvestre* leaves resulted in a marked fall in the blood glucose level in normal, glucose-fed hyperglycaemic, insulin-treated and streptozotocin induced diabetic rats. So they concluded that the extract potentiated insulin release from pancreatic beta cells.

Miyatake *et al.* (1993) isolated a compound called conduritol A from an extract of *Gymnema sylvestre* dried leaves. This compound was also tested for its effect on intestinal glucose absorption in rats. The results showed that the absorption of glucose *in vitro* was completely inhibited in the presence of 0.2 mg/ml and that the blood sugar level in rats was effectively depressed by administering conduritol A at 10 mg/kg of rat body weight.

Ota and Ariyoshi (1995) reported that gurmarin, the sweetness suppressing polypeptide with 35 amino acid residues consisted of 3 intramolecular disulfide bond. The exact positions of these disulfide bonds were also located.

Six known gymnemic acids and four new triterpenoid saponins, named gymnemasins A, B, C and D, were isolated from the leaves of *Gymnema sylvestre* by Sahu *et al.* (1996). A new compound called gymnemanol was the aglycone component of these saponins.

Gymnema sylvestre, being a member of the family Asclepiadaceae seldom sets seeds and hence seeds cannot be used as propagules for large scale multiplication. *In vitro* mass propagation methods were also attempted by Nazeem *et al.* (1991) with limited success and a viable protocol for mass multiplication is yet to be standardised. Callusing could be induced in mature leaf explants cultured on MS medium supplemented with Kinetin 1 ppm and NAA 2 ppm. The friable light green callus however failed to differentiate to form vascular tissues. Anu (1993) reported that the main limitation in establishing *in vitro* cultures of *Gymnema sylvestre* was microbial interference by the fungus *Colletotrichum sp.*

Gurmar is usually propagated by rooted vine cuttings, but rooting success is generally low. It is difficult to propagate vegetatively at present so the rooted cuttings are being sold at a very high price.

Information regarding the nature of vine cuttings to be used, the effect of growth regulators and their optimum concentration for rooting; conducive environmental conditions for rooting and other related aspects will help to standardise the propagation through cuttings in *Gymnema sylvestre*. This may also help to boost up large scale multiplication of elite planting material at cheaper cost.

As such literature available on the vegetative multiplication aspects of this crop is scanty. However, information pertinent to this study on other medicinal and aromatic plants are also reviewed hereunder.

2.1 Vegetative propagation through stem cuttings

Vegetative or asexual propagation is used to produce a large number of genetically identical plants in a relatively short interval of time. This is not a natural phenomenon for most plant species and special techniques have been developed to facilitate propagation.

In the case of propagation by stem cuttings, segments of shoots containing lateral or terminal buds develop adventitious roots under conducive conditions and thus produce independent plants.

Several factors such as the type of wood, stage of growth, treatment with growth regulators, exposure to misting, retention of leaves and the time of the year in which cuttings are taken influence the rooting of cuttings in plants (Hartman *et al.*, 1993).

2.1.1 Effect of type of wood and number of nodes

In *Rauvolfia serpentina*, Chandra (1956) reported that the hard wood cuttings of size 5" to 8" length produced roots within 15 days after planting with hormonal treatment. Sahu (1979) also reported that 7.5 cm long stem cuttings with two buds was the best material for vegetative propagation and noticed 66 per cent germination. Gauniyal *et al.* (1988) suggested that the stem cuttings of 6-7 cm length with 2 buds is best suited for propagation of *Rauvolfia serpentina* and the hard wood cuttings performed better than softwood cuttings.

Shanthamalliah *et al.* (1974) reported that in black pepper semiherbaceous cuttings taken from the middle portion of the stem rooted better than the herbaceous cuttings from soft terminal or hardwood cuttings. According to

Nambiar *et al.* (1977) for rapid multiplication of the hybrid pepper cv. Panniyur-1, two noded cuttings could be used. Nambiar *et al.* (1978) compared the rooting behaviour of two, four and six noded cuttings and found that two noded cuttings were the best planting material resulting in maximum rooting and field establishment.

Bavappa and Gurusinghe (1980) showed that pepper cuttings with even one node could be successfully propagated similar to cuttings with several nodes. Hegde (1983) found that three noded cuttings of Panniyur-1 pepper rooted better than one or two noded cuttings.

Swamy and Kalyanasundaram (1960) reported that geranium could be successfully propagated through stem cuttings. The rooting percentage was found to be high in middle cuttings than in basal cuttings (Duraiswamy and Arumugam, 1980).

El-Keltawi and Croteau (1985) reported the use of single node cuttings to propagate several species of mint. Mitra and Kushari (1985) observed that in *Solanum khasianum* the rooting percentage was the highest with four noded cuttings and the lowest with single noded cuttings.

Rao and Selvarajan (1982) reported that herbaceous cuttings of *Pogostemon cablin* were found to be the ideal planting material with 100 per cent rooting and survival. In *Rosmarinus officinalis,* rooting per cent was the highest when 9-12 cm long cuttings taken from one year old shoots were used.

Vegetative propagation studies conducted by Philip *et al.* (1991) revealed that hardwood cuttings of 20 cm length in *Sida retusa*, semi hardwood cuttings of 15-20 cm length in *Vitex negundo* and six noded semi hardwood cuttings in

Piper longum showed better response to rooting. Higher the number of nodes, greater will be the rooting percentage and survival rate.

Sudhadevi (1992) compared the rooting behaviour of softwood, semi hardwood and hardwood cuttings of *Alstonia venenata*. The results revealed that softwood cuttings failed to root. Semi hardwood cuttings recorded maximum sprouting compared to hardwood cuttings. She also reported that in *Coscinium fenestratum* the percentage of sprouting was only 40 in stem cuttings on 60th day and they failed to root within this period.

Pal *et al.* (1993a) studied the rooting of shoot cuttings of Datura and found that semi hardwood cuttings were difficult to root while the softwood cuttings rooted easily.

2.1.2 Effect of hormones and their concentrations

The practical use of synthetic auxin in stimulating root formation from the basal part of cuttings was demonstrated about five decades ago by several workers (Thimann and Went, 1934; Cooper, 1935; Hitchcock and Zimmermann, 1940). The discovery of naturally occurring auxins like IAA (indole acetic acid) and synthetic auxins like IBA (indole-3-butyric acid) was a milestone in the history of propagation and was of real value in stimulating the production of adventitious roots in stem cuttings in several horticultural plants (Linder, 1939).

Mitra and Kushari (1985) observed that in *Solanum khasianum*, cuttings were moderately easy-to-root and treatment with indole-3-butyric acid (IBA) increased the per cent of rooting as well as root growth.

Hurov (1967) reported that black pepper cuttings when dipped in 0.2 per cent IBA and placed in rooting media recorded the best results. Pillai *et al.* (1982) conducted studies on rooting of two noded cuttings of pepper dipped in 1000 ppm IBA for 15-60 seconds. Cuttings dipped for 45 seconds recorded the highest percentage of rooting on 20th and 90th day of observation.

In *Tylophora indica*, cuttings prepared from lateral shoots showed maximum rooting success, when treated with IBA at 1000 ppm concentration (Pal *et al.*, 1993b).

Fouda and Schmidt (1994) observed that in Rose hybrid cv. Red Success, the best rooting (86.25%) was produced by treatment of cuttings with IBA at 1000 ppm.

Thespesia populnea stem cuttings responded better to IAA than NAA or IBA (Basak *et al.*, 1995).

In neem (*Azadirachta indica*) leafy stem cuttings treated with 0.2-0.4 per cent IBA gave the best results for root development (Kamaluddin and Ali, 1996).

2.1.3 Effect of mist on rooting of cuttings

Intermittent mist has been used in propagation since 1940. An increase in the relative humidity and retention of a film of water on the leaf surface help to reduce the transpiration rate of cuttings kept for rooting within a mist chamber. This prevents desiccation of cuttings and provides more favourable environmental conditions for root formation.

Garner (1944) reported that the prevailing environment inside the rooting structure would decide the extent of initiation and development of new roots and intermittent mist at high light intensity has a favourable influence on rooting.

Beneficial effect of mist on rooting in a wide variety of difficult to root plant species has been recorded by Erickson and Bitters (1953).

Mitra and Kushari (1985) observed that mist propagation under plastic resulted in high percentage of rooted cuttings in many plant species.

2.1.4 Effect of retention of leaves

It has long been known that the presence of leaves on cuttings exerts a strong stimulatory influence on root initiation.

Arumugam and Kumar (1980) studied the effect of leaves on rooting of stem cuttings of bergamot mint and reported that stem cuttings with two leaves retained on it could be used for large scale propagation. Ivanova and Gladun (1986) observed that two noded cuttings with upper two leaves retained was the best planting material for propagation of *Rosa damacena*.

Modak *et al.* (1990) reported that in *Adhatoda vasica* better rooting occurred in foliated cuttings than in defoliated cuttings.

Sudhadevi (1992) observed that retention or removal of leaves had no influence on the rooting of cuttings in *Alstonia venenata*.

2.1.5 Field survival of rooted cuttings

Factors which determine the root initiation and development of cuttings can also influence field establishment of successfully rooted cuttings.

Olive cuttings treated with IBA at 5000 ppm and kept under intermittent mist showed maximum rooting (65-70%) and field survival (80-100%)(Gautam and Chauhan, 1990).

In *Tylophora indica* stem cuttings treated with IBA at 1000 ppm and kept in mist chamber showed the maximum rooting success and percentage survival of rooted cuttings (Pal *et al.*, 1993b).

Noor-Aini and Ling (1993) reported that in *Shorea parvifolia,* rooted terminal cuttings survived best in the field whereas the rooted basal cuttings showed the lowest survival rate.

2.2 Anatomical changes during rooting of cuttings

The root initiation and development in any plant is largely governed by the anatomy of the stem (Hartmann *et al.*, 1993). The developmental anatomical changes that occur in adventitious root formation from stem cuttings are dedifferentiation and formation of callus at the base, root initiation, subsequent development into root primordia, emergence of the primordia outwards and formation of vascular connections (Hartmann *et al.*, 1993).

The precise site of origin of adventitious roots differ with plant species. In woody perennial plants, adventitious roots usually originate from living parenchymatous cells primarily in the young secondary phloem. Medullary rays, cambium, lenticels and pith have also been reported to give rise to adventitious roots (Nanda, 1975).

Also, within a species itself, different tissues can function as the site of origin of roots.

2.3 Biochemical changes during rooting

Physiological as well as biochemical changes occurring within the regenerating organ greatly influence the successful formation of root initials on cuttings.

A balance between carbohydrate and nitrogen reserves appear to be better for root development. Sen *et al.* (1965) has shown that a high C/N ratio is beneficial for rooting of cuttings. Stock plants with low C/N ratio produced cuttings with low rooting potential (Hartmann, 1956).

A decrease in the carbohydrate content in the cuttings is generally observed during the first few days of the rooting period (Haissig, 1982). However, a net accumulation of carbohydrates normally occurs until the roots emerge from the cuttings (Davis and Potter, 1981). Accumulation of sugars and starch in cuttings begins in the leaves, at a later point in the stem and lastly in the base of the cutting (Haissig *et al.*, 1988).

Basu *et al.* (1967) recorded that total nitrogen in the bark and wood decreased during root formation. The net synthesis of proteins has also been found to fall down during the root initiation (Roychoudhary, 1971). However, Kamineck (1968) pointed out that protein synthesis was a prerequisite for root formation. New proteins were synthesised during root initiation.

A decrease in the total sugar, total carbohydrate and polyphenols and an increase in total nitrogen were recorded in the girdled tissues of *Thespesia populnea* cuttings at the time of root initiation and development. These changes were further pronounced in those cuttings treated with auxins (Basak *et al.*, 1995). They further observed that C/N ratio decreased and protein-nitrogen activity increased during root formation.

Materials and Methods

MATERIALS AND METHODS

The present studies on standardisation of propagation through cuttings in *Gymnema sylverstre* R.Br. were carried out in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara, Trichur during the period from May 1997 to January 1998.

Vellanikkara is situated 10° 31' N latitude and 75° 13' E longitude, with an elevation of about 40 M above the Mean Sea Level. The area is characterised with heavy rains during June-September (South West Monsoon) and October-November (North East Monsoon) months followed by a summer season from March to May. The meteorological data for the experimental period as recorded by the Agrometeorological observatory at the College of Horticulture, Vellanikkara are presented in Fig.1 and Appendix I.

The details of the materials used and methodology followed for the investigation are described in this session.

3.1 Type of cutting and its preparation

The plants for preparing the cuttings were collected from the forests and a few homesteads of Vellanikkara (Trichur District) during May-June 1997.

3.1.1 Type of cutting

Softwood, semihard wood and hard wood cuttings of diameter 0.5 cm, 1.0 cm and 2.0 cm respectively, having 3 nodes and 15-17 cm length were

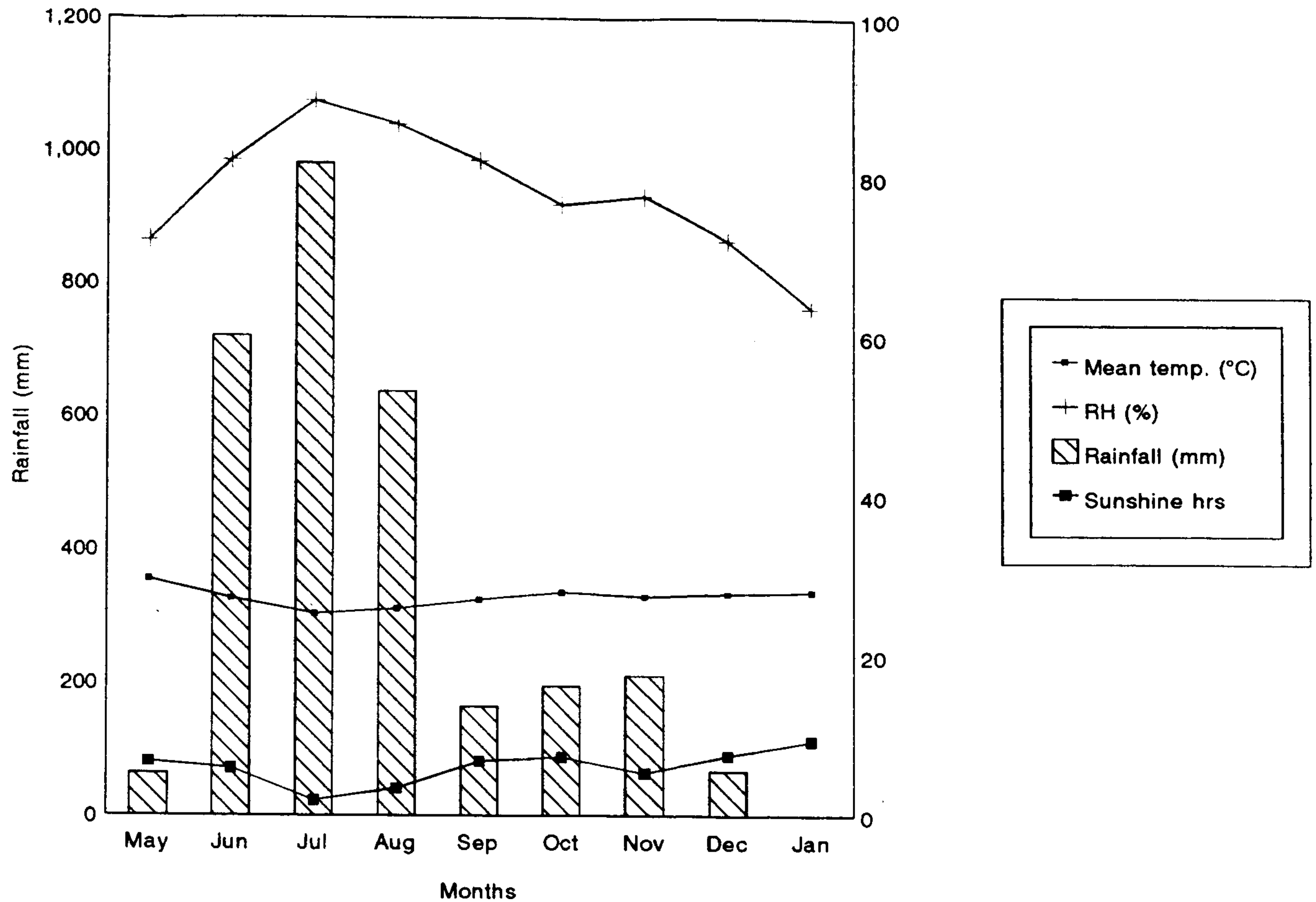


Fig.1. Meteorological data (monthly average) for the crop period (May 1997 to January 1998)

prepared. Four leaves were normally retained for all the three types of cuttings. The very soft apical bud at the tip of the softwood cuttings was retained.

3.1.2 Preparation of cutting

Softwood cuttings were prepared from the top succulant portions of the vines. The axillary sprouts and lateral shoots arising from the nodes were also used for the purpose.

Semihard wood cuttings were prepared from the medium matured middle portions of the vine, leaving 50 cm from the tip and base of vines.

Hardwood cuttings were prepared from the mature and woody basal portions of the vines.

3.2 Growth regulator treatment

3.2.1 Type of hormone

Three different auxins were used, viz., Indole Acetic Acid (IAA), Indole-3-Butyric Acid (IBA) and Naphthalene Acetic Acid (NAA) for treating the cuttings.

3.2.2 Concentration of hormone

Each of these hormones was used at 500 mg l^{-1} for treating the softwood cuttings, 750 mg l^{-1} for treating the semihard wood cuttings and 1000 mg l^{-1} for treating the hardwood cuttings. In addition to this each type of cutting had a control treated with distilled water. The different treatment combinations were

i) T_1 - Softwood cuttings treated with IAA 500 mg l^{-1}

ii) T_2 - Softwood cuttings treated with IBA 500 mg l^{-1}

iii) T_3 - Softwood cuttings treated with NAA 500 mg l^{-1}

iv) T_0 - Softwood cuttings treated with distilled water (control)

v) S_1 - Semihard wood cuttings treated with IAA 750 mg l^{-1}

vi) S_2 - Semihard wood cuttings treated with IBA 750 mg l^{-1}

vii) S_3 - Semihard wood cuttings treated with NAA 750 mg l^{-1}

viii) S_0 - Semihard wood cuttings treated with dist. H_2O (control)

ix) H_1 - Hardwood cuttings treated with IAA at 1000 mg l^{-1}

x) H_2 - Hardwood cuttings treated with IBA at 1000 mg l^{-1}

xi) H_3 - Hardwood cuttings treated with NAA at 1000 mg l^{-1}

xii) H_0 - Hardwood cuttings treated with distilled water (control)

3.2.3 Method of hormone dip

The prepared softwood, semihard wood and hardwood cuttings were dipped in each of the growth regulator solutions for 5 minutes.

3.3 Planting of the treated cuttings

The growth regulator treated cuttings were planted immediately in polythene bags filled with potting mixture of sand, soil and farmyard manure in the ratio 2:1:1.

3.4 Environment provided

All the 12 treatment combinations were kept for rooting under two environmental conditions, viz., under open natural shade and within a mist chamber, provided with automatic misting facility. The misting was done at the rate of two minutes for every 15 minutes interval.

3.5 Layout of the experiment

The experiment was laid out in a Completely Randomized Design (CRD) with two replications. Treatments included combinations of three types of cuttings, four levels of hormone treatments (including control) and two types of environmental conditions. Thus there were twenty four treatment combinations each having 100 cuttings kept for rooting.

The twenty four treatment combinations were

1)	MT_1	13)	OT_1
2)	MS_1	14)	OS_1
3)	MH_1	15)	OH_1
4)	MT_2	16)	OT_2
5)	MS_2	17)	OS_2
6)	MH_2	18)	OH_2
7)	MT_3	19)	OT_3
8)	MS_3	20)	OS_3
9)	MH_3	21)	OH_3
10)	MT_0	22)	OT_0
11)	MS_0	23)	OS_0
12)	MH_0	24)	OH_0

denotes

'M' - misting environment provided

'O' - open natural shade provided

In addition to the above, twenty cuttings were maintained for each treatment in order to fix the interval for taking those observations which need destructive sampling and also to carry out the anatomical studies related with root development.

3.6 Crop management

The cuttings kept under open natural shade were irrigated occasionally to avoid desiccation. Occurrence of fungal rot to the cuttings placed within the mist chamber was controlled by spraying 0.1 per cent Bavistin.

3.7 Leafy Vs leafless cuttings

Leaves of twenty cuttings per treatment combination were excised and observations of these leafless cuttings were recorded separately.

3.8 Observations

3.8.1 Preliminary observations

3.8.1.1 Days to sprout and sprouting percentage

Days taken for sprouting was noted separately for each treatment. The number of cuttings which produced visible sprouts was counted separately for each treatment and their percentage was worked out.

3.8.1.2 Days to root

The number of days taken for root emergence was observed for each treatment by destructive sampling of the additional twenty cuttings maintained for each treatment.

3.8.1.3 Percentage of rooting success

The number of cuttings which successfully rooted and produced new shoots was counted separately for each treatment and their percentage was worked out.

3.8.2 Biometric observations

Five successfully rooted cuttings were selected from each treatment for taking the biometric observations at monthly interval.

3.8.2.1 Length of the new shoot

The length of the new shoot produced was measured and expressed in centimetre.

3.8.2.2 Number of new leaves produced

The number of new leaves produced on the successfully established cuttings was counted separately for each treatment.

3.8.2.3 Number of new shoots

The total number of new shoots produced in the rooted cuttings was counted.

3.8.2.4 Diameter of the main vine

The diameter of the main vine was recorded from each observational plant at 2 cm from its base using a non elastic twine, measured in scale and recorded in centimetre.

The observational plants were carefully uprooted without causing any damage to their root system three months after planting. Then the following observations were made.

3.8.2.5 Length of the longest root

The length of the longest root was measured from the base of the cutting to the tip of the root for each observational plant and expressed in centimetre.

3.8.2.6 Volume of roots

The entire roots from each observational plant was dipped in water taken in a measuring cylinder. The original level and the final level of water was noted and the rise in water level was expressed in millilitre.

3.8.2.7 Fresh weight of aerial parts (stem and leaf)

Fresh weight of stem and leaves was recorded separately for each observational plant and expressed in gram.

3.8.2.8 Fresh weight of roots

After cleaning the roots, fresh weight was recorded separately for each observational plant and expressed in gram.

3.8.2.9 Dry weight of aerial parts (stem and leaf)

The stem and leaf samples, after recording the fresh weight were dried in an oven maintained at 70°C to a constant weight and expressed in gram.

3.8.2.10 Dry weight of roots

After taking the fresh weight, the root samples were dried in a hot air oven at 70°C to a constant weight and expressed in gram.

3.8.2.11 Driage

Based on the fresh weight and dry weight of each plant part viz., leaf, stem and roots, their respective driage was worked out in percentage.

3.9 Percentage of field establishment

Those treatment combinations which recorded high rooting success were observed for their field survival rate. The number of rooted cuttings successfully established in the field was counted separately for each treatment and their percentage was worked out.

3.10 Anatomical studies

Softwood, semihard wood and hardwood cuttings, which were planted in polythene bags filled with potting mixture formed the material for study.

Hand sections were taken daily from the base of the cuttings from 10th day of planting onwards. These sections were stained for two minutes by keeping them in Saffranin stain prepared by dissolving 1 ml in 5 ml of water. Excess stain was then washed out in water for 1 minute. The stained sections were mounted on a glass slide and were covered with a cover slip. These sections were then observed through a light microscope to analyse different stages of root development.

Photomicrographs of the sections showing different stages of adventitious root development were taken using a microscope (Leitz Biomed Leic Wild MPS 28/32 model) at magnification of 4x, 10x or 100x.

3.11 Biochemical studies

Preliminary anatomical studies were made to ascertain the approximate time of various stages of rooting. Based on this, biochemical studies were undertaken at the time of planting, 20 days after planting (at the time of root initiation), 24 days after planting (at the time of root primordia formation) and 27 days after planting (at root emergence) of the cuttings. This was done for each treatment combination. Leaf samples (2nd and 3rd leaves from tip) and stem base were collected at all the above four stages and analysed for the following aspects.

3.11.1 Total carbohydrates

The total carbohydrates present in the leaves and base of the stem during different stages of root development were analysed by Anthrone method (Dubois *et al.*, 1951).

3.11.2 Total nitrogen

Estimation of total nitrogen in the leaves and base of stem during root development stages was done by Microkjeldhal mehtod (Jackson, 1973).

3.11.3 C/N ratio

From the above two observations C/N ratio was worked out.

3.11.4 Protein content

From the total N content, the protein content in the leaves and base of stem during different stages of root development were found out by multiplying the N content by 6.25 (Simpson *et al.*, 1965).

3.12 Statistical analysis

The data were subjected to statistical analysis by applying `Analysis of variance' technique for a factorial experiment conducted in Completely Randomised Design, with appropriate transformations wherever necessary (Panse and Sukhatme, 1995). The treatment means were compared using Duncan's Multiple Range Test (Federer, 1977).

Results

RESULTS

The results of the investigation on standardisation of propagation through cuttings in *Gymnema sylvestre* R.Br. are presented in this chapter.

4.1 Preliminary observations

4.1.1 Days to sprout

The influence of environment, hormone treatments and type of cutting on sprouting was observed (Table 1). Each of these factors significantly influenced the number of days taken for sprouting. Vine cuttings placed in mist chamber sprouted earlier (on an average within 14.8 days after planting) compared to cuttings placed under open natural shade which took 16.8 days to sprout.

It was observed that treatment with hormones (IAA, IBA and NAA) in general induced early sprouting of cuttings. Effects of IAA (15.07 days), IBA (15.50 days) and NAA (15.67 days) on days taken for sprouting were on par, but differed significantly from untreated cuttings, which took 17 days for sprouting.

Type of cuttings differed significantly with one another with respect to days taken for sprouting. Hardwood cuttings took only 13.60 days to sprout, whereas semi-hardwood cuttings took 15.78 days and terminal (softwood) cuttings took 18.04 days for sprouting.

However, the interaction effects of these factors were found to be insignificant (Table 1, 2 and 3).

Table 1. Effect of environment, hormone treatments and type of wood on days to sprout and percentage sprouting of cuttings

Treatments	Days to sprout			Sprouting percentage		
	Mist	Open	Mean	Mist	Open	Mean
IAA	14.445^{d}	15.695^{c}	15.070^{b}	37.667^{a} (0.645)	20.667^{bc} (0.446)	29.167^{a} (0.545)
IBA	14.722^{d}	16.278^{bc}	15.500^{b}	18.000^{c} (0.419)	8.333^{de} (0.284)	13.167^{b} (0.352)
NAA	14.445^{d}	16.890^{b}	15.668^{b}	33.000^{a} (0.599)	23.667^{b} (0.484)	28.333^{a} (0.545)
Control	15.723^{c}	18.278^{a}	17.001^{a}	11.667^{d} (0.334)	7.000^{e} (0.260)	9.333^{c} (0.297)
Softwood	17.334^{b}	18.751^{a}	18.043^{a}	48.750^{a} (0.774)	28.000^{b} (0.541)	38.375^{a} (0.657)
Semi hardwood	14.584^{c}	16.980^{b}	15.782^{b}	16.250^{c} (0.403)	11.000^{d} (0.326)	13.625^{b} (0.364)
Hardwood	12.584^{d}	14.625^{c}	13.604^{c}	10.250^{d} (0.321)	5.750^{e} (0.237)	8.000^{c} (0.279)
Mean	14.834^{b}	16.785^{a}		25.083^{a} (0.499)	14.917^{b} (0.368)	

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

Table 2. Interaction effect of environment, hormone and type of wood on days to sprout, days to root and root volume

Treatment combination	Days to sprout	Days to root	Root volume (ml)
T_1	17.000^{bc}	27.500^{b}	1.225^{a}
S_1	15.043^{efg}	27.050^{bc}	0.575^{bcd}
H_1	13.168^{hi}	26.600^{c}	0.420^{bcd}
T_2	18.000^{b}	27.850^{b}	0.700^{b}
S_2	15.750^{de}	27.450^{bc}	0.350^{cd}
H_2	12.750^{i}	27.800^{b}	0.275^{cd}
T_3	17.418^{bc}	27.850^{b}	1.075^{a}
S_3	15.585^{def}	27.250^{bc}	0.425^{bcd}
H_3	14.000^{gh}	27.100^{bc}	0.338^{cd}
T_0	19.753^{a}	28.900^{a}	0.600^{bc}
S_0	16.750^{cd}	27.800^{b}	0.325^{cd}
H_0	14.500^{fg}	27.750^{b}	0.225^{d}

Treatment means that are compared and having common letters as their superscripts do not differ significantly

Table 3. Interaction effect of environment, hormone and type of wood on days of sprout, days to root, diameter of vine and root volume in cuttings

Treatment combination	Days to sprout	Days to root	Diameter of vine (cm)	Root volume (ml)
MT_1	16.000^{cde}	27.000^{efgh}	7.100^{a}	1.550^{a}
MS_1	14.000^{fg}	26.300^{h}	4.000^{defghi}	0.700^{bcde}
MH_1	13.335^{gh}	26.200^{h}	3.900^{efghij}	0.550^{bcde}
MT_2	17.665^{bc}	27.100^{efgh}	4.800^{bcde}	0.800^{bc}
MS_2	14.500^{efg}	26.900^{efgh}	4.000^{defghi}	0.350^{cde}
MH_2	12.000^{h}	26.600^{gh}	4.000^{efghi}	0.300^{cde}
MT_3	17.000^{bcd}	27.300^{defgh}	5.500^{b}	1.350^{a}
MS_3	14.335^{efg}	26.500^{gh}	4.300^{cdefg}	0.550^{bcde}
MH_3	12.000^{h}	26.400^{h}	4.000^{efghi}	0.400^{bcde}
MT_0	18.670^{b}	27.900^{cdef}	4.700^{bcdef}	0.750^{bcd}
MS_0	15.500^{def}	26.600^{fgh}	4.200^{cdefg}	0.350^{cde}
MH_0	13.000^{gh}	26.000^{h}	4.000^{efghi}	0.200^{e}
OT_1	18.000^{b}	28.000^{cde}	5.000^{bc}	0.900^{b}
OS_1	16.080^{cde}	27.800^{cdefg}	4.100^{defgh}	0.450^{bcde}
OH_1	13.000^{gh}	27.000^{efgh}	3.600^{ghij}	0.290^{cde}
OT_2	18.335^{b}	28.600^{bc}	3.100^{ij}	0.600^{bcde}
OS_2	17.000^{bcd}	28.000^{cde}	3.200^{hij}	0.350^{cde}
OH_2	13.500^{gh}	29.000^{abc}	3.100^{ij}	0.250^{de}
OT_3	17.835^{bc}	28.400^{bcd}	4.900^{bcd}	0.800^{bc}
OS_3	16.833^{bcd}	28.000^{cde}	3.800^{fghij}	0.300^{cde}
OH_3	16.000^{cde}	27.800^{cdefg}	3.800^{fghij}	0.275^{de}
OT_0	20.835^{a}	29.900^{a}	3.700^{ghij}	0.450^{bcde}
OS_0	18.000^{b}	29.000^{abc}	3.200^{hij}	0.300^{cde}
OH_0	16.000^{cde}	29.500^{ab}	3.050^{j}	0.250^{de}

Treatment means that are compared and having common letters as their superscripts do not differ significantly

4.1.2 Percentage of sprouting

The three factors viz. environment, hormone treatment and type of cutting were found to significantly influence the sprouting percentage of the cuttings (Table 1).

Cuttings recorded maximum sprouting when placed under mist (25.08%). Sprouting percentage was maximum for cuttings treated with IAA (29.17) and was on par with sprouting of the NAA treated cuttings (28.33). IBA treated cuttings showed a significantly lower percentage of sprouting (13.17) whereas the untreated cuttings showed the least sprouting (9.33%).

Among the three types of cuttings, terminal cuttings recorded the highest sprouting percentage (38.38) followed by semi-hardwood cuttings (13.63). The hardwood cuttings recorded the lowest sprouting (8.01%).

All the 2-factor and 3-factor interactions were found to be significant (Table 1, 4 and 5).

Among the 2-factor interactions involving environment and hormone treatments, cuttings treated with either IAA or NAA and placed under mist showed a sprotuing efficiency of 37.67 per cent and 33 per cent respectively (Table 1). These treatment combinations were on par and were the most significant ones with respect to sprouting. Similarly softwood cuttings placed under mist showed the highest sprouting (48.75%) and the least sprouting was recorded by hardwood cuttings placed in open condition (5.75%). With respect to interaction involving type of cuttings and hormone treatments, softwood cuttings treated with IAA was the most significant one which showed maximum sprouting (62%). Softwood cuttings treated with NAA recorded 50 per cent sprouting and the least sprouting of five per cent was shown by untreated hardwood cuttings (Table 4).

Among the 3-factor interactions softwood cuttings treated with IAA and placed under mist was the most significant one and it recorded a sprouting as high as 81 per cent (Table 5).

4.1.3 Days to root

Days taken for rooting the cuttings of *Gymnema* was found to be significantly influenced by the environment, hormone treatment and the type of cuttings used (Table 6).

Effect of mist was significantly superior to open shaded condition. The cuttings placed in mist chamber rooted earlier (within 26.7 days on an average) when compared to cuttings kept in open shaded condition (Table 6). Among treatment with hormones, IAA treated cuttings rooted most earlier than all other treatments and took on an average 27.05 days for rooting. With respect to the types of cuttings, hardwood and semi-hardwood cuttings were on par for days taken for rooting and took on an average 27.31 days and 27.39 days respectively, whereas softwood cutting took 28.02 days for rooting (Table 6).

The interaction effects of these three factors were found to be insignificant (Table 2, 3 and 6).

4.1.4 Rooting percentage

The data related to the percentage rooting success as influenced by the treatments (factors) are presented in Table 6. Out of the two environments provided, cuttings kept in mist chamber showed a higher rooting success of 23.43 percentage as against 14.25 percentage rooting recorded by cuttings kept in open conditions. Among the hormone treatments, cuttings, treated with IAA and NAA recorded 28.50 and 27.17 percentage of rooting success respectively, which were on par and significantly superior to other treatments. Among the planting material the rooting success recorded by softwood cuttings was the most significant (37.38%).

The different interactions among the treatments were found to be highly significant (Table 4, 5 and 6).

Cuttings kept in mist after treating with IAA was the most significant one for the two factor interactions involving environment and hormone treatment. It recorded a rooting success of 36.67 percentage (Table 6). So also the softwood cuttings kept in mist was significantly superior to all the other two factor interactions involving type of cutting and environment provided. It recorded a rooting success as high as 47.5 percentage. Among the two factor interaction involving type of cutting and hormone treatment, maximum rooting was recorded in softwood cuttings treated with IAA (62%) followed by softwood cutting treated with NAA (49%) (Table 4).

Softwood cuttings treated with IAA and kept in mist (MT_1) showed the most significant three factor interaction and showed a rooting success of 81 per cent (Table 5). It was followed by softwood cuttings treated with NAA and kept in mist (49%). The least significant rooting was observed for hardwood cuttings, which were not given any hormone treatment and kept in open shaded condition (3%).

Table 4. Interaction effect of environment, hormone and type of wood on biometric characters

Treatment combination	% sprouting	% rooting	Length of the new shoot (1 MAP) (cm)	Length of the new shoot (2 MAP) (cm)	Length of the new shoot (3 MAP) (cm)	No.of leaves produced (2 MAP)	No. of leaves produced (3 MAP)
T_1	62.000^{a} (0.918)	62.000^{a} (0.918)	2.650^{a}	34.650^{a}	54.150^{a}	13.150^{a}	22.800^{a}
S_1	16.000^{d} (0.407)	15.550^{d} (0.401)	0.950^{e}	21.600^{c}	33.500^{d}	8.750^{c}	12.800^{cd}
H_1	9.500^{ef} (0.311)	8.000^{e} (0.286)	0.450^{f}	16.750^{e}	28.900^{ef}	5.000^{ef}	8.500^{fg}
T_2	24.500^{c} (0.507)	23.000^{c} (0.492)	1.850^{d}	27.300^{b}	37.550^{c}	8.000^{c}	13.500^{c}
S_2	8.500^{efg} (0.294)	6.500^{ef} (0.257)	0.800^{e}	18.400^{de}	28.700^{ef}	5.850^{ef}	9.750^{ef}
H_2	6.500^{fg} (0.253)	5.000^{fg} (0.224)	0.380^{f}	13.550	25.750^{gh}	3.650^{gh}	7.500^{g}
T_3	50.000^{b} (0.785)	49.000^{b} (0.775)	2.400^{b}	33.550^{a}	50.850^{b}	11.250^{b}	19.000^{b}
S_3	24.000^{c} (0.510)	24.000^{c} (0.510)	0.900^{e}	21.150^{cd}	34.750^{d}	7.350^{cd}	11.000^{de}
H_3	11.000^{e} (0.329)	8.500^{e} (0.291)	0.300^{f}	13.450^{f}	27.050^{fg}	4.600^{fgh}	7.500^{g}
T_0	17.000^{d} (0.420)	15.550^{d} (0.398)	2.100^{c}	24.950^{b}	37.850^{c}	8.300^{c}	14.500^{c}
S_0	6.000^{fg} (0.246)	5.000^{fg} (0.224)	0.700^{e}	18.700^{de}	29.800^{e}	6.500^{de}	10.250^{ef}
H_0	5.000^{g} (0.224)	4.000^{g} (0.198)	0.200^{f}	13.950^{f}	24.025^{h}	3.500^{h}	6.500^{g}

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

MAP - Months after planting

Table 5. Interaction effect of environment, hormone and type of wood on biometric characters

Treatment combination	% sprouting	% rooting	Length of new shoot (3 MAP) (cm)	No. of leaves (2 MAP)	Driage % (stem)	Driage % (leaf)	Driage % (root)
1	2	3	4	5	6	7	8
MT_1	81.00^{a}	81.00^{a}	61.00^{a}	16.70^{a}	30.093^{b}	26.308^{b}	34.603^{b}
	(1.120)	(1.120)			(0.581)	(0.539)	(0.629)
MS_1	21.00^{efg}	20.00^{e}	36.80^{e}	10.50^{c}	28.541^{de}	24.657^{ef}	32.118^{ef}
	(0.476)	(0.464)			(0.564)	(0.520)	(0.603)
MH_1	11.00^{hij}	9.00^{gh}	31.80^{fgh}	5.00^{defg}	27.777^{fg}	24.000^{gh}	31.125^{ghi}
	(0.338)	(0.304)			(0.555)	(0.512)	(0.592)
MT_2	35.00^{cd}	32.00^{d}	41.90^{d}	10.00^{c}	27.900^{efg}	23.995^{gh}	30.890^{ij}
	(0.632)	(0.601)			(0.556)	(0.512)	(0.589)
MS_2	10.00^{hijk}	7.00^{ghi}	32.60^{fg}	6.50^{de}	25.959^{k}	23.859^{h}	30.385^{jk}
	(0.322)	(0.261)			(0.535)	(0.510)	(0.584)
MH_2	9.00^{hijkl}	6.00^{hi}	29.00^{hij}	4.00^{fg}	25.322^{l}	24.245^{fgh}	31.716^{fg}
	(0.304)	(0.247)			(0.527)	(0.515)	(0.598)
MT_3	57.00^{b}	56.00^{b}	57.60^{b}	13.00^{b}	29.632^{bc}	25.470^{cd}	33.258^{cd}
	(0.856)	(0.846)			(0.576)	(0.529)	(0.615)
MS_3	27.00^{de}	27.00^{d}	36.90^{e}	10.00^{c}	26.680^{ij}	24.663^{ef}	31.433^{ghi}
	(0.546)	(0.546)			(0.543)	(0.520)	(0.595)
MH_3	15.00^{fgh}	11.00^{fg}	30.90^{fgh}	5.00^{defg}	26.594^{ijk}	24.070^{fgh}	31.206^{ghi}
	(0.396)	(0.338)			(0.542)	(0.513)	(0.593)
MT_0	22.00^{ef}	21.00^{e}	41.80^{d}	10.00^{c}	28.100^{ef}	23.963^{gh}	31.056^{ghi}
	(0.487)	(0.473)			(0.559)	(0.512)	(0.591)
MS_0	7.00^{jkl}	6.00^{hi}	34.00^{ef}	7.00^{d}	27.003^{hi}	24.080^{fgh}	30.955^{hij}
	(0.267)	(0.247)			(0.546)	(0.513)	(0.590)
MH_0	6.00^{jkl}	5.00^{ij}	27.00^{ijk}	4.00^{fg}	24.597^{m}	24.038^{fgh}	30.108^{kl}
	(0.247)	(0.224)			(0.519)	(0.512)	(0.581)
OT_1	43.00^{c}	43.00^{c}	47.30^{c}	10.00^{c}	30.928^{a}	27.090^{a}	36.162^{a}
	(0.715)	(0.715)			(0.590)	(0.547)	(0.645)
OS_1	11.00^{hij}	11.00^{fg}	30.20^{ghi}	7.00^{d}	29.100^{cd}	24.670^{ef}	33.225^{cd}
	(0.338)	(0.338)			(0.570)	(0.520)	(0.640)
OH_1	8.00^{ijkl}	7.00^{ghi}	26.00^{jkl}	5.00^{defg}	27.444^{gh}	24.928^{de}	33.333^{c}
	(0.285)	(0.267)			(0.551)	(0.523)	(0.615)
OT_2	14.00^{ghi}	14.00^{f}	33.20^{fg}	6.00^{def}	26.100^{jk}	25.000^{de}	32.619^{de}
	(0.383)	(0.383)			(0.536)	(0.524)	(0.608)

Contd.

Table 5. Continued

1	2	3	4	5	6	7	8
OS_2	7.00^{jkl} (0.267)	6.00^{hi} (0.247)	24.80^{kl}	5.00^{defg}	24.449^{ijk} (0.540)	24.528^{efg} (0.518)	30.000^{kl} (0.580)
OH_2	4.00^{l} (0.201)	4.00^{ij} (0.201)	22.50^{lm}	3.30^{g}	27.028^{hi} (0.547)	24.198^{fgh} (0.514)	29.492^{l} (0.574)
OT_3	43.00^{c} (0.715)	42.00^{c} (0.705)	44.10^{cd}	9.50^{c}	30.139^{b} (0.581)	26.966^{a} (0.596)	34.891^{b} (0.632)
OS_3	21.00^{efg} (0.475)	21.00^{e} (0.475)	32.60^{fg}	4.70^{efg}	28.143^{ef} (0.559)	25.977^{bc} (0.535)	32.529^{e} (0.607)
OH_3	7.00^{jkl} (0.262)	6.00^{hi} (0.244)	23.20^{lm}	4.20^{fg}	26.522^{ijk} (0.544)	25.144^{de} (0.525)	31.238^{ghi} (0.593)
OT_0	12.00^{hij} (0.353)	10.00^{fg} (0.322)	33.90^{ef}	6.60^{de}	28.478^{e} (0.563)	24.892^{de} (0.522)	31.595^{fgh} (0.597)
OS_0	5.00^{kl} (0.224)	4.00^{ij} (0.201)	25.60^{jkl}	6.00^{def}	26.522^{ijk} (0.541)	24.667^{ef} (0.520)	31.000^{hij} (0.591)
OH_0	4.00^{l} (0.201)	3.00^{j} (0.172)	21.05^{m}	3.00^{g}	26.047^{jk} (0.536)	23.209^{i} (0.503)	22.575^{m} (0.495)

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

MAP - Months after planting

Table 6. Effect of environment, hormone treatments and type of wood on days to root and percentage rooting

Treatments	Days to root			Rooting percentage		
	Mist	Open	Mean	Mist	Open	Mean
IAA	26.500^{d}	27.600^{c}	27.050^{c}	36.667^{a} (0.629)	20.333^{c} (0.440)	28.500^{a} (0.535)
IBA	26.867^{d}	28.533^{b}	27.700^{ab}	15.000^{d} (0.372)	8.000^{f} (0.277)	11.500^{b} (0.324)
NAA	26.733^{d}	28.067^{bc}	27.400^{bc}	31.333^{b} (0.576)	23.000^{c} (0.475)	27.167^{a} (0.526)
Control	26.833^{d}	29.467^{a}	28.150^{a}	10.667^{e} (0.315)	5.667^{g} (0.232)	8.167^{c} (0.273)
Softwood	27.325^{b}	28.725^{a}	28.025^{a}	47.500^{a} (0.760)	27.250^{b} (0.531)	37.375^{a} (0.645)
Semi hardwood	26.575^{c}	28.200^{a}	27.388^{b}	15.000^{c} (0.381)	10.500^{d} (0.315)	12.750^{b} (0.348)
Hardwood (H)	26.300^{c}	28.325^{a}	27.312^{b}	7.750^{e} (0.278)	5.000^{f} (0.221)	6.375^{c} (0.250)
Mean	26.733^{b}	28.417^{a}		23.417^{a} (0.473)	14.250^{b} (0.356)	

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

4.2 Biometric observations

4.2.1 Length of the new shoot

The data related to the length of vine taken at monthly interval as influenced by the treatments are presented in Table 7. The data clearly reveal that length of new vine is significantly influenced by environment provided, hormone treatment and type of cuttings selected.

Vines kept in mist recorded maximum length at all stages of observation with 1.23 cm, 24.08 cm and 38.85 cm at 1, 2 and 3 months respectively after planting. Among the hormones, cuttings treated with IAA recorded the maximum vine length of 1.35 cm and 24.33 cm at one and two months after planting. However, effects of IAA and NAA on vine length were on par at 3 months after planting (38.85 cm and 37.55 cm, respectively). Softwood cuttings had the maximum vine length of 2.25 cm, 30.11 cm and 45.10 cm at 1, 2 and 3 months respectively after planting followed by semi-hardwood cuttings and the least vine length at all stages of observations was recorded in hardwood cuttings.

Among the two-factor interactions, interaction between environment and type of cutting as well as interaction between type of cutting and hormone treatment were significant at all stages of observations. Softwood cuttings kept in mist and softwood cuttings treated with IAA were the significantly superior treatment combinations at all stages of observations (Table 7 and Table 4).

Out of the twenty-four treatment combinations of three-factor interaction effects, softwood cuttings treated with IAA and kept in mist (MT_1) showed significantly superior vine length (61 cm) at 3 months after planting (Table 5).

Table 7. Effect of environment, hormone and type of wood on length of new shoot

Treatments	Length of the shoot (cm)								
	1 MAP			2 MAP			3 MAP		
	Mist	Open	Mean	Mist	Open	Mean	Mist	Open	Mean
IAA	1.467[a]	1.233[bc]	1.350[a]	28.233[a]	20.433[c]	24.333[a]	43.200[a]	34.500[b]	38.850[a]
IBA	1.033[cd]	0.987[d]	1.010[c]	21.600[c]	17.900[d]	19.750[c]	34.500[b]	26.833[c]	30.667[b]
NAA	1.300[ab]	1.100[bcd]	1.200[b]	25.767[b]	19.667[cd]	22.717[b]	41.800[a]	33.300[b]	37.550[a]
Control	1.000[bcd]	0.900[d]	1.000[c]	20.773[c]	17.667[d]	19.200[c]	34.267[b]	26.850[c]	30.558[b]
Softwood	2.425[a]	2.075[b]	2.250[a]	35.600[a]	24.625[b]	30.112[a]	50.575[a]	39.625[b]	45.100[a]
Semi-hardwood	0.875[c]	0.800[c]	0.838[b]	21.750[c]	18.175[d]	19.963[b]	35.075[c]	28.300[d]	31.687[b]
Hardwood	0.375[d]	0.290[d]	0.333[c]	14.900[e]	13.950[e]	14.425[c]	29.675[d]	23.188[e]	26.431[c]
Mean	1.225[a]	1.055[b]		24.083[a]	18.917[b]		38.850[a]	30.371[b]	

MAP - Months after planting

Treatment means that are compared and having common letters as their superscripts do not differ significantly

4.2.2 Number of new leaves

Data presented in Table 8 reveal that cuttings kept in mist recorded maximum number of leaves at all stages of observation. Among the hormone treatments cuttings treated with IAA and NAA were on par with respect to number of leaves produced at one month after planting (1.08 and 1.03 respectively). However, cuttings treated with IAA was significantly superior to all the other treatments in the number of leaves produced at two and three months after planting (9.03 and 14.7 respectively). Softwood cutting produced maximum number of leaves at all stages of observations (Table 8).

Interaction between environment and hormonal treatments was not significant. However, significant interaction between environment and type of cuttings was observed (Table 8). Softwood cuttings kept in mist showed significant effect at all stages of observations.

Type of cuttings and hormonal treatments were found to be significantly interacting with regard to the number of leaves produced at two and three months after planting (Table 4). Softwood cuttings treated with IAA produced maximum leaves of 13.35 and 22.80 at two and three months after planting.

The three factor interaction was generally found insignificant except for the number of leaves produced at two months after planting. Softwood cuttings treated with IAA and kept in mist showed maximum leaves (16.70) at this stage (Table 5).

Table 8. Effect of environment, hormone and type of wood on the number of leaves

Treatments	Length of the shoot (cm)								
	1 MAP			2 MAP			3 MAP		
	Mist	Open	Mean	Mist	Open	Mean	Mist	Open	Mean
IAA	1.400^{a}	0.767^{b}	1.083^{a}	10.733^{a}	7.333^{c}	9.033^{a}	16.767^{a}	12.633^{c}	14.700^{a}
IBA	1.167^{a}	0.367^{d}	0.767^{c}	6.833^{c}	4.833^{e}	5.833^{c}	12.000^{c}	8.500^{e}	10.250^{c}
NAA	1.350^{a}	0.700^{bc}	1.025^{ab}	9.333^{b}	6.133^{cd}	7.333^{b}	14.667^{b}	10.333^{d}	12.500^{b}
Control	1.200^{a}	0.467^{cd}	0.833^{bd}	7.000^{c}	5.200^{de}	6.100^{c}	12.667^{c}	8.167^{e}	10.417^{c}
Softwood	1.825^{a}	0.750^{cd}	1.287^{a}	12.425^{a}	8.025^{b}	10.225^{a}	20.825^{a}	14.075^{b}	17.450^{a}
Semi-hardwood	1.125^{b}	0.625^{d}	0.875^{b}	8.500^{b}	5.725^{c}	7.112^{b}	13.000^{b}	8.900^{c}	10.950^{b}
Hardwood	0.888^{c}	0.350^{e}	0.619^{c}	4.500^{d}	3.875^{d}	4.188^{c}	8.250^{c}	6.750^{d}	7.500^{c}
Mean	1.279^{a}	0.575^{b}		8.475^{a}	5.875^{b}		14.025^{a}	9.908^{b}	

MAP - Months after planting

Treatment means that are compared and having common letters as their superscripts do not differ significantly

4.2.3 Number of shoots

Data on the number of shoots produced by the rooted cuttings, as influenced by different treatments are presented in Table 9. Data clearly showed that environment had no significant influence on the number of shoots produced. However, hormone treatments and type of cuttings selected have significant influence on the number of shoots. Cuttings treated with NAA produced maximum number of shoots (1.43), followed by cuttings treated with IAA (1.22). Cuttings treated with IBA and untreated cuttings were on par and produced one shoot each. Among the type of cuttings, softwood cuttings produced maximum number of shoots (1.43). Semi-hardwood and hardwood cuttings were on par with regard to the number shoots produced.

No significant interaction was observed, except for the interaction between type of cuttings and hormonal treatment. Softwood cuttings treated with NAA was significantly superior to all other treatment combinations and produced an average of 2.05 number of shoots per rooted cutting (Table 10).

4.2.4 Diameter of main vine

Data on the diameter of main vine as influenced by different treatments is shown in Table 9. The data reveal that diameter of main vine is significantly influenced by different treatments.

Cuttings kept in mist produced vine with maximum girth (4.54 mm). Similarly softwood cuttings had maximum diameter for the vine (4.85 mm), whereas semi-hardwood and hardwood cuttings were on par and showed a vine girth of 3.85 and 3.68 mm respectively. Among hormone treatments cuttings treated with IAA and NAA were on par and produced vines with diameter of 4.62 mm and

Table 9. Effect of environment, hormone and type of wood on the number of shoots and diameter of main vine

Treatments	Number of shoots per vine			Diameter of vine (cm)		
	Mist	Open	Mean	Mist	Open	Mean
IAA	1.267^{abc}	1.167^{bc}	1.217^{b}	5.000^{a}	4.233^{b}	4.617^{a}
IBA	1.000^{c}	1.000^{c}	1.000^{c}	4.267^{b}	3.133^{c}	3.700^{b}
NAA	1.533^{a}	1.333^{ab}	1.433^{a}	4.600^{ab}	4.167^{b}	4.383^{a}
Control	1.000^{c}	1.000^{c}	1.000^{c}	4.300^{b}	3.317^{c}	3.808^{b}
Softwood	1.475^{a}	1.375^{a}	1.425^{a}	5.525^{a}	4.175^{b}	4.850^{a}
Semi hardwood	1.000^{b}	1.000^{b}	1.000^{b}	4.125^{b}	3.575^{c}	3.850^{b}
Hardwood	1.125^{b}	1.000^{b}	1.063^{b}	3.975^{b}	3.387^{c}	3.681^{b}
Mean	1.200^{a}	1.125^{a}		4.542^{a}	3.712^{b}	

Treatment means that are compared and having common letters as their superscripts do not differ significantly

Table 10. Interaction effect of environment, hormone and type of wood on biometric characters of rooted cuttings

Treatment combination	No.of shoots produced/ vine	Diameter of main vine (cm)	Length of longest root (cm)	Driage (%)		
				Stem	Leaf	Root
T_1	1.650[b]	6.050[a]	20.400[a]	30.510[a] (0.585)	26.699[a] (0.593)	35.383[a] (0.637)
S_1	1.000[c]	4.050[cd]	14.125[c]	28.821[c] (0.567)	24.614[d] (0.520)	32.672[c] (0.608)
H_1	1.000[c]	3.750[cd]	12.275[def]	27.610[e] (0.553)	24.464[de] (0.517)	32.229[d] (0.604)
T_2	1.000[c]	3.950[cd]	14.600[c]	27.000[f] (0.546)	24.497[de] (0.518)	31.755[ef] (0.599)
S_2	1.000[c]	3.600[cd]	12.700[de]	26.204[h] (0.537)	24.193[e] (0.514)	30.192[i] (0.582)
H_2	1.000[c]	3.550[d]	10.450[gh]	26.175[h] (0.537)	24.221[de] (0.515)	30.604[hi] (0.586)
T_3	2.050[a]	5.200[a]	19.075[b]	29.885[b] (0.578)	26.218[b] (0.538)	34.074[b] (0.623)
S_3	1.000[c]	4.050[cd]	13.450[cd]	27.412[e] (0.551)	25.320[c] (0.527)	31.981[de] (0.601)
H_3	1.250[c]	3.900[cd]	11.425[efg]	26.558[gh] (0.541)	24.607[de] (0.519)	31.222[g] (0.593)
T_0	1.000[c]	4.200[c]	13.625[cd]	28.289[d] (0.561)	24.428[de] (0.517)	31.325[fg] (0.594)
S_0	1.000[c]	3.700[cd]	11.300[fg]	26.762[fg] (0.544)	24.373[de] (0.516)	30.977[gh] (0.590)
H_0	1.000[c]	3.525[d]	9.900[h]	25.322[i] (0.527)	23.624[f] (0.508)	26.341[j] (0.538)

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

4.38 mm respectively. Cuttings treated with IBA and untreated cuttings were on par and showed a vine diameter of 3.70 mm and 3.80 mm respectively (Table 9).

Among the interactions, only the interaction between environment and type of cutting and interaction between type of cutting and hormonal treatment were significant. Softwood cuttings kept in mist produced the maximum vine diameter of 5.53 mm (Table 9). So also softwood cuttings treated with IAA showed vines with the maximum girth of 6.05 mm as revealed from Table 10.

The three-factor interactions were insignificant (Table 3).

4.2.5 Length of the longest root

The effect of treatments on root length was found to be significant (Table 11). Maximum root length was observed for cuttings kept in mist (14.08 cm) which was significantly superior to that of cuttings kept in open (13.14 cm). Effect of hormone treatments was found to be significant. Hormone treatments in general resulted in increased root length compared to untreated cuttings. Maximum root length was observed for IAA treated cuttings (15.60 cm) followed by NAA treated cuttings (14.65 cm) (Plates 1 and 2). Mean root length of IBA treated cuttings was 12.58 cm and that of untreated cuttings was the least (11.61 cm). Among the type of cuttings, softwood cuttings were significantly superior and produced a root length of 16.92 cm, followed by semi-hardwood (12.89 cm) and hardwood cuttings (11.01 cm) (Plates 3 and 4).

Only the interaction between hormone treatment and type of cuttings was significant with respect to root length. IAA treated softwood cuttings produced maximum root length (16.65 cm) and were significantly superior to all other treatment combinations (Table 10).

Plate 1. Effect of hormone treatment on rooting of cutting kept in mist chamber

(4) Treated with IAA
(5) Treated with IBA
(6) Treated with NAA
(7) Control

Plate 2. Effect of hormone treatment on rooting of cutting kept under natural shade


4
5
6
7

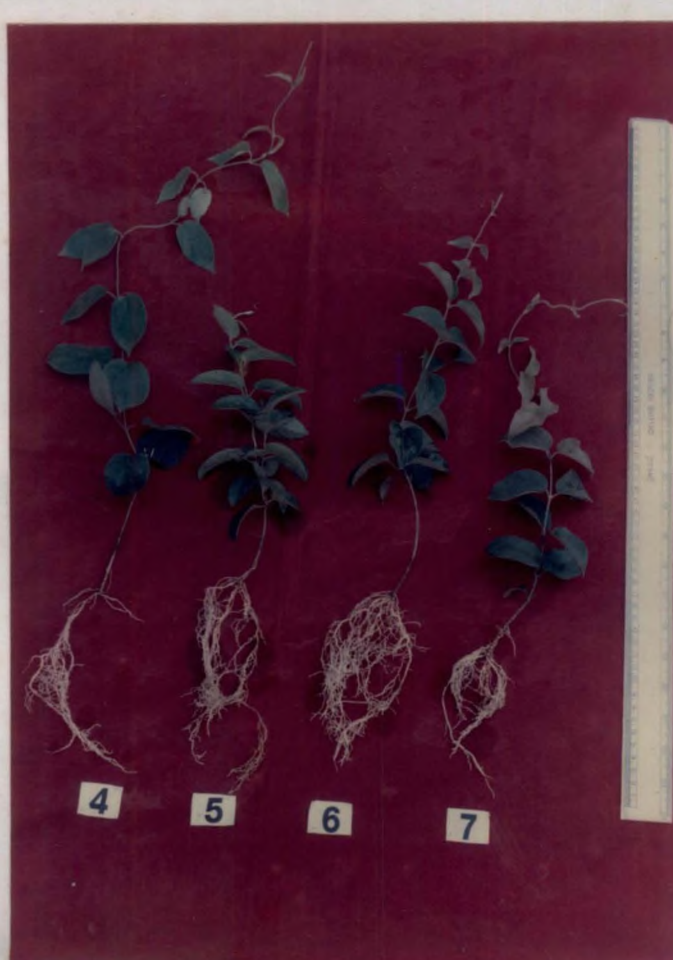

4
5
6
7

Plate 3. Types of cutting and rooting pattern (cuttings kept in mist)

(2) Softwood
(3) Semi-hardwood
(4) Hardwood

Plate 4. Types of cutting and rooting pattern (cuttings kept under natural shade)


2
3
4


2
3
4

Table 11. Effect of environment, hormone and type of wood on the root characters

Treatment	Length of the longest root (cm)			Root volume (ml)		
	Mist	Open	Mean	Mist	Open	Mean
IAA	16.650[a]	14.550[b]	15.600[a]	0.933[a]	0.547[bc]	0.740[a]
IBA	12.667[c]	12.500[c]	12.583[c]	0.483[c]	0.400[c]	0.442[bc]
NAA	15.100[b]	14.200[b]	14.650[b]	0.767[ab]	0.458[c]	0.613[ab]
Control	11.917[cd]	11.300[d]	11.608[d]	0.433[c]	0.333[c]	0.383[c]
Softwood	17.531[a]	16.313[b]	19.925[a]	1.112[a]	0.688[b]	0.900[a]
Semi hardwood	13.138[c]	12.650[c]	12.894[b]	0.488[bc]	0.350[b]	0.419[b]
Hardwood	11.575[d]	10.450[e]	11.013[c]	0.363[c]	0.266[c]	0.314[b]
Mean	14.083[a]	13.138[b]		0.654[a]	0.435[b]	

Treatment means that are compared and having common letters as their superscripts do not differ significantly

4.2.6 Root volume

Data on the root volume of cuttings as influenced by different treatments are presented in Table 11. The data showed that cuttings kept in mist produced the maximum root volume (0.65 ml), and were significantly superior to cuttings kept under open conditions (0.44 ml). Among the different hormones treated, IAA was significantly superior and produced a root volume of 0.74 ml, followed by cuttings treated with NAA (0.61 ml) and the least value for root volume was observed for untreated cuttings (0.38 ml). Softwood cuttings produced the maximum root volume (0.90 ml) and were significantly superior to semi-hardwood cuttings (0.42 ml) and hardwood cuttings (0.31 ml).

The different interaction effects were not found to be significant with regard to root volume (Table 2, 3 and 11).

4.2.7 Fresh weight of stem

Data presented in Table 12 revealed that fresh weight of stem was not influenced by environment provided for successful rooting of cuttings. However, treatments with hormones and type of cuttings greatly influenced the fresh weight. Cuttings treated with IAA and NAA are on par and significantly superior to other treatments with fresh weights of 2.86 g and 2.76 g respectively. Among the type of cuttings, softwood cuttings in which the fresh weight of stem was 3.20 g was the most significant treatment.

The different interaction effects of treatment combinations were not significant (Table 12 to 14).

4.2.8 Dry weight of stem

Data related to dry weight of stem are presented in Table 12. The data showed that environment did not significantly influence dry weight. At the same time, treatment with hormones and type of cuttings were found to significantly influence the character under study. IAA and NAA treated cuttings were on par and significantly superior to other treatments. They recorded values of 0.83 g and 0.78 g for dry weight of stem. With regard to the type of cuttings softwood cuttings had maximum value for dry weight of stem (0.93 g) and the hardwood cuttings had the least value (0.58 g).

The interaction effect of different treatments were not significant (Table 12 to 14).

4.2.9 Driage percentage of stem

Data related to driage (%) of stem are presented in Table 12. It showed that the characters under study were significantly influenced by the treatments. Cuttings kept in open environment recorded maximum value (27.74%) and were significantly superior to cuttings kept in mist. With regard to hormone treatments cuttings treated with IAA was significantly superior to all other treatments (28.98%). Among the type of cuttings, softwood cuttings recorded maximum driage for stem (28.92%).

Interaction effect

Data presented in Table 10 revealed that among the two factor interactions involving hormone treatments and type of cuttings, softwood cuttings treated with IAA (T_1) was the most significant one with a driage percentage of

Table 12. Effect of environment, hormone and type of wood on the fresh weight, dry weight and driage percentage of stem in rooted cuttings

Treatments	Fresh weight of stem (g)			Dry weight of stem (g)			Driage percentage		
	Mist	Open	Mean	Mist	Open	Mean	Mist	Open	Mean
IAA	2.797^{ab}	2.920^{a}	2.858^{a}	0.810^{ab}	0.858^{a}	0.834^{a}	28.804^{b} (0.566)	29.157^{a} (0.570)	28.980^{a} (0.568)
IBA	2.285^{c}	2.442^{bc}	2.363^{b}	0.607^{d}	0.647^{cd}	0.627^{b}	26.394^{f} (0.539)	26.526^{f} (0.541)	26.460^{gh} (0.540)
NAA	2.627^{abc}	2.885^{a}	2.756^{a}	0.733^{bc}	0.825^{ab}	0.779^{a}	27.635^{d} (0.553)	28.268^{c} (0.560)	27.952^{cd} (0.557)
Control	2.477^{bc}	2.467^{bc}	2.472^{b}	0.662^{cd}	0.670^{cd}	0.666^{b}	26.567r (0.541)	27.015^{e} (0.546)	26.791^{fg} (0.544)
Softwood	3.061^{a}	3.339^{a}	3.200^{a}	0.889^{a}	0.971^{a}	0.930^{a}	28.931^{a} (0.568)	28.911^{a} (0.567)	28.921^{a} (0.568)
Semi-hardwood	2.480^{b}	2.428^{b}	2.454^{b}	0.672^{b}	0.671^{b}	0.672^{b}	27.046^{c} (0.547)	27.554^{b} (0.553)	27.300^{b} (0.550)
Hardwood	2.098^{c}	2.269^{bc}	2.183^{c}	0.547^{c}	0.608^{bc}	0.578^{c}	26.073^{d} (0.536)	26.760^{c} (0.544)	26.416^{c} (0.540)
Mean	2.546^{a}	2.678^{a}		0.703^{a}	0.750^{a}		27.350^{b} (0.550)	27.742^{a} (0.555)	

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

30.51. Two-factor interactions involving other factors were found to be insignificant (Table 12).

Data shown in Table 5 revealed that among the three factor interactions, the most significant combination was softwood cuttings treated with IAA and kept in open (OT_1) which recorded the highest driage percentage (30.09) followed by treatment combinations OT_3, MT_1 and MT_3 which were on par with driage percentages of 30.14, 30.09 and 29.63 respectively.

4.2.10 Fresh weight of leaves

Table 15 shows data related to the fresh weight of leaves in each successfully rooted cutting as influenced by different treatments. Environment had no significant effect on the character under study. However, the other two factors, viz., hormone treatment and type of cutting had significant effect on fresh weight of leaves. Among hormones, effect of treatment with IAA and NAA were on par and they were the most significant ones with fresh weight of 6.64 g and 6.37 g, respectively. Among the type of cuttings, softwood cuttings recorded maximum fresh weight for leaves (7.21 g).

The different interaction effects were not significant (Table 13 to 15).

4.2.11 Dry weight of leaves

Data presented in Table 15 reveal that environment provided has no significant effect on the character under study. Hormone treatments had significant effect and IAA treated cuttings and IBA treated cuttings which were on par were the most significant treatments with dry weight values of 1.69 g and 1.62 g respectively. Among type of cuttings, softwood cuttings recorded highest value (1.84 g) and was the most significant one.

Interaction effects were generally not significant (Table 13 to 15).

4.2.12 Driage percentage of leaves

Data related to driage percentage of leaves for different treatments are shown in Table 15. Environment was found to significantly influence the character under study and cuttings kept under open environment recorded maximum driage percentage (25.11). Similarly hormones greatly influenced the driage percentage of leaves, with cuttings treated in the NAA and IAA, being the most significant treatments which were on par (25.38 and 25.28%). Among the different types of cuttings softwood cutting was the most significant treatment with a driage percentage of 25.46 and hardwood cuttings recorded the least value (24.23%).

Interaction effect

All the different two factor interactions were found to be significant (Table 10 and Table 15). Softwood cuttings kept in open condition was the most significant treatment combination for interaction involving environment and type of cuttings. It recorded a driage of 25.99 per cent. Among treatment combinations for interaction involving hormone treatment and type of cuttings, softwood cuttings treated with IAA (T_1) recorded the highest value (26.70%) and was the most significant combination (Table 10). For interaction involving environment and hormone treatment, cuttings treated with NAA and kept in open recorded the highest value of 26.03 per cent and was the most significant combination (Table 15).

Table 13. Interaction effect of environment, hormone and type of wood on the stem, leaf and root characters

Treatment combination	Stem		Leaf		Root	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
T_1	3.498^{a}	1.068^{a}	7.712^{a}	2.060^{a}	1.955^{ab}	0.692^{a}
S_1	2.750^{bcd}	0.793^{b}	6.525^{b}	1.610^{b}	1.315^{cde}	0.430^{bcd}
H_1	2.328^{de}	0.642^{cd}	5.690^{cd}	1.390^{cde}	1.050^{efg}	0.340^{def}
T_2	2.827^{bc}	0.763^{bc}	6.742^{b}	1.652^{b}	1.640^{abc}	0.523^{b}
S_2	2.185^{e}	0.573^{d}	5.500^{cd}	1.330^{de}	0.803^{fg}	0.242^{ef}
H_2	2.078^{e}	0.545^{d}	5.038^{d}	1.220^{e}	0.698^{g}	0.213^{f}
T_3	3.612^{a}	1.080^{a}	7.650^{a}	2.007^{a}	2.000^{a}	0.683^{a}
S_3	2.480^{bcde}	0.680^{bcd}	6.087^{bc}	1.540^{bc}	1.173^{def}	0.375^{cde}
H_3	2.175^{e}	0.578^{d}	5.357^{d}	1.317^{de}	0.955^{efg}	0.297^{def}
T_0	2.863^{b}	0.810^{b}	6.740^{b}	1.648^{b}	1.555^{bcd}	0.487^{bc}
S_0	2.400^{cde}	0.642^{cd}	6.125^{bc}	1.493^{bcd}	1.025^{efg}	0.318^{def}
H_0	2.153^{e}	0.545^{d}	5.110^{d}	1.207^{e}	0.862^{fg}	0.230^{f}

Treatment means that are compared and having common letters as their superscripts do not differ significantly

Table 14. Interaction effect of environment, hormone and type of wood on stem, leaf and root characters

Treatment combination	Stem		Leaf		Root	
	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)	Fresh weight (g)	Dry weight (g)
MT$_1$	3.390^{ab}	1.020^{ab}	7.470^{ab}	1.965^{abc}	1.850^{abc}	0.640^{ab}
MS$_1$	2.750^{bcd}	0.785^{cd}	6.550^{bcd}	1.615^{de}	1.260^{cdef}	0.405^{defgh}
MH$_1$	2.250^{def}	0.625^{defgh}	6.125^{cdef}	1.470^{defghi}	0.900^{ef}	0.280^{efghij}
MT$_2$	2.705^{cde}	0.755^{cdef}	6.585^{bcd}	1.580^{defg}	1.440^{bcde}	0.445^{cdef}
MS$_2$	2.215^{def}	0.575^{efgh}	5.700^{defg}	1.360^{efghij}	0.805^{ef}	0.245^{efghij}
MH$_2$	1.935^{f}	0.490^{h}	4.950^{g}	1.200^{ij}	0.615^{f}	0.195^{ij}
MT$_3$	3.375^{ab}	1.000^{ab}	7.420^{ab}	1.890^{bc}	1.850^{abc}	0.615^{abc}
MS$_3$	2.455^{cdef}	0.655^{cdefgh}	6.305^{cde}	1.555^{defg}	1.130^{def}	0.355^{defghij}
MH$_3$	2.050^{ef}	0.545^{gh}	5.505^{defg}	1.325^{fghij}	0.885^{ef}	0.275^{efghij}
MT$_0$	2.775^{bcd}	0.780^{cd}	6.510^{bcd}	1.560^{defg}	1.400^{cde}	0.435^{cdefg}
MS$_0$	2.500^{cdef}	0.675^{cdefgh}	6.250^{cde}	1.505^{defgh}	1.050^{ef}	0.325^{efghij}
MH$_0$	2.155^{def}	0.530^{gh}	5.200^{efg}	1.250^{hij}	0.930^{ef}	0.280^{efghij}
OT$_1$	3.605^{a}	1.115^{a}	7.955^{a}	2.155^{a}	2.060^{ab}	0.745^{a}
OS$_1$	2.750^{bcd}	0.800^{cd}	6.500^{bcd}	1.605^{def}	1.370^{cde}	0.455^{bcde}
OH$_1$	2.405^{cdef}	0.660^{cdefgh}	5.255^{efg}	1.310^{ghij}	1.200^{def}	0.400^{defgh}
OT$_2$	2.950^{bc}	0.770^{cde}	6.900^{abc}	1.725^{cd}	1.840^{abc}	0.600^{abc}
OS$_2$	2.155^{def}	0.570^{efgh}	5.300^{efg}	1.300^{ghij}	0.800^{ef}	0.240^{ghij}
OH$_2$	2.220^{def}	0.600^{defgh}	5.125^{fg}	1.240^{hij}	0.780^{ef}	0.230^{hij}
OT$_3$	3.850^{a}	1.160^{a}	7.850^{a}	2.125^{ab}	2.150^{a}	0.750^{a}
OS$_3$	2.505^{cdef}	0.705^{cdefg}	5.870^{cdefg}	1.525^{defgh}	1.215^{cdef}	0.395^{defghi}
OH$_3$	2.300^{cdef}	0.610^{defgh}	5.210^{efg}	1.310^{ghij}	1.025^{ef}	0.320^{efghij}
OT$_0$	2.950^{bc}	0.840^{bc}	6.970^{abc}	1.735^{cd}	1.110^{abcd}	0.540^{bcd}
OS$_0$	2.300^{cdef}	0.610^{defgh}	6.000^{cdefg}	1.480^{defghi}	1.000^{ef}	0.310^{efghij}
OH$_0$	2.150^{def}	0.560^{fgh}	5.020^{fg}	1.165^{j}	0.795^{ef}	0.180^{j}

Treatment means that are compared and having common letters as their superscripts do not differ significantly

Table 15. Effect of environment, hormone and type of wood on leaf fresh weight, dry weight and driage percentage of stem in rooted cuttings

Treatments	Fresh weight of leaf (g)			Dry weight of leaf (g)			Driage percentage		
	Mist	Open	Mean	Mist	Open	Mean	Mist	Open	Mean
IAA	6.715^{a}	6.570^{ab}	6.642^{a}	1.683^{a}	1.690^{a}	1.687^{a}	24.988^{c} (0.523)	25.563^{b} (0.530)	25.276^{a} (0.527)
IBA	5.745^{c}	5.775^{c}	5.760^{c}	1.380^{c}	1.422^{c}	1.401^{b}	24.033^{f} (0.512)	24.575^{de} (0.519)	24.304^{b} (0.516)
NAA	6.410^{ab}	6.320^{abc}	6.365^{ab}	1.590^{ab}	1.653^{a}	1.622^{a}	24.734^{cd} (0.521)	26.029^{a} (0.535)	25.382^{a} (0.528)
Control	5.987^{bc}	5.997^{bc}	5.992^{bc}	1.438^{c}	1.460^{bc}	1.449^{b}	24.027^{f} (0.512)	24.256^{ef} (0.515)	24.141^{b} (0.514)
Softwood	6.996^{a}	7.426^{a}	7.211^{a}	1.749^{b}	1.935^{a}	1.842^{a}	24.934^{b} (0.523)	25.987^{e} (0.535)	25.461^{a} (0.529)
Semi-hardwood	6.201^{b}	5.917^{bc}	6.059^{b}	1.509^{c}	1.478^{c}	1.493^{b}	24.315^{c} (0.516)	24.961^{b} (0.523)	24.638^{b} (0.519)
Hardwood	5.445^{cd}	5.152^{d}	5.299^{c}	1.311^{d}	1.256^{d}	1.284^{c}	24.088^{c} (0.513)	24.370^{c} (0.516)	24.229^{c} (0.515)
Mean	6.214^{a}	6.165^{a}		1.523^{a}	1.556^{a}		24.446^{b} (0.517)	25.106^{a} (0.525)	

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

Among different treatment combinations for three factor interaction, treatment combinations OT_1 and OT_3 were on par and were the most significant combinations with driage percentages of 27.09 and 26.97 respectively (Table 5). The least significant combination was OH_0 (23.21%).

4.2.13 Fresh weight of root

Table 16 shows fresh weight of root as influenced by different treatments. The data reveal that environment has no significant effect on fresh weight of root, whereas hormone treatments and type of cuttings significantly influenced the character under study. Among different hormone treatments, IAA and NAA treated cuttings showed values of 1.44 g and 1.38 g respectively which were on par and were the most significant treatments. Among the type of cuttings, softwood cuttings exhibited the most significant effect and recorded the highest value of 1.79 g for fresh weight of root.

However, the interaction effects were not significant (Table 13, 14 and 16).

4.2.14 Dry weight of roots

Data presented in Table 16 indicate that environment, hormone treatment and type of cuttings have high significant effect on the character under study. Cuttings kept in open showed maximum dry weight for roots (0.43 g). Similarly softwood cuttings recorded the highest dry weight of 0.60 g. Cuttings treated with IAA and NAA were on par with dry weights of 0.49 g and 0.45 g respectively.

The different interaction effects were insignificant (Table 13, 14 and 16).

4.2.15 Driage percentage for roots

Data presented in Table 16 clearly reveal that environment provided had no significant effect on the character under study, whereas the hormone treatments and type of cuttings had significant influence on driage of roots. Among hormone treatments, cuttings treated with IAA recorded maximum value of 33.43 per cent, followed by NAA treated cuttings (32.43%). Among the type of cuttings softwood cuttings showed maximum driage for roots (33.13%), followed by semi-hardwood cuttings (31.46%) and the least value of 30.10 per cent was recorded by hardwood cuttings.

Interaction effect

Data shown in Table 10 and Table 16 indicate that the different two-factor interactions were significant. Cuttings treated with IAA and kept in open was the most significant combination among interaction involving hormone treatment and environment. It recorded the highest value of 34.24 per cent for the character under study. So also among interactions involving environment and type of cuttings, the maximum value of 33.82 per cent was recorded for softwood cuttings kept in open environment. The most significant combination for interaction between hormone treatment and type of cuttings was softwood cuttings treated with IAA, having a driage percentage of 35.38 and the least value was for untreated hardwood cuttings (26.34 per cent).

The three factor interaction was also found to be significant (Table 5). Softwood cuttings treated with IAA and kept in open condition (OT_1) recorded the highest value (36.16%), followed by treatment combinations OT_3 and MT_1, which were on par with values of 34.89 per cent and 34.60 per cent. The least value was recorded by untreated hardwood cuttings kept in open (22.58%).

Table 16. Effect of environment, hormone and type of wood on fresh weight, dry weight and driage percentage of root

Treatments	Fresh weight of root (g)			Dry weight of root (g)			Driage percentage		
	Mist	Open	Mean	Mist	Open	Mean	Mist	Open	Mean
IAA	1.337^{ab}	1.543^{a}	1.440^{a}	0.442^{abc}	0.533^{a}	0.487^{a}	32.615^{b} (0.608)	34.240^{a} (0.625)	33.428^{a} (0.616)
IBA	0.957^{c}	1.140^{bc}	1.047^{b}	0.295^{d}	0.357^{cd}	0.326^{b}	30.997^{d} (0.590)	30.704^{d} (0.587)	30.850^{c} (0.589)
NAA	1.288^{abc}	1.463^{ab}	1.376^{a}	0.415^{bc}	0.488^{ab}	0.452^{a}	31.965^{c} (0.601)	32.886^{b} (0.611)	32.426^{b} (0.606)
Control	1.127^{bc}	1.168^{bd}	1.147^{b}	0.347^{cd}	0.343^{cd}	0.345^{b}	30.706^{d} (0.587)	28.390^{e} (0.561)	29.548^{d} (0.574)
Softwood	1.635^{b}	1.946^{a}	1.787^{a}	0.534^{b}	0.659^{a}	0.596^{a}	32.452^{b} (0.606)	33.817^{a} (0.620)	33.134^{a} (0.613)
Semi-hardwood	1.061^{c}	1.096^{c}	1.079^{b}	0.332^{c}	0.350^{c}	0.341^{b}	31.223^{d} (0.593)	31.689^{c} (0.598)	31.456^{b} (0.595)
Hardwood	0.833^{c}	0.950^{c}	0.891^{b}	0.258^{c}	0.282^{c}	0.270^{c}	31.039^{b} (0.591)	29.159^{e} (0.569)	30.099^{c} (0.580)
Mean	1.176^{a}	1.329^{a}		0.375^{b}	0.430^{a}		31.571^{a} (0.597)	31.555^{b} (0.596)	

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

4.3 Leafy Vs leafless cuttings

Data on the percentage of sprouting, percentage of rooting and length of the longest root for leafy as well as leafless cuttings for different treatments are presented in Table 17. Presence and absence of leaves showed significant influence on these characters, which were significantly higher for leafy cuttings (Table 17 and Plate 5).

Leafy softwood cuttings recorded maximum sprouting percentage (38.38%) and maximum rooting percentage (37.38%), whereas the corresponding values for leafless softwood cuttings were 22 per cent and 20.94 per cent respectively. Root length was also maximum for leafy softwood cutting (16.99 cm) compared to that of leafless softwood cuttings (8.59 cm).

The observational values were significantly higher for these characters in the case of leafy cuttings, showing the comparitive superiority of leafy over leafless cuttings.

4.4 Field establishment rate

Selected treatment combinations which recorded maximum rooting success in the case of softwood, semi-hardwood and hardwood cuttings, for each of the two environments provided (mist and open) were carefully planted in the field and their field establishment rate was studied after one month (Plate 6). The treatment combinations were MT_1, MS_3, MH_3, OT_1, OS_3 and OH_1. The data are presented in Table 18. The data clearly indicated that neither the environment nor the type of cuttings had any significant effect on the field survival rate.

Plate 5. Effect of retention of leaves on root and shoot growth of cutting

(2) Leafy cutting
(3) Leafless cutting

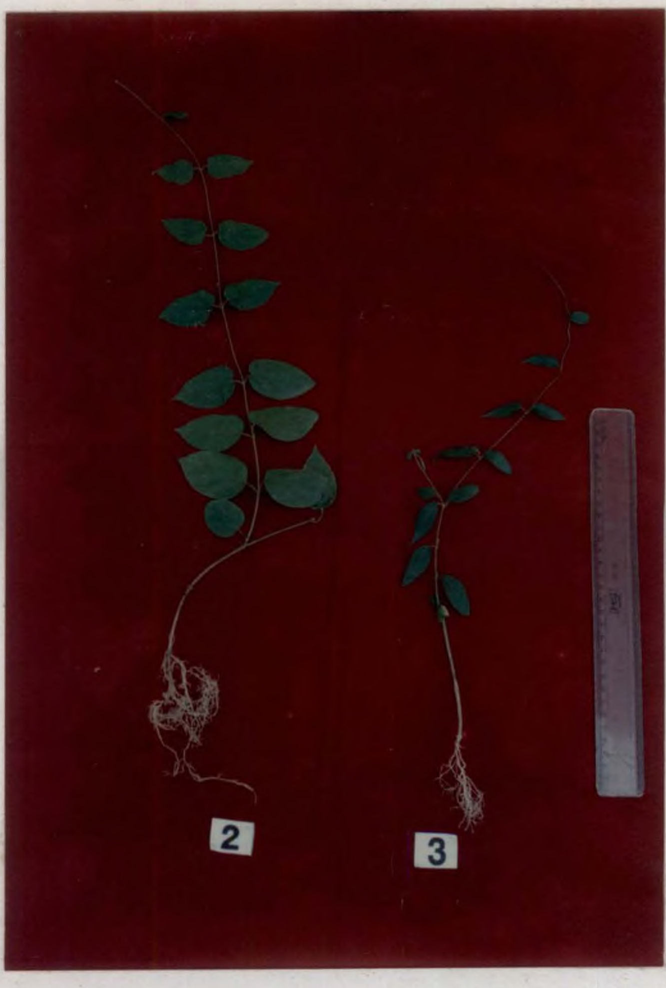


Table 17. Effect of leafiness on successful rooting of cuttings

Treatments	Mist						Open						Mean					
	% sprouting		% rooting		Length of the longest root (cm)		% sprouting		% rooting		Length of the longest root (cm)		% sprouting		% rooting		Length of the longest root (cm)	
	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless
IAA	37.667^{a}	21.000^{a}	36.667^{a}	20.000^{a}	16.650^{a}	8.233^{a}	20.667^{bc}	10.167^{b}	20.333^{c}	9.667^{b}	14.550^{b}	7.467^{bc}	29.167^{a}	15.583^{a}	28.500^{a}	14.833^{a}	15.600^{a}	7.850^{a}
	(0.645)	(0.445)	(0.629)	(0.430)			(0.446)	(0.304)	(0.440)	(0.295)			(0.545)	(0.374)	(0.535)	(0.362)		
IBA	18.000^{c}	11.000^{b}	15.000^{d}	9.833^{b}	12.833^{c}	7.067^{bc}	8.333^{de}	4.500^{c}	8.000^{f}	4.333^{c}	12.500	6.217^{e}	13.167^{b}	7.750^{b}	11.500^{b}	7.083^{b}	12.667^{c}	6.642^{c}
	(0.419)	(0.308)	(0.372)	(0.289)			(0.284)	(0.202)	(0.277)	(0.197)			(0.352)	(0.255)	(0.324)	(0.243)		
NAA	33.000^{a}	20.500^{a}	31.333^{b}	18.333^{a}	15.100^{a}	7.717^{ab}	23.667^{b}	11.667^{b}	23.000^{c}	11.000^{b}	14.200^{b}	6.950^{cd}	28.333^{a}	16.083^{a}	27.167^{a}	14.667^{a}	14.650^{b}	7.333^{b}
	(0.599)	(0.449)	(0.576)	(0.416)			(0.484)	(0.331)	(0.475)	(0.316)			(0.542)	(0.390)	(0.526)	(0.366)		
Control	11.667^{d}	5.333^{c}	10.667^{e}	5.000^{c}	11.917^{cd}	6.383^{cd}	7.000^{e}	3.667^{c}	5.667^{g}	3.167^{c}	11.300^{d}	4.867^{f}	9.333^{c}	4.500^{c}	8.167^{c}	4.083^{c}	11.608^{d}	5.625^{d}
	(0.334)	(0.217)	(0.315)	(0.208)			(0.259)	(0.182)	(0.232)	(0.174)			(0.297)	(0.200)	(0.273)	90.191)		
Soft wood	48.750^{a}	29.875^{a}	47.500^{a}	28.125^{a}	17.662^{a}	8.875^{a}	28.000^{b}	14.125^{b}	27.250^{b}	13.750^{b}	16.313^{b}	8.312^{b}	38.375^{a}	22.000^{a}	37.375^{a}	20.938^{a}	16.987^{a}	8.594^{a}
	(0.774)	(0.566)	(0.760)	90.546)			(0.541)	(0.376)	(0.531)	(0.370)			(0.657)	(0.471)	(0.645)	(0.458)		
Semi-hardwood	16.250^{c}	8.750^{c}	15.000^{c}	8.250^{c}	13.138^{c}	6.837^{c}	11.000^{d}	5.500^{d}	10.500^{d}	5.125^{d}	12.650^{c}	6.050^{d}	13.625^{b}	7.125^{b}	12.750^{b}	6.688^{b}	12.894^{b}	6.444^{b}
	(0.403)	(0.286)	(0.381)	(0.277)			(0.326)	(0.227)	(0.315)	(0.219)			(0.364)	(0.256)	(0.348)	(0.248)		
Hard-wood	10.250^{d}	4.750^{d}	7.750^{e}	3.500^{e}	11.575^{d}	6.337^{cd}	5.750^{e}	2.875^{e}	5.000^{f}	2.250^{f}	10.450^{e}	4.762^{e}	8.000^{c}	3.813^{c}	6.375^{c}	2.875^{c}	11.013^{c}	5.550^{c}
	(0.321)	(0.212)	(0.278)	(0.183)			(0.237)	(0.162)	(0.221)	(0.147)			(0.279)	(0.187)	(0.250)	(0.165)		
Mean	25.083^{a}	14.458^{a}	23.417^{a}	13.292^{a}	14.125^{a}	7.350^{a}	14.917^{b}	7.500^{b}	14.250^{b}	7.042^{b}	13.138^{b}	6.375^{b}						
	(0.499)	(0.355)	(0.473)	(0.335)			(0.368)	(0.255)	(0.356)	(0.245)								

Values in parenthesis indicate angular transformed values

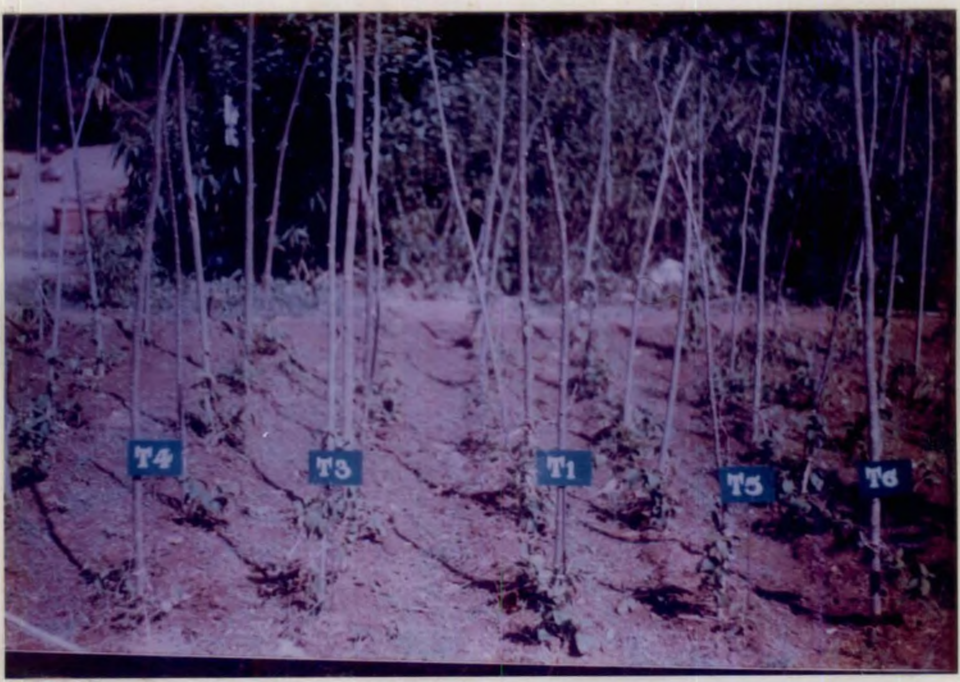
Treatment means that are compared and having common letters as their superscripts do not differ significantly

Table 18. Field survival percentage of successful treatments

Treatments	Mist	Open	Mean	
Softwood	85.09 (1.17)	95.00 (1.41)	94.04 (1.29)	NS
Semi hardwood	89.42 (1.24)	95.00 (1.41)	92.21 (1.33)	NS
Hardwood	90.00 (1.25)	95.00 (1.41)	92.50 (1.33)	NS
Mean	88.17 (1.22)	95.00 (1.41)		
	NS	NS		

Values in parenthesis indicate angular transformed values
NS - Non significant

Plate 6. A general view of the plants established in field


T4
T3
T1
T5
T6

Among cuttings, which were initially kept in mist chamber for rooting, hardwood cuttings recorded maximum field survival rate (90.00%) followed by semi-hardwood cuttings (89.42%) and the least value was recorded by softwood cutting (85.09%). However, cuttings initially kept in open environment for rooting did not significantly vary in their survival rate and all the three treatment combinations OT_1, OS_3 and OH_1) recorded 95 per cent survival, one month after field planting.

4.5 Histological studies

Anatomical studies to locate the precise genesis of root initials revealed that in all the three types of cuttings, the initials were found to originate from the young secondary phloem cells. The stages of rooting were identified as follows:

Root initiation - 20 days after planting (Plate 7)
Root primordia formation - 24 days after planting (Plate 8)
Root emergence - 27 days after planting (Plate 9 and 10)
Callus formation at the base of the stem was observed simultaneously (Plate 11)

The duration for the physiological stages in rooting was similar in all the three types of cuttings.

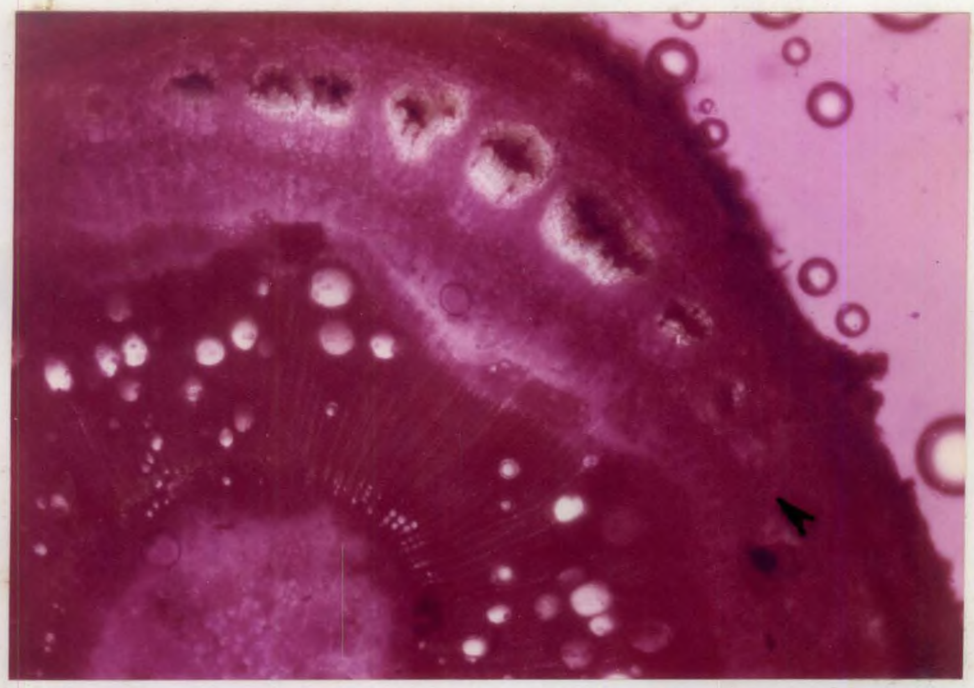
4.6 Biochemical studies

4.6.1 Total carbohydrate content

Total carbohydrate content in leaves as well as the base of stem at three different stages of root development viz., root initiation, root primordia formation and root emergence stage (as influenced by treatments and their combinations) are presented in Tables 19, 20 and 21. The data reveal that the carbohydrate content in general increases from root initiation stage upto root primordia formation. The

Plate 7. Cross section of stem base of *Gymnema sylvestre* showing root initiation

Plate 8. Cross section of stem base of *Gymnema sylvestre* showing root primordia formation



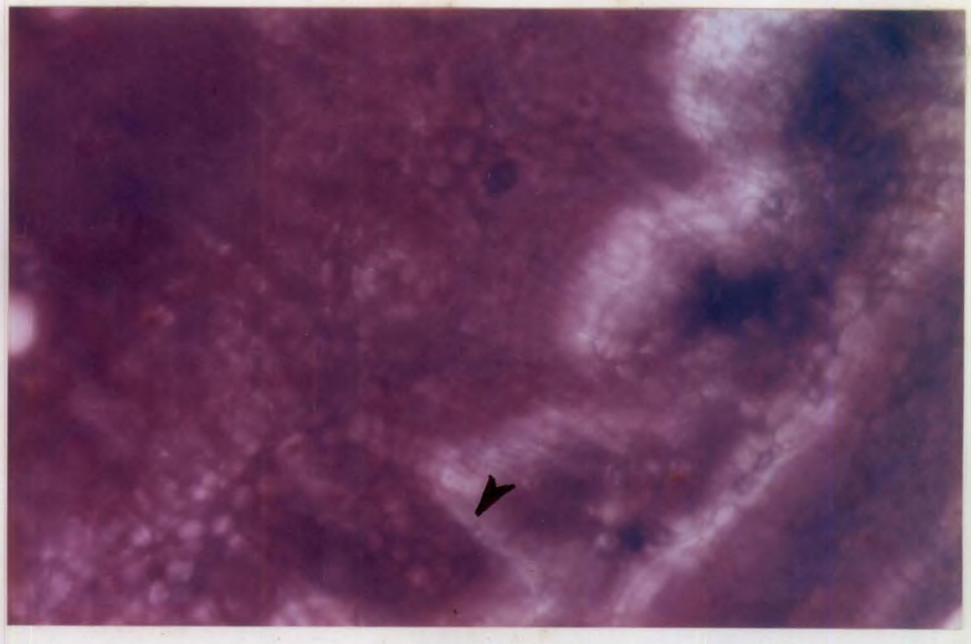
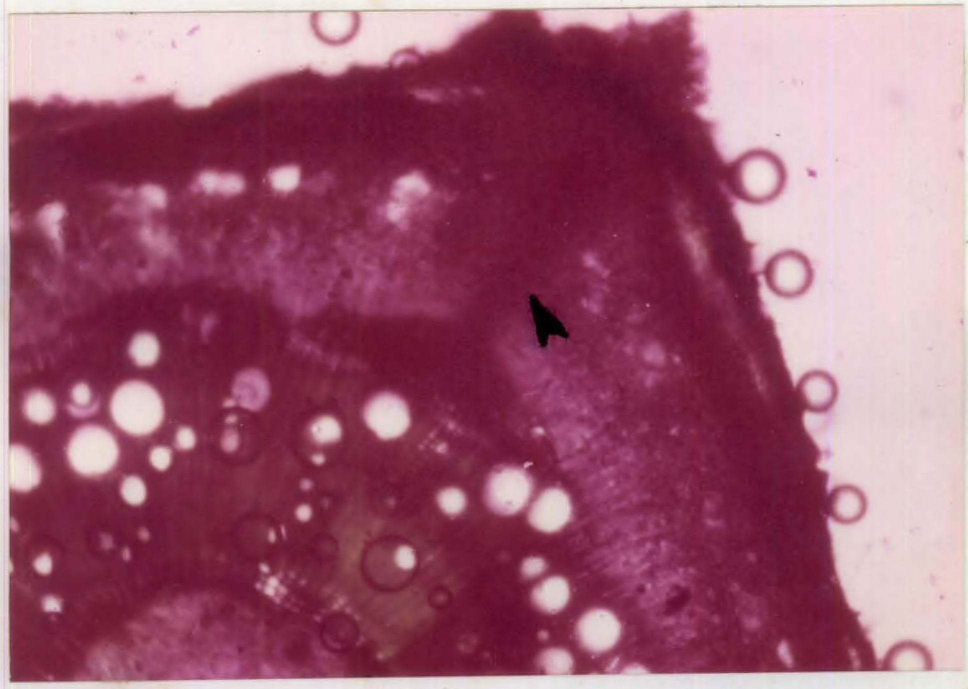


Plate 9. Cross section of stem base of *Gymnema sylvestre* showing root emergence stage (10x)

Plate 10. Cross section of stem base of *Gymnema sylvestre* showing root emergence stage (100x)



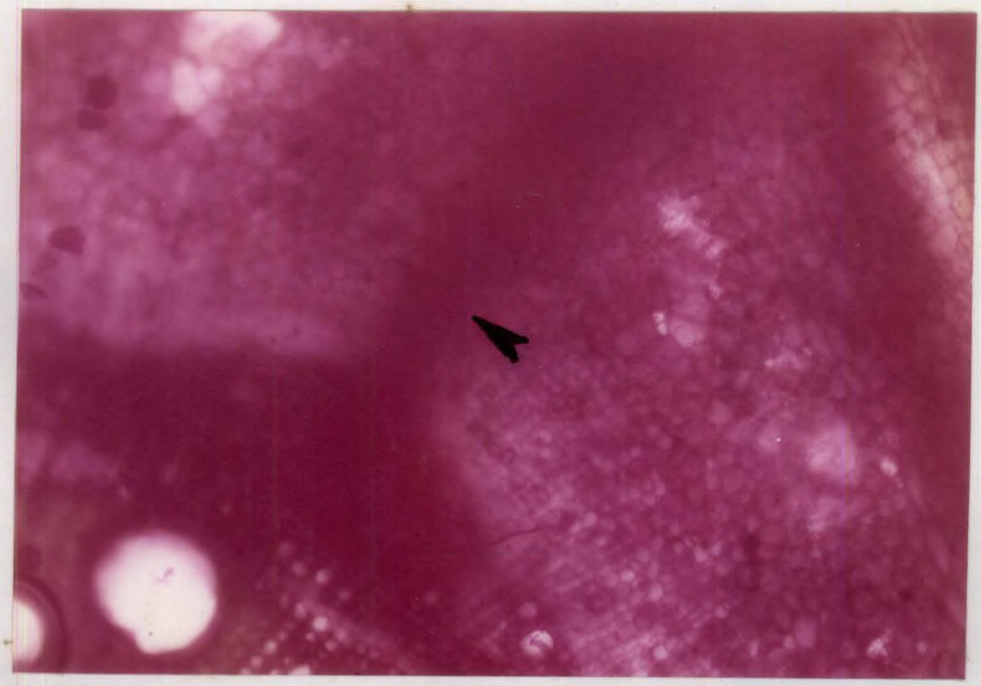


Plate 11. Callus formation at the base of the stem of *Gymnema sylvestre*

carbohydrate content thus reaches a peak during root primordia formation. However, a sudden decrease in carbohydrate content is noticed during root emergence stage and this decrease in carbohydrate content was more pronounced at the base of the stem (Fig. 2).

The data also indicate that the carbohydrate content in the leaf as well as at the base of the stem in different treatments is significantly influenced by the different factors viz., the environment provided, the hormone treatments and the type of cuttings. With respect to the environment provided, cuttings kept in mist showed maximum carbohydrate content compared to cuttings kept in open at all stages of root development in leaf as well as stem. Among hormone treatments, cuttings treated with IAA recorded maximum content of carbohydrate upto root primordia formation. The NAA treated cuttings contained more carbohydrate during root emergence stage than that of IAA treated cuttings. Among the different type of cuttings, softwood cuttings showed maximum content of carbohydrate at all stages in leaves as well as stem (Table 19).

The different interaction effects were also found to be significant and the most significant three factor interaction at all stages for leaf as well as stem was the treatment combination MT_1 (Table 20).

4.6.2 The nitrogen content

Data presented in Tables 22, 23 and 24 show the nitrogen content in leaves and base of stem at different stages of root development for different treatments and their combinations as influenced by different factors of rooting under study.

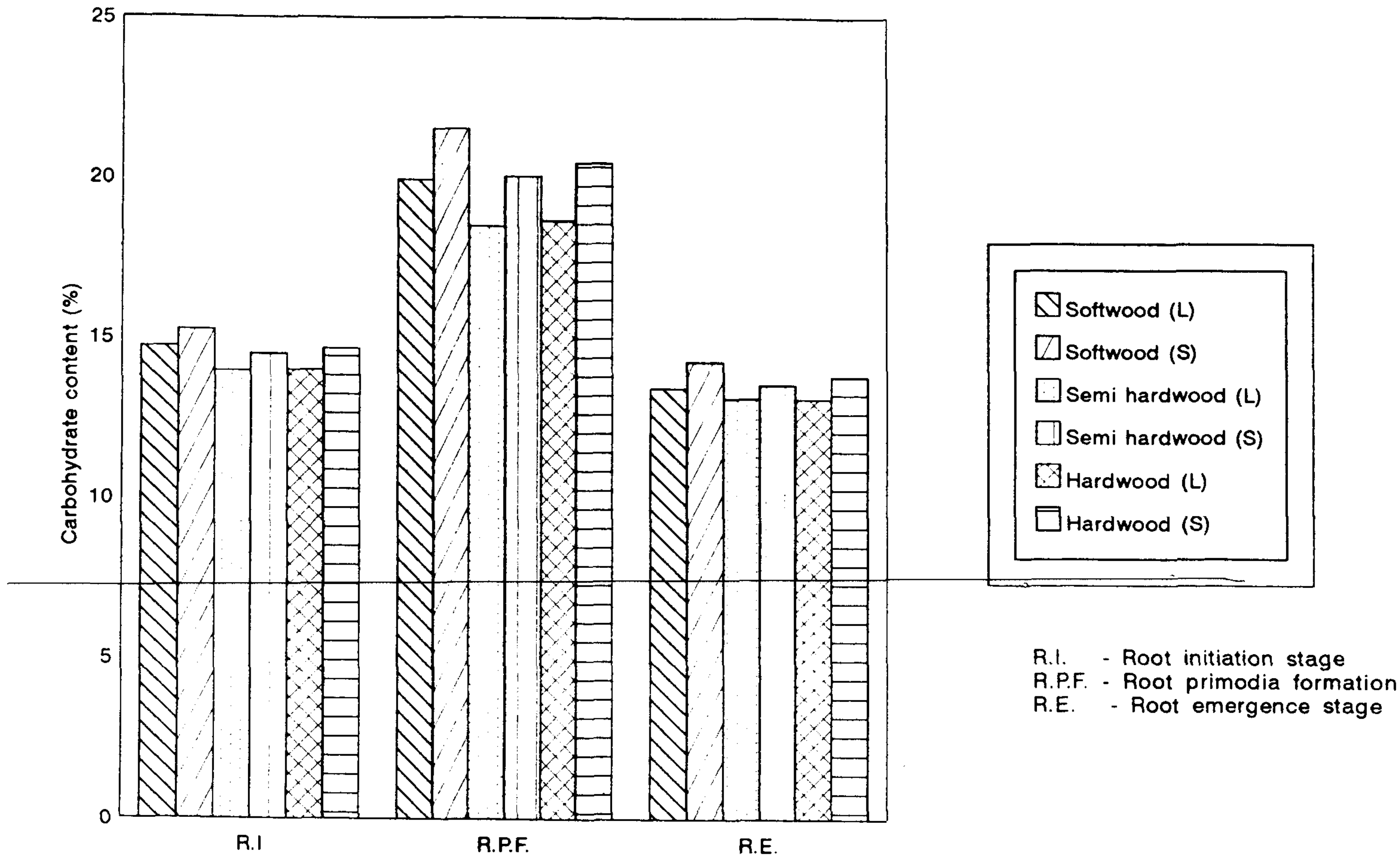


Fig.2. Changes in carbohydrate content in leaf (L) as well as stem (S) during root development

Table 19. Effect of environment, hormone and type of wood on the content of total carbohydrates at different stages of rooting (Per cent on dry weight basis)

Treat-ments	Mist						Open						Mean					
	R.I		R.P.F.		R.E.		R.I.		R.P.F.		R.E.		R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
IAA	14.625^{ab}	15.293^{a}	19.705^{b}	22.955^{a}	12.585^{b}	13.970^{bc}	14.400^{bc}	15.040^{a}	20.192^{a}	21.172^{b}	13.153^{d}	13.815^{c}	14.513^{a}	15.167^{a}	19.948^{a}	22.060^{a}	12.870^{b}	13.893^{b}
	(0.392)	(0.402)	(0.460)	(0.499)	(0.363)	(0.383)	(0.389)	(0.378)	(0.466)	(0.478)	(0.371)	(0.381)	(0.391)	(0.400)	(0.463)	(0.489)	(0.367)	(0.382)
IBA	13.835^{d}	14.738^{bc}	18.798^{c}	20.595^{c}	12.558^{e}	13.488^{d}	13.518^{e}	14.020^{e}	18.490^{d}	19.890^{d}	12.657^{e}	12.907^{e}	13.677^{c}	14.379^{d}	18.664^{b}	20.240^{c}	12.507^{c}	13.198^{c}
	(0.381)	(0.394)	(0.448)	(0.471)	(0.362)	(0.376)	(0.377)	(0.384)	(0.444)	(0.462)	(0.364)	(0.367)	(0.379)	(0.389)	(0.446)	(0.467)	(0.368)	(0.372)
NAA	14.772^{a}	15.020^{ab}	19.883^{ab}	21.345^{b}	13.760^{b}	14.013^{bc}	14.403^{bc}	14.738^{c}	19.897^{ab}	21.022^{b}	13.447^{c}	13.980^{bc}	14.588^{a}	14.879^{b}	19.890^{a}	21.183^{b}	13.603^{a}	13.996^{b}
	(0.394)	(0.398)	(0.462)	(0.480)	(0.380)	(0.384)	(0.389)	(0.394)	(0.462)	(0.476)	(0.375)	(0.383)	(0.392)	(0.396)	(0.462)	(0.478)	(0.378)	(0.383)
Control	14.303^{c}	15.062^{a}	17.963^{e}	19.608^{d}	14.037^{a}	14.518^{a}	13.817^{d}	14.288^{d}	17.125^{f}	18.638^{e}	13.507^{bd}	14.240^{b}	14.060^{b}	14.675^{c}	17.544^{c}	19.123^{d}	13.772^{a}	14.377^{a}
	(0.388)	(0.398)	(0.438)	(0.459)	(0.384)	(0.391)	(0.381)	(0.388)	(0.427)	(0.446)	90.375)	(0.387)	(0.384)	(0.393)	(0.432)	(0.453)	(0.380)	(0.389)
Soft	15.053^{a}	15.629^{a}	19.815^{a}	22.551^{a}	13.608^{a}	14.714^{a}	14.368^{b}	14.801^{b}	19.987^{a}	20.435^{bc}	13.266^{b}	13.805^{b}	14.710^{a}	15.215^{a}	19.901^{a}	21.493^{a}	13.437^{a}	14.259
wood	(0.398)	(0.406)	(0.461)	(0.494)	(0.378)	(0.394)	(0.389)	(0.395)	(0.463)	(0.469)	(0.373)	(0.380)	(0.394)	(0.401)	(0.462)	(0.482)	(0.375)	(0.387)
Semi-	14.221^{bc}	14.635^{bc}	18.871^{b}	20.196^{c}	13.352^{b}	13.389^{c}	13.660^{e}	14.311^{d}	18.065^{d}	19.854^{d}	12.880^{c}	13.691^{b}	13.941^{b}	14.473^{c}	18.966^{c}	20.030^{c}	13.116^{b}	13.540^{c}
hardwood	(0.387)	(0.393)	(0.449)	(0.466)	(0.374)	(0.375)	(0.379)	(0.388)	(0.439)	(0.462)	(0.367)	(0.379)	(0.383)	(0.390)	(0.444)	(0.464)	(0.371)	(0.377)
Hard-	13.878^{de}	14.821^{b}	18.576^{c}	20.630^{b}	12.745^{c}	13.890^{b}	14.076^{cd}	14.452^{cd}	18.725^{bc}	20.253^{c}	13.926^{ab}	13.705^{b}	13.977^{b}	14.657^{b}	18.651^{b}	20.441^{b}	13.090^{b}	13.797^{b}
wood	(0.382)	(0.395)	(0.446)	(0.471)	(0.365)	(0.382)	(0.385)	(0.390)	(0.447)	(0.467)	(0.375)	(0.379)	(0.383)	(0.393)	(0.446)	(0.469)	(0.370)	(0.381)
Mean	14.384^{a}	15.028^{a}	19.088^{a}	21.126^{a}	13.235^{a}	13.998^{a}	14.035^{b}	14.552^{b}	18.926^{b}	20.180^{b}	13.191^{b}	13.734^{b}						
	(0.389)	(0.398)	(0.452)	(0.477)	(0.372)	(0.383)	(0.384)	(0.391)	(0.450)	(0.466)	(0.392)	(0.380)						

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage

R.P.F. - Root primordia formation

R.E. - Root emergence stage

Table 20. Interaction effect of environment, hormone and type of wood on total carbohydrate content (%)

Treatment combination	R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
T_1	15.280^{a}	15.810^{a}	21.403^{a}	23.990^{a}	12.813^{e}	14.288^{b}
	(0.402)	(0.409)	(0.481)	(0.511)	(0.366)	(0.388)
S_1	14.413^{b}	14.858^{cd}	19.545^{b}	21.130^{c}	13.158^{cd}	13.725^{def}
	(0.389)	(0.396)	(0.458)	(0.478)	(0.371)	(0.380)
H_1	13.845^{c}	14.832^{cd}	18.898^{c}	21.070^{c}	12.637^{ef}	13.665^{def}
	(0.381)	(0.395)	(0.450)	(0.477)	(0.363)	(0.379)
T_2	13.825^{c}	14.453^{e}	18.860^{c}	20.165^{de}	12.822^{e}	12.960^{h}
	(0.381)	(0.390)	(0.444)	(0.966)	(0.366)	(0.360)
S_2	13.402^{d}	14.170^{e}	18.370^{d}	20.020^{e}	12.652^{ef}	13.110^{gh}
	(0.375)	(0.386)	(0.443)	(0.464)	(0.364)	(0.370)
H_2	13.803^{c}	14.515^{de}	18.702^{cd}	20.543^{d}	12.347^{f}	13.523^{ef}
	(0.381)	(0.391)	(0.447)	(0.470)	(0.359)	(0.377)
T_3	15.295^{a}	15.442^{b}	21.412^{a}	21.935^{b}	14.247^{a}	14.735^{a}
	(0.402)	(0.404)	(0.481)	(0.487)	(0.387)	(0.394)
S_3	14.110^{bc}	14.350^{e}	18.860^{c}	20.532^{d}	13.162^{d}	13.420^{fg}
	(0.385)	(0.388)	(0.449)	(0.470)	(0.371)	(0.375)
H_3	14.358^{b}	14.845^{cd}	19.398^{b}	21.082^{c}	13.400^{cd}	13.832^{de}
	(0.389)	(0.395)	(0.456)	(0.477)	(0.375)	(0.381)
T_0	14.440^{b}	15.155^{bc}	17.930^{e}	19.882^{e}	13.865^{b}	15.055^{a}
	(0.390)	(0.400)	(0.437)	(0.462)	(0.381)	(0.398)
S_0	13.837^{c}	14.515^{de}	17.098^{f}	18.418^{g}	13.492^{c}	13.905^{cd}
	(0.381)	(0.391)	(0.426)	(0.444)	(0.376)	(0.382)
H_0	13.903^{c}	14.355^{e}	17.605^{f}	19.070^{f}	13.957^{ab}	14.170^{bc}
	(0.382)	(0.3890	(0.433)	(0.452)	(0.383)	(0.386)

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage
R.P.F. - Root primordia formation
R.E. - Root emergence stage

Table 21. Interaction effect of environment, hormone and type of wood on total carbohydrate content (%)

Treatment combination	R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	2	3	4	5	6	7
MT_1	15.610^{a} (0.406)	16.005^{a} (0.412)	20.635^{b} (0.472)	26.420^{a} (0.540)	12.105^{l} (0.355)	14.590^{bc} (0.392)
MS_1	14.765^{cd} (0.394)	15.210^{bcd} (0.401)	19.870^{c} (0.462)	21.485^{cd} (0.482)	13.455^{def} (0.316)	13.880^{defgh} (0.382)
MH_1	13.500^{ij} (0.376)	14.665^{efgh} (0.393)	18.610^{hij} (0.446)	20.960^{de} (0.476)	12.195^{kl} (0.357)	13.440^{hij} (0.375)
MT_2	14.010^{fghi} (0.384)	14.895^{cdefg} (0.396)	19.070^{efgh} (0.950)	20.840^{ef} (0.474)	13.070^{fghij} (0.370)	13.630^{fghi} (0.378)
MS_2	13.495^{ij} (0.376)	14.330^{hi} (0.388)	18.42^{ijk} (0.444)	20.135^{gh} (0.465)	12.615^{ijk} (0.363)	13.160^{ij} (0.371)
MH_2	14.000^{fghi} (0.383)	14.990^{cdef} (0.398)	18.905^{fghi} (0.450)	20.810^{ef} (0.474)	11.990^{l} (0.354)	13.675^{efghi} (0.379)
MT_3	15.500^{ab} (0.405)	15.810^{a} (0.404)	20.685^{b} (0.472)	22.220^{b} (0.491)	14.465^{ab} (0.390)	14.860^{b} (0.396)
MS_3	14.610^{cde} (0.392)	13.990^{i} (0.383)	19.680^{cd} (0.460)	20.245^{fgh} (0.467)	13.625^{cde} (0.378)	13.015^{j} (0.369)
MH_3	14.205^{efg} (0.386)	15.260^{bc} (0.401)	19.285^{def} (0.455)	21.570^{c} (0.483)	13.190^{efgh} (0.372)	14.165^{cde} (0.386)
MT_0	15.090^{bc} (0.399)	15.805^{a} (0.409)	18.870^{fghij} (0.449)	20.725^{ef} (0.473)	14.790^{a} (0.394)	15.775^{a} (0.468)
MS_0	14.015^{fghi} (0.384)	15.010^{cdef} (0.398)	17.515^{m} (0.432)	18.920^{k} (0.450)	13.715^{cd} (0.379)	13.500^{ghij} (0.376)
MH_0	13.805^{ghij} (0.381)	14.370^{hi} (0.389)	17.505^{lm} (0.432)	19.180^{jk} (0.453)	13.605^{cde} (0.378)	14.280^{cd} (0.388)
OT_1	14.950^{cd} (0.397)	15.615^{ab} (0.406)	22.170^{a} (0.490)	21.560^{c} (0.483)	13.520^{def} (0.377)	13.985^{defg} (0.383)
OS_1	14.060^{fgh} (0.384)	14.505^{fghi} (0.391)	19.220^{defg} (0.454)	20.775^{ef} (0.473)	12.860^{ghij} (0.367)	13.570^{fghi} (0.377)
OH_1	14.190^{efg} (0.386)	15.000^{cdef} (0.398)	19.185^{defg} (0.453)	21.180^{cde} (0.478)	13.080^{fghi} (0.370)	13.890^{defgh} (0.380)
OT_2	13.640^{hij} (0.378)	14.010^{i} (0.384)	18.650^{ghij} (0.447)	19.490^{ij} (0.457)	12.575^{jk} (0.362)	12.290^{k} (0.358)

Contd.

Table 21. Continued

1	2	3	4	5	6	7
OS_2	13.310^{j}	14.010^{i}	18.320^{jk}	19.905^{hi}	12.690^{hij}	13.060^{j}
	(0.373)	(0.384)	(0.442)	(0.462)	(0.364)	(0.390)
OH_2	13.605^{hij}	14.040^{i}	18.500^{hijk}	20.275^{fgh}	12.705^{hij}	13.370^{hij}
	(0.378)	(0.384)	(0.445)	(0.467)	(0.364)	(0.374)
OT_3	15.090^{bc}	15.075^{cde}	22.140^{a}	21.650^{c}	14.030^{bc}	14.610^{bc}
	(0.399)	(0.399)	(0.490)	(0.484)	(0.384)	(0.392)
OS_3	13.610^{hij}	14.710^{defgh}	18.040^{kl}	20.820^{ef}	12.700^{hij}	13.825^{defgh}
	(0.378)	(0.394)	(0.439)	(0.474)	(0.364)	(0.381)
OH_3	14.510^{def}	14.430^{ghi}	19.510^{cde}	20.595^{efg}	13.610^{cde}	13.500^{ghij}
	(0.391)	(0.390)	(0.457)	(0.471)	(0.378)	(0.376)
OT_0	13.790^{ghij}	14.505^{fghi}	16.990^{n}	19.040^{jk}	12.940^{ghij}	14.335^{cd}
	(0.380)	(0.391)	(0.425)	(0.452)	(0.368)	(0.388)
OS_0	13.660^{hij}	14.020^{i}	16.680^{n}	17.915^{l}	13.270^{defg}	14.310^{cd}
	(0.379)	(0.384)	(0.421)	(0.437)	(0.373)	(0.388)
OH_0	14.000^{fghi}	14.340^{hi}	17.705^{lm}	18.960^{jk}	14.310^{ab}	14.060^{def}
	(0.383)	(0.388)	(0.434)	(0.451)	(0.388)	(.0384)

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage

R.P.F. - Root primordia formation

R.E. - Root emergence stage

Table 22. Effect of environment, hormone and type of wood on the nitrogen content of cuttings at different stages of rooting (Per cent on dry weight basis)

Treatments	Mist						Open						Mean					
	R.I		R.P.F.		R.E.		R.I.		R.P.F.		R.E.		R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
IAA	1.739^{ab}	0.301^{b}	2.002^{a}	0.417^{abc}	1.431^{c}	0.354^{bc}	1.725^{b}	0.301^{b}	2.047^{a}	0.391^{c}	1.481^{c}	0.284^{e}	1.732^{b}	0.301^{b}	2.025^{a}	0.404^{a}	1.456^{b}	0.319^{b}
	(0.132)	(0.054)	(0.142)	(0.064)	(0.120)	(0.059)	(0.132)	(0.055)	(0.144)	(0.062)	(0.122)	(0.053)	(0.132)	(0.054)	(0.143)	(0.063)	(0.121)	(0.056)
IBA	1.852^{a}	0.334^{ab}	1.995^{a}	0.434^{abc}	1.451^{c}	0.340^{bcd}	1.770^{ab}	0.318^{ab}	2.017^{a}	0.401^{bc}	1.585^{b}	0.298^{de}	1.811^{a}	0.326^{ab}	2.006^{a}	0.418^{a}	1.518^{b}	0.319^{b}
	(0.136)	(0.057)	(0.142)	(0.065)	(0.121)	(0.058)	(0.133)	(0.056)	(0.142)	(0.063)	(0.126)	(0.054)	(0.135)	(0.056)	(0.142)	(0.064)	(0.123)	(0.056)
NAA	1.737^{ab}	0.351^{ab}	2.015^{a}	0.438^{abc}	1.437^{c}	0.411^{a}	1.793^{ab}	0.341^{ab}	2.047^{a}	0.448^{ab}	1.575^{b}	0.313^{bcd}	1.765^{ab}	0.346^{a}	2.031^{a}	0.443^{a}	1.506^{b}	0.362^{a}
	(0.132)	(0.059)	(0.142)	(0.065)	(0.120)	(0.064)	(0.134)	(0.058)	(0.143)	(0.662)	(0.126)	(0.056)	(0.133)	(0.058)	(0.143)	(0.066)	(0.123)	(0.060)
Control	1.810^{ab}	0.368^{a}	2.093^{a}	0.471^{a}	1.710^{a}	0.357^{b}	1.783^{ab}	0.301^{b}	2.059^{a}	0.389^{c}	1.695^{a}	0.305^{cde}	1.797^{ab}	0.335^{ab}	2.076^{a}	0.430^{a}	1.702^{a}	0.331^{b}
	(0.135)	(0.060)	(0.145)	(0.068)	(0.131)	(0.059)	(0.134)	(0.054)	(0.144)	(0.062)	(0.130)	(0.055)	(0.134)	(0.057)	(0.145)	(0.065)	(0.131)	(0.057)
Soft wood	1.666^{d}	0.219^{d}	1.881^{c}	0.293^{d}	1.358^{c}	0.268^{c}	1.697^{cd}	0.223^{d}	1.973^{b}	0.281^{d}	1.476^{b}	0.216^{d}	1.682^{c}	0.221^{c}	1.927^{c}	0.287^{c}	1.417^{b}	0.242^{c}
	(0.129)	(0.047)	(0.138)	(0.054)	(0.117)	(0.052)	(0.131)	(0.047)	(0.141)	(0.053)	(0.122)	(0.047)	(0.130)	(0.047)	(0.139)	(0.054)	(0.119)	(0.049)
Semi-hardwood	1.912^{a}	0.321^{c}	2.185^{a}	0.418^{c}	1.607^{a}	0.329^{b}	1.817^{b}	0.337^{c}	2.151^{a}	0.446^{c}	1.633^{a}	0.325^{b}	1.864^{a}	0.329^{b}	2.168^{a}	0.432^{b}	1.620^{a}	0.327^{b}
	(0.139)	(0.057)	(0.148)	(0.065)	(0.127)	(0.057)	(0.135)	(0.058)	(0.147)	(0.067)	(0.128)	(0.057)	(0.137)	(0.057)	(0.148)	(0.066)	(0.128)	(0.057)
Hardwood	1.774^{bc}	0.476^{a}	2.013^{b}	0.609^{a}	1.557^{ab}	0.510^{a}	1.789^{b}	0.386^{b}	2.003^{b}	0.495^{b}	1.643^{a}	0.358^{b}	1.782^{b}	0.431^{a}	2.008^{b}	0.552^{a}	1.600^{a}	0.430^{a}
	(0.134)	(0.069)	(0.142)	(0.078)	(0.125)	(0.071)	(0.134)	(0.062)	(0.142)	(0.070)	(0.128)	(0.060)	(0.104)	(0.066)	(0.142)	(0.074)	(0.127)	(0.065)
Mean	1.784^{a}	0.338^{a}	2.026^{b}	0.440^{a}	1.507^{b}	0.366^{a}	1.768^{b}	0.315^{b}	2.042^{a}	0.407^{b}	1.584^{a}	0.300^{b}						
	(0.134)	(0.057)	(0.143)	(0.666)	(0.123)	(0.060)	(0.133)	(0.056)	(0.143)	(0.063)	(0.126)	(0.054)						

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage

R.P.F. - Root primordia formation

R.E. - Root emergence stage

Table 23. Interaction effect of environment, hormone and type of wood on the nitrogen content of cuttings (%)

Treatment combination	R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
T_1	1.620^{f}	0.206^{e}	1.889^{c}	0.280^{e}	1.305^{e}	0.215^{f}
	(0.128)	(0.045)	(0.138)	(0.058)	(0.114)	(0.046)
S_1	1.817^{abcde}	0.316^{d}	2.225^{a}	0.436^{cd}	1.562^{b}	0.329^{cd}
	(0.135)	(0.056)	(0.150)	(0.066)	(0.125)	(0.057)
H_1	1.759^{abcde}	0.382^{bc}	1.960^{bc}	0.497^{bc}	1.501^{bc}	0.414^{ab}
	(0.133)	(0.062)	(0.146)	(0.070)	(0.123)	(0.064)
T_2	1.725^{cdef}	0.221^{e}	1.885^{c}	0.281^{e}	1.375^{de}	0.245^{ef}
	(0.132)	(0.047)	(0.138)	(0.053)	(0.117)	(0.049)
S_2	1.882^{ab}	0.316^{d}	2.140^{a}	0.421^{d}	1.597^{b}	0.289^{de}
	(0.137)	(0.056)	(0.147)	(0.065)	(0.127)	(0.054)
H_2	1.825^{abcd}	0.440^{ab}	1.992^{bc}	0.551^{ab}	1.582^{b}	0.424^{a}
	(0.135)	(0.066)	(0.142)	(0.074)	(0.126)	(0.065)
T_3	1.680^{ef}	0.236^{e}	1.954^{c}	0.301^{e}	1.424^{cd}	0.285^{de}
	(0.130)	(0.049)	(0.140)	(0.053)	(0.120)	(0.053)
S_3	1.860^{abc}	0.351^{cd}	2.163^{a}	0.456^{cd}	1.598^{b}	0.360^{bc}
	(0.131)	(0.059)	(0.148)	(0.067)	(0.127)	(0.060)
H_3	1.754^{bcde}	0.451^{a}	1.975^{bc}	0.571^{a}	1.496^{bc}	0.442^{a}
	(0.135)	(0.061)	(0.147)	(0.076)	(0.123)	(0.066)
T_0	1.702^{def}	0.221^{e}	1.980^{bc}	0.286^{e}	1.565^{b}	0.225^{f}
	(0.131)	(0.47)	(0.141)	(0.053)	(0.125)	(0.047)
S_0	1.898^{a}	0.331^{cd}	2.143^{a}	0.416^{d}	1.722^{a}	0.330^{cd}
	(0.138)	(0.058)	(0.147)	(0.065)	(0.132)	(0.057)
H_0	1.790^{abcde}	0.451^{a}	2.104^{ab}	0.588^{a}	1.820^{a}	0.439^{a}
	(0.134)	(0.067)	(0.146)	(0.077)	(0.135)	(0.066)

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage

R.P.F. - Root primordia formation

R.E. - Root emergence stage

Table 24. Interaction effect of environment, hormone and type of wood on the nitrogen content (%)

Treatment combination	R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	2	3	4	5	6	7
MT_1	1.613^{h}	0.201^{fg}	1.830^{fg}	0.310^{g}	1.199^{efg}	0.231^{f}
	(0.127)	(0.045)	(0.136)	(0.056)	(0.110)	(0.0489)
MS_1	1.850^{def}	0.300^{a}	2.252^{e}	0.400^{bcd}	1.597^{bc}	0.331^{bcd}
	(0.136)	(0.055)	(0.151)	(0.063)	(0.127)	(0.058)
MH_1	1.753^{bc}	0.402^{defg}	1.925^{bcd}	0.541^{de}	1.497^{a}	0.502^{bcdef}
	(0.133)	(0.063)	(0.139)	(0.074)	(0.123)	(0.071)
MT_2	1.700^{h}	0.190^{g}	1.811^{g}	0.261^{fg}	1.288^{defg}	0.250^{cdef}
	(0.131)	(0.044)	(0.135)	(0.051)	(0.114)	(0.050)
MS_2	2.050^{cde}	0.331^{ab}	2.213^{de}	0.441^{bcd}	1.597^{bcde}	0.301^{a}
	(0.144)	(0.058)	(0.149)	(0.066)	(0.127)	(0.55)
MH_2	1.805^{ab}	0.481^{defg}	1.959^{abc}	0.601^{de}	1.468^{a}	0.470^{bcdef}
	(0.135)	(0.069)	(0.140)	(0.078)	(0.121)	(0.069)
MT_3	1.650^{fgh}	0.251^{defg}	1.901^{fg}	0.302^{efg}	1.348^{bc}	0.351^{def}
	(0.129)	(0.050)	(0.138)	(0.055)	(0.166)	(0.054)
MS_3	1.815^{defg}	0.291^{abcde}	2.080^{ef}	0.381^{de}	1.498^{bc}	0.351^{bcde}
	(0.135)	(0.054)	(0.145)	(0.062)	(0.123)	(0.059)
MH_3	1.744^{a}	0.510^{abcde}	2.064^{ab}	0.630^{de}	1.467^{a}	0.531^{bcdef}
	(0.132)	(0.072)	(0.144)	(0.079)	(0.121)	(0.073)
MT_0	1.702^{fgh}	0.231^{cdefg}	1.982^{fg}	0.301^{bcd}	1.598^{efg}	0.240^{cdef}
	(0.131)	(0.048)	(0.141)	(0.055)	(0.127)	(0.049)
MS_0	1.933^{cd}	0.361^{abc}	2.193^{de}	0.451^{ab}	1.737^{bc}	0.331^{ab}
	(0.140)	(0.060)	(0.149)	(0.067)	(0.132)	(0.058)
MH_0	1.795^{a}	0.511^{abcd}	2.104^{a}	0.662^{a}	1.795^{a}	0.501^{bcdef}
	(0.134)	(0.072)	(0.146)	(0.081)	(0.134)	(0.071)
OT_1	1.627^{h}	0.210^{defg}	1.949^{g}	0.250^{def}	1.411^{g}	0.198^{ef}
	(0.128)	(0.046)	(0.140)	(0.050)	(0.119)	(0.045)
OS_1	1.783^{cd}	0.332^{abc}	2.197^{de}	0.471^{cde}	1.527^{bcd}	0.328^{bcdef}
	(0.134)	(0.058)	(0.149)	(0.069)	(0.114)	(0.057)
OH_1	1.764^{cd}	0.362^{bcdefg}	1.996^{de}	0.452^{de}	1.506^{bcd}	0.327^{bcdef}
	(0.133)	(0.060)	(0.142)	(0.067)	(0.123)	(0.057)
OT_2	1.749^{efgh}	0.251^{defg}	1.958^{fg}	0.301^{de}	1.462^{efg}	0.239^{bcdef}
	(0.133)	(0.060)	(0.140)	(0.055)	(0.121)	(0.049)

Contd.

Table 24. Continued

1	2	3	4	5	6	7
OS_2	1.715^{def}	0.302^{abcde}	2.066^{e}	0.402^{bcd}	1.598^{cdef}	0.278^{cdef}
	(0.131)	(0.055)	(0.144)	(0.063)	(0.127)	(0.053)
OH_2	1.845^{bc}	0.400^{bcdef}	2.025^{cd}	0.500^{abc}	1.697^{b}	0.377^{bcd}
	(0.136)	(0.063)	(0.143)	(0.071)	(0.131)	(0.061)
OT_3	1.710^{gh}	0.220^{bcdefg}	2.008^{fg}	0.301^{de}	1.501^{fg}	0.219^{cdef}
	(0.131)	(0.047)	(0.142)	(0.055)	(0.123)	(0.047)
OS_3	1.905^{bc}	0.412^{a}	2.247^{bcd}	0.531^{abc}	1.698^{b}	0.368^{abc}
	(0.138)	(0.064)	(0.150)	(0.073)	(0.131)	(0.061)
OH_3	1.763^{bc}	0.391^{efg}	1.886^{cd}	0.512^{cde}	1.525^{bc}	0.352^{bcdef}
	(0.133)	(0.063)	(0.138)	(0.072)	(0.124)	(0.059)
OT_0	1.701^{h}	0.210^{cdefg}	1.979^{g}	0.271^{cd}	1.531^{fg}	0.209^{cdef}
	(0.131)	(0.046)	(0.141)	(0.052)	(0.124)	(0.046)
OS_0	1.863^{def}	0.302^{abcde}	2.093^{ef}	0.381^{ab}	1.708^{bcd}	0.328^{abc}
	(0.137)	(0.055)	(0.145)	(0.062)	(0.131)	(0.057)
OH_0	1.785^{bc}	0.392^{abcd}	2.104^{cd}	0.515^{a}	1.845^{b}	0.377^{bcdef}
	(0.134)	(0.063)	(0.146)	(0.072)	(0.136)	(0.061)

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage
R.P.F. - Root primordia formation
R.E. - Root emergence stage

The nitrogen content showed a gradual increase upto root primordia formation and then there was a fall during root emergence in leaf as well as stem samples for all the treatments. This decline in nitrogen content was more steep in the case of leaf samples (Fig.3).

The data also reveal that though the nitrogen content for different treatments was significantly influenced by environment, hormone treatments and type of cuttings, there was no uniformity in their effects. The interaction effects also showed a similar trend.

4.6.3 C/N ratio

As in the case of carbohydrates and nitrogen, the C/N ratio increased upto root primordia formation and then declined during root emergence (Table 25).

The data presented in Table 25 also indicate that environment provided, hormone treatment given and type of wood selected significantly influenced the C/N ratio in leaf and stem at all stages of root development. Cuttings kept in mist showed higher C/N ratio (8.11, 9.48, 8.87 for leaf and 49.88, 53.22, 41.69 for stem) than those kept in open (7.96, 9.30, 8.37 for leaf and 49.15, 53.12, 48.35 for stem) at all the three stages of root development (Table 25). Similarly softwood cuttings recorded maximum C/N ratio at all stages (8.76, 10.34, 9.53 for leaf and 69.56, 75.10, 60.36 for stem). Effect of hormones on C/N ratio was not uniform for various treatments. However, the fall in C/N ratio during root emergence stage was more sharp in hormone treated cuttings than in control.

The different interaction effects were also found to be significant (Tables 26 and 27). Among interaction involving environment and type of cutting, softwood cuttings kept in mist was superior to all other treatment combinations (9.05, 10.55,

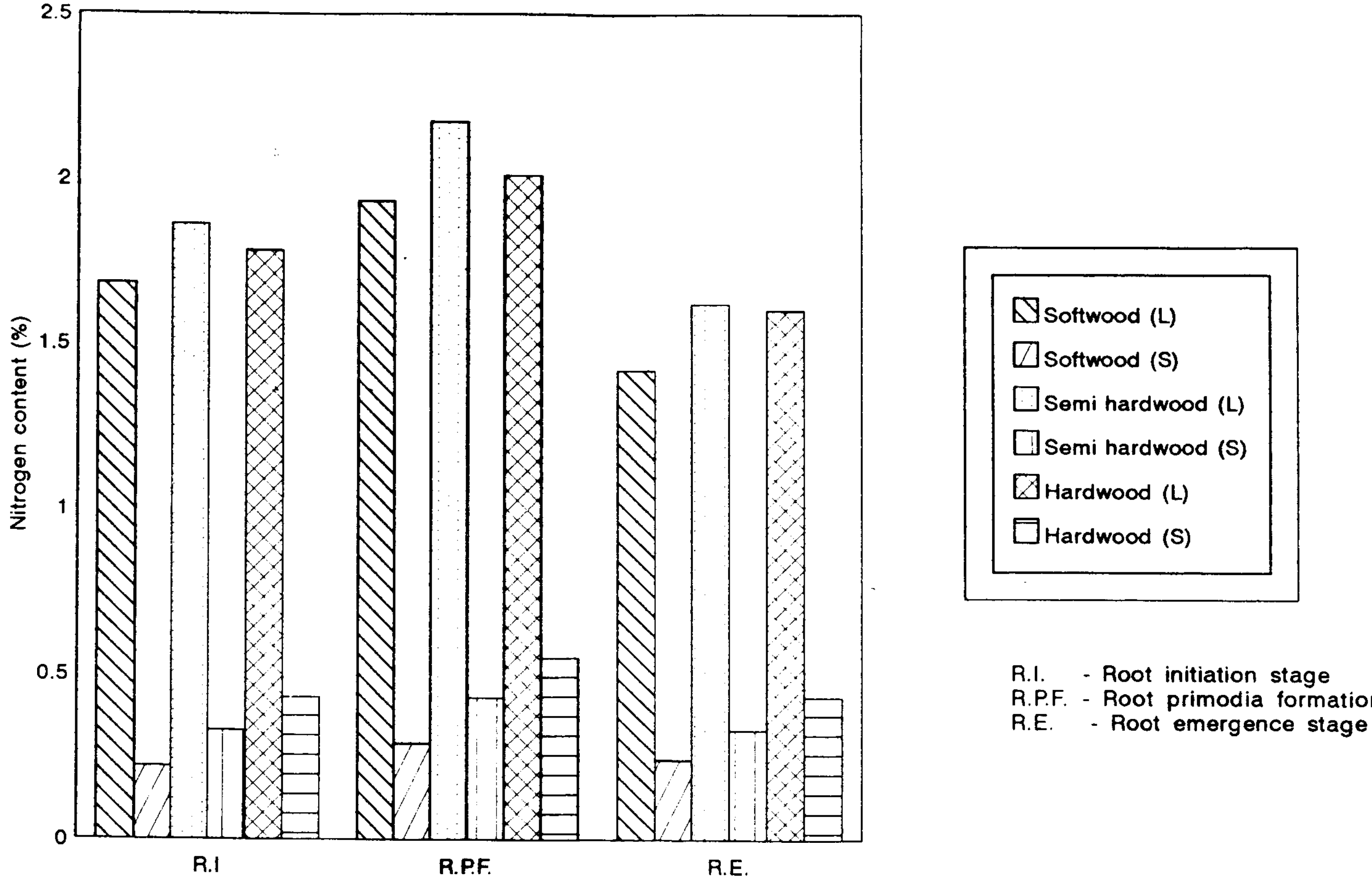


Fig.3. Changes in nitrogen content in leaf (L) as well as stem (S) during root development

Table 25. Effect of environment, hormone and type of wood on the C/N ratio of cuttings at different stages of rooting

Treatments	Mist						Open						Mean					
	R.I		R.P.F.		R.E.		R.I.		R.P.F.		R.E.		R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
IAA	8.954^{b}	55.618^{a}	9.922^{a}	59.159^{a}	8.890^{b}	44.010^{e}	8.371^{c}	53.125^{b}	9.913^{a}	59.054^{a}	8.895^{b}	51.458^{a}	8.412^{a}	54.372^{a}	9.917^{a}	59.106^{a}	8.893^{b}	47.734^{a}
IBA	7.528^{h}	50.904^{c}	9.500^{d}	53.394^{c}	8.738^{c}	42.435^{f}	7.645^{g}	45.795^{e}	9.175^{e}	51.626^{d}	8.012^{f}	44.608^{d}	7.587^{d}	48.350^{b}	9.337^{c}	52.510^{b}	8.375^{c}	43.522^{c}
NAA	8.529^{a}	46.986^{d}	9.895^{b}	53.706^{b}	9.608^{a}	35.358^{g}	8.066^{d}	47.029^{d}	9.800^{c}	50.454^{f}	8.583^{d}	47.494^{c}	8.297^{b}	47.007^{c}	9.848^{b}	52.080^{c}	9.095^{a}	41.426^{d}
Control	7.935^{e}	46.011^{e}	8.609^{f}	46.638^{g}	8.244^{e}	44.961^{d}	7.761^{f}	50.664^{c}	8.323^{g}	51.345^{e}	7.992^{f}	49.839^{b}	7.848^{c}	48.338^{b}	8.466^{d}	48.992^{d}	8.118^{d}	47.400^{b}
Soft wood	9.045^{a}	72.271^{a}	10.552^{a}	76.901^{a}	10.056^{a}	56.452^{b}	8.479^{b}	66.857^{b}	10.129^{b}	73.295^{b}	8.996^{b}	65.258^{a}	8.762^{a}	69.564^{a}	10.340^{a}	75.098^{a}	9.526^{a}	60.355^{a}
Semi-hardwood	7.467^{e}	45.943^{c}	8.649^{e}	48.628^{c}	8.331^{c}	40.867^{d}	7.530^{b}	43.108^{d}	8.403^{f}	44.952^{d}	7.903^{e}	42.404^{c}	7.498^{c}	44.525^{d}	8.526^{c}	46.790^{b}	8.117^{c}	41.636^{b}
Hard-wood	7.823^{c}	31.426^{f}	9.242^{d}	34.144^{f}	8.220^{d}	27.755^{f}	7.873^{c}	37.495^{e}	9.376^{c}	41.113^{e}	8.213^{d}	38.388^{e}	7.848^{b}	34.460^{c}	9.311^{b}	37.628^{c}	8.217^{b}	33.071^{c}
Mean	8.111^{a}	49.880^{a}	9.482^{a}	53.224^{a}	8.870^{a}	41.691^{b}	7.961^{b}	49.153^{b}	9.303^{b}	53.120^{b}	8.371^{b}	48.350^{a}						

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage

R.P.F. - Root primordia formation

R.E. - Root emergence stage

Table 26. Interaction effect of environment, hormone and type of wood on the C/N ratio

Treatment combination	R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
T_1	9.433^{a}	76.905^{a}	11.327^{a}	85.664^{a}	9.839^{b}	66.877^{a}
S_1	7.932^{f}	47.228^{e}	8.786^{h}	48.854^{e}	8.422^{f}	41.686^{f}
H_1	7.872^{f}	38.982^{i}	9.640^{e}	42.801^{i}	8.417^{f}	34.640^{h}
T_2	8.020^{e}	67.003^{c}	10.026^{c}	72.300^{c}	9.374^{c}	52.972^{c}
S_2	7.174^{j}	44.914^{f}	8.594^{j}	47.643^{f}	7.923^{h}	45.351^{d}
H_2	7.566^{h}	33.132^{j}	9.392^{f}	37.588^{j}	7.828^{i}	32.242^{j}
T_3	9.108^{b}	65.756^{d}	10.954^{b}	72.814^{b}	10.039^{a}	54.479^{b}
S_3	7.598^{h}	41.890^{h}	8.745^{i}	46.208^{g}	8.289^{g}	37.298^{g}
H_3	8.187^{d}	33.375^{j}	9.844^{d}	37.218^{k}	8.958^{d}	32.502^{ij}
T_0	8.487^{c}	68.592^{b}	9.054^{g}	69.614^{d}	8.852^{e}	67.092^{a}
S_0	7.289^{i}	44.069^{g}	7.978^{l}	44.456^{h}	7.835^{i}	42.208^{e}
H_0	7.768^{g}	32.352^{k}	8.366^{k}	32.905^{l}	7.666^{j}	32.900^{i}

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage
R.P.F. - Root primordia formation
R.E. - Root emergence stage

Table 27. Interaction effect of environment, hormone and type of wood on the C/N ratio

Treatment combination	R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
MT_1	9.681[a]	79.629[a]	11.276[b]	85.089[b]	10.096[b]	63.298[e]
MS_1	7.981[gh]	50.700[h]	8.823[n]	53.646[i]	8.425[k]	41.934[jk]
MH_1	7.699[k]	36.526[m]	9.668[g]	38.743[q]	8.149[l]	26.800[q]
MT_2	8.241[e]	78.189[b]	10.527[e]	79.848[c]	10.144[b]	54.520[f]
MS_2	6.587[o]	43.359[k]	8.324[r]	45.710[l]	7.902[m]	43.721[i]
MH_2	7.756[gk]	31.164[p]	9.650[g]	34.626[s]	8.168[l]	29.065[p]
MT_3	9.391[b]	62.989[f]	10.881[d]	73.700[d]	10.731[a]	42.397[j]
MS_3	8.050[fg]	48.076[i]	9.462[j]	53.206[i]	9.099[f]	37.028[n]
MH_3	8.145[ef]	29.892[q]	9.344[k]	34.211[s]	8.994[g]	26.651[q]
MT_0	8.866[d]	68.275[e]	9.523[i']	68.969[g]	9.255[e]	65.593[d]
MS_0	7.244[mn]	41.637[l]	7.987t	41.951[n]	7.898[m]	40.786[l]
MH_0	7.691[k]	28.121[r]	8.318[r]	28.995t	7.577[o]	28.503[p]
OT_1	9.186[c]	74.181[c]	11.378[a]	86.240[a]	9.582	70.957[a]
OS_1	7.883[hi]	43.756[k]	8.758[o]	44.062[m]	8.419[k]	41.438[kl]
OH_1	8.044[fg]	41.438[l]	9.612[h]	46.859[k]	8.685[i]	42.481[j]
OT_2	7.799[ijk]	55.817[g]	9.525[i]	64.751[h]	8.604[j]	51.423[g]
OS_2	7.761[ijk]	46.469[j]	8.865[m]	49.577[j]	7.944[m]	46.981[h]
OH_2	7.376[l]	35.100[o]	9.134[l]	40.550[o]	7.489[p]	35.419[o]
OT_3	8.825[d]	68.523[de]	11.026[c]	71.928[e]	9.347[d]	66.561[c]
OS_3	7.146[n]	35.705[n]	8.029s	39.209[p]	7.479[p]	37.569[n]
OH_3	8.228[e]	36.859[m]	10.345[f]	40.2250	8.922[h]	38.353[m]
OT_0	8.107[f]	68.908[d]	8.858[p]	70.259[f]	8.449[k]	68.590[b]
OS_0	7.330[lm]	46.502[j]	7.969t	46.960[k]	7.772[n]	43.630[i]
OH_0	7.845[ij]	36.582[m]	8.415[q]	36.816[r]	7.754[n]	37.297[n]

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage
R.P.F. - Root primordia formation
R.E. - Root emergence stage

10.06 for leaf and 72.27, 76.90, 56.45 for stem) at different stages of root development, except at root emergence in stem, at which softwood cuttings kept in open had the maximum C/N ratio of 64.26 (Table 25).

The three factor *interactions though significant did not give uniform* results. However, the fall in C/N ratio during root emergence was more sharp in hormone treated softwood cuttings kept in mist than in any other treatment combinations (Table 27).

4.6.4 Protein content

The protein content showed similar results as those of nitrogen content at all stages of root development in leaf as well as stem (Tables 28, 29, 30 and Fig.4).

The protein content also showed an increase upto root primordia formation and there was a decrease during root emergence. This decrease in protein content was more pronounced in the case of leaf samples (Fig.4).

The data also show that though the protein content was significantly influenced by environment, hormone treatments and type of wood, they did not give any uniform results. However, cuttings kept in mist were significantly superior in their protein content in stem (2.12, 2.75, 2.29%) at all stages than those in open (1.97, 2.54, 1.88%) (Table 28). Other main effects and their interaction were significant enough, but did not follow any uniform pattern (Table 28, 29 and 30).

Table 28. Effect of environment, hormone and type of wood on the protein content of cuttings at different stages of rooting
(Per cent on dry weight basis)

Treat-ments	Mist						Open						Mean					
	R.I		R.P.F.		R.E.		R.I.		R.P.F.		R.E.		R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
IAA	10.867^{cd}	1.880^{e}	12.515^{c}	2.608^{cd}	8.943^{d}	2.215^{b}	10.781^{b}	1.883^{e}	12.795^{b}	2.445^{e}	9.259^{c}	1.777^{d}	10.824^{c}	1.882^{c}	12.655^{b}	2.527^{c}	9.101^{c}	1.996^{b}
	(0.336)	(0.136)	(0.361)	(0.161)	(0.303)	(0.148)	(0.334)	(0.137)	(0.366)	(0.156)	(0.309)	(0.133)	(0.335)	(0.137)	(0.363)	(0.158)	(0.306)	(0.140)
IBA	11.573^{a}	2.087^{cd}	12.466^{c}	2.714^{bc}	9.069^{cd}	2.128^{b}	11.059^{bc}	1.984^{d}	12.604^{bc}	2.505^{de}	9.907^{b}	1.864^{cd}	11.316^{a}	2.036^{b}	12.535^{b}	2.609^{bc}	9.488^{b}	1.996^{b}
	(0.347)	(0.143)	(0.361)	(0.163)	(0.306)	(0.145)	(0.339)	(0.141)	(0.363)	(0.158)	(0.320)	(0.136)	(0.343)	(0.142)	(0.362)	(0.161)	(0.313)	(0.141)
NAA	10.853^{cd}	2.193^{ab}	12.594^{bc}	2.734^{bc}	8.983^{d}	2.570^{a}	11.204^{b}	2.132^{bc}	12.794^{b}	2.800^{ab}	9.843^{b}	1.957^{c}	11.029^{b}	2.162^{a}	12.694^{b}	2.767^{a}	9.413^{b}	2.264^{a}
	(0.336)	(0.147)	(0.363)	(0.164)	(0.304)	(0.160)	(0.341)	(0.145)	(0.366)	(0.167)	(0.319)	(0.140)	(0.338)	(0.146)	(0.364)	(0.166)	(0.312)	(0.150)
Control	11.314^{b}	2.298^{a}	13.081^{a}	2.944^{a}	10.688^{a}	2.234^{b}	11.144^{b}	1.883^{e}	12.867^{ab}	2.432^{e}	10.593^{a}	1.904^{c}	11.229^{a}	2.091^{ab}	12.974^{a}	2.688^{b}	10.640^{a}	2.069^{b}
	(0.343)	(0.150)	(0.370)	(0.170)	(0.333)	(0.148)	(0.340)	(0.137)	(0.367)	(0.155)	(0.331)	(0.137)	(0.342)	(0.143)	(0.368)	(0.163)	(0.332)	(0.143)
Soft	10.414^{d}	1.366^{e}	11.756^{d}	1.834^{e}	8.490^{e}	1,674^{d}	10.605^{d}	1.394^{e}	12.334^{c}	1.755^{e}	9.227^{d}	1.353^{e}	10.510^{c}	1.380^{c}	12.045^{c}	1.794^{c}	8.858^{b}	1.514^{c}
wood	(0.329)	(0.117)	(0.350)	(0.136)	(0.295)	(0.129)	(0.332)	(0.118)	(0.359)	(0.133)	(0.309)	(0.117)	(0.330)	(0.118)	(0.354)	(0.134)	(0.302)	(0.123)
Semi-	11.951^{a}	2.003^{d}	13.653^{a}	2.613^{d}	10.043^{b}	2.054^{c}	11.354^{b}	2.104^{c}	13.443^{a}	2.790^{c}	10.204^{ab}	2.034^{c}	11.652^{a}	2.054^{b}	13.548^{a}	2.702^{b}	10.123^{a}	2.044^{b}
hardwood	(0.353)	(0.142)	(0.378)	(0.162)	(0.322)	(0.144)	(0.344)	(0.145)	(0.375)	(0.167)	(0.325)	(0.143)	(0.348)	90.144)	(0.377)	(0.165)	(0.324)	(0.143)
Hard-	11.090^{c}	2.975^{a}	12.582^{b}	3.803^{a}	9.729^{c}	3.132^{a}	11.182^{bc}	2.415^{b}	12.518^{bc}	3.092^{b}	10.271^{a}	2.240^{b}	11.136^{b}	2.695^{a}	12.550^{b}	3.448^{a}	10.000^{a}	2.686^{a}
wood	(0.339)	(0.173)	(0.363)	(0.196)	(0.317)	(0.178)	(0.341)	(0.156)	(0.362)	(0.177)	(0.326)	(0.150)	(0.340)	(0.165)	(0.362)	(0.186)	(0.321)	(0.164)
Mean	11.152^{a}	2.115^{a}	12.664^{b}	2.750^{a}	9.421^{b}	2.287^{a}	11.047^{b}	1.971^{b}	12.765^{a}	2.546^{b}	9.901^{a}	1.876^{b}						
	(0.340)	(0.144)	(0.364)	(0.165)	(0.312)	(0.150)	(0.339)	(0.140)	(0.365)	(0.159)	(0.320)	(0.137)						

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage

R.P.F. - Root primordia formation

R.E. - Root emergence stage

Table 29. Interaction effect of environment, hormone and type of wood on the protein content of cuttings (%)

Treatment combination	R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
T_1	10.125[g] (0.324)	1.286[f] (0.114)	11.808[e] (0.359)	1.752[g] (0.133)	8.156[g] (0.289)	1.341[g] (0.116)
S_1	11.355[bc] (0.344)	1.973[d] (0.141)	13.903[a] (0.352)	2.725[de] (0.166)	9.764[c] (0.318)	2.058[d] (0.144)
H_1	10.992[de] (0.338)	2.386[b] (0.155)	12.253[d] (0.358)	3.103[c] (0.177)	9.383[d] (0.311)	2.589[b] (0.161)
T_2	10.718[ef] (0.334)	1.380[ef] (0.117)	11.780[e] (0.350)	1.756[fg] (1.330)	8.594[f] (0.297)	1.528[f] (1.124)
S_2	11.766[a] (0.356)	1.975[d] (0.141)	13.373[bc] (0.314)	2.631[e] (0.163)	9.981[c] (0.325)	1.809[e] (0.135)
H_2	11.405[bc] (0.344)	2.753[a] (0.167)	12.452[d] (0.361)	3.441[b] (0.186)	9.889[c] (0.320)	2.650[ab] (0.163)
T_3	10.502[f] (0.330)	1.472[e] (0.122)	12.216[d] (0.357)	1.883[e] (0.138)	8.903[e] (0.303)	1.781[e] (0.133)
S_3	11.623[ab] (0.348)	2.197[c] (0.148)	13.522[b] (0.376)	2.848[d] (0.169)	9.986[c] (0.321)	2.248[c] (0.151)
H_3	10.961[de] (0.337)	2.819[a] (0.168)	12.344[d] (0.359)	3.570[ab] (0.190)	9.350[d] (0.311)	2.761[a] (0.166)
T_0	10.634[f] (0.332)	1.381[ef] (0.118)	12.377[d] (0.359)	1.786[fg] (0.134)	9.780[c] (0.318)	1.405[g] (0.119)
S_0	11.866[a] (0.352)	2.069[cd] (0.144)	13.394[bc] (0.375)	2.602[e] (0.162)	10.763[b] (0.334)	2.059[d] (0.144)
H_0	11.186[cd] (0.341)	2.822[a] (0.168)	13.152[c] (0.371)	3.677[a] (0.193)	11.378[a] (0.344)	2.744[a] (0.166)

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage
R.P.F. - Root primordia formation
R.E. - Root emergence stage

Table 30. Interaction effect of environment, hormone and type of wood on the protein content (%)

Treatment combination	R.I.		R.P.F.		R.E.	
	Leaf	Stem	Leaf	Stem	Leaf	Stem
1	2	3	4	5	6	7
MT_1	10.078^{j}	1.256^{ij}	11.438^{hi}	1.941^{h}	7.494I	1.441^{ghi}
	(0.323)	(0.112)	(0.345)	(0.140)	(0.277)	(0.120)
MS_1	11.563^{cde}	1.875^{ef}	14.075^{a}	2.503^{g}	9.981^{d}	2.069^{de}
	(0.347)	(0.137)	(0.385)	(0.159)	(0.321)	(0.144)
MH_1	10.959^{fgh}	2.509^{b}	12.031^{fg}	3.381^{c}	9.353^{e}	3.134^{ab}
	(0.331)	(0.159)	(0.354)	(0.185)	(0.311)	(0.178)
MT_2	10.625^{hi}	1.91^{j}	11.322^{i}	1.631^{j}	8.053^{h}	1.563^{g}
	(0.332)	(0.109)	(0.343)	(0.128)	(0.288)	(0.125)
MS_2	12.812^{a}	2.066^{de}	13.831^{a}	2.753^{f}	9.978^{d}	1.881^{ef}
	(0.366)	(0.144)	(0.381)	(0.167)	(0.321)	(0.138)
MH_2	11.281^{def}	3.006^{a}	12.244^{efg}	3.756^{b}	9.175^{ef}	2.941^{b}
	(0.343)	(0.174)	(0.357)	(0.195)	(0.308)	(0.172)
MT_3	10.316^{ij}	1.569^{g}	11.881^{g}	1.884^{hi}	8.425^{g}	2.191^{cd}
	(0.327)	(0.126)	(0.352)	(0.138)	(0.294)	(0.149)
MS_3	11.344^{def}	1.819^{ef}	13.000^{bc}	2.378^{g}	9.359^{e}	2.197^{cd}
	(0.344)	(0.135)	(0.369)	(0.155)	(0.311)	(0.149)
MH_3	10.900^{fgh}	3.191^{a}	12.900^{bcd}	3.941^{ab}	9.166^{ef}	3.222^{a}
	(0.336)	(0.180)	(0.369)	(0.200)	(0.308)	(0.183)
MT_0	10.637^{hi}	1.447^{gh}	12.384^{ef}	1.878^{hi}	9.987^{d}	1.503^{gh}
	(0.332)	(0.121)	(0.360)	(0.137)	(0.322)	(0.123)
MS_0	12.084^{b}	2.253^{cd}	13.706^{a}	2.819^{f}	10.853^{bc}	2.069^{de}
	(0.355)	(0.151)	(0.379)	(0.169)	(0.336)	(0.144)
MH_0	11.219^{def}	3.194^{a}	13.153^{b}	4.134^{a}	11.222^{ab}	3.131^{ab}
	(0.342)	(0.180)	(0.371)	(0.205)	(0.342)	(0.178)
OT_1	10.172^{j}	1.316^{hij}	12.178^{efg}	1.563^{j}	8.819^{f}	1.241^{j}
	(0.325)	(0.115)	(0.356)	(0.125)	(0.302)	(0.112)
OS_1	11.147^{efg}	2.072^{de}	13.731^{a}	2.947^{ef}	9.547^{e}	2.047^{de}
	(0.340)	(0.144)	(0.380)	(0.173)	(0.314)	(0.144)
OH_1	11.025^{fgh}	2.262^{cd}	12.475^{def}	2.825^{f}	9.412^{e}	2.044^{de}
	(0.338)	(0.151)	(0.361)	(0.169)	(0.312)	(0.143)
OT_2	10.931^{fgh}	1.569^{g}	12.237^{efg}	1.881^{hi}	9.134^{ef}	1.494^{gh}
	(0.331)	(0.126)	(0.357)	(0.138)	(0.307)	(0.123)

Contd.

Table 30. Continued

1	2	3	4	5	6	7
OS_2	11.719^{ghi}	1.884^{ef}	12.916^{bcd}	2.509^{g}	9.984^{d}	1.737^{f}
	(0.334)	(0.138)	(0.368)	(0.159)	(0.321)	(0.132)
OH_2	11.528^{cde}	2.500^{b}	12.659^{bcde}	3.125^{de}	10.603^{c}	2.359^{c}
	(0.346)	(0.159)	(0.364)	(0.178)	(0.332)	(0.154)
OT_3	10.688^{ghi}	1.375^{hi}	12.550^{cde}	1.881^{hi}	9.381^{e}	1.372^{hij}
	(0.333)	(0.118)	(0.362)	(0.138)	(0.311)	(0.117)
OS_3	11.903^{bc}	2.575^{b}	14.044^{a}	3.319^{cd}	10.612^{c}	2.300^{c}
	(0.352)	(0.161)	(0.384)	(0.183)	(0.332)	(0.152)
OH_3	11.022^{fgh}	2.447^{bc}	11.787^{gh}	3.200^{cd}	9.534^{e}	2.200^{cd}
	(0.338)	(0.157)	90.350)	(0.180)	(0.314)	(0.149)
OT_0	10.631^{hi}	1.316^{hij}	12.369^{ef}	1.694^{ij}	9.572^{de}	1.306^{ij}
	(0.332)	(0.115)	(0.359)	(0.131)	(0.315)	(0.115)
OS_0	11.647^{bcd}	1.884^{ef}	13.081^{b}	2.384^{g}	10.672^{c}	2.050^{de}
	(0.348)	(0.138)	(0.370)	(0.155)	(0.333)	(0.144)
OH_0	111.153^{efg}	2.450^{bc}	13.150^{b}	3.219^{cd}	11.534^{a}	2.356^{c}
	(0.341)	(0.157)	(0.311)	(0.180)	(0.347)	(0.154)

Values in parenthesis indicate angular transformed values

Treatment means that are compared and having common letters as their superscripts do not differ significantly

R.I. - Root initiation stage

R.P.F. - Root primordia formation

R.E. - Root emergence stage

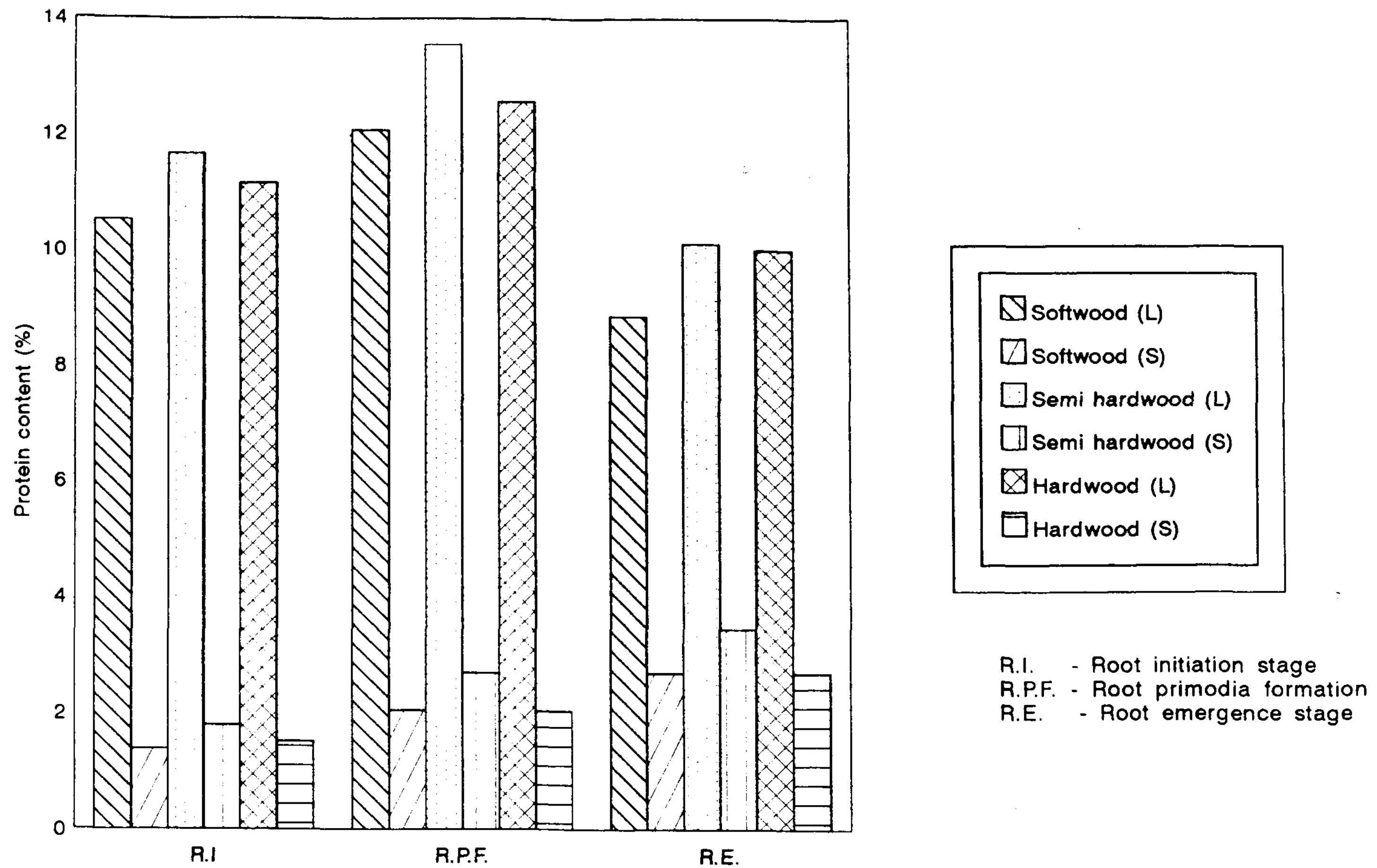


Fig.4. Changes in protein content in leaf (L) as well as stem (S) during root development

Discussion

DISCUSSION

The results of the investigation carried out to standardise propagation through cuttings in *Gymnema sylvestre* R.Br. are discussed in this chapter.

Propagation through stem cuttings

Propagation using stem cuttings is preferred as a tool for large scale multiplication of elite genotypes of a crop, as it has some distinct advantages over other conventional propagation methods viz., grafting and budding. The method is, inexpensive, rapid, simple and free from any compatibility problems commonly encountered when plants are raised by grafting or budding. It also enables one to raise many plants on a limited space from a few stock plants. So this method offers tremendous scope for conservation, domestication and large scale multiplication of those medicinal plants which are rare as well as much sought after in the traditional system of Indian medicine.

Type of cuttings and rhizogenesis

The type of wood used for cuttings is immaterial in the species of plants which are easy-to-root, but it is a matter of great importance in the difficult-to-root material. *Gymnema sylvestre* is a laticiferous twining shrub which is difficult-to-root.

While comparing the type of cuttings, it was found that although hardwood and semi-hardwood cuttings were slightly early in sprouting and rooting (Table 1 and 6), the percentage of sprouting and rooting and other qualitative aspects such as root volume, length and girth of the longest root, number of shoots and leaves produced and length of the shoot were high in softwood cuttings.

Softwood cuttings recorded the maximum number of shoots (1.43), maximum girth of shoot (4.85 mm), longest root length (16.92 cm) and the maximum root volume (0.90 ml). They also recorded the highest values for fresh weight of stem (3.20 g), leaf (7.21 g) and root (1.79 g) as well as for dry weight of stem (0.93 g), leaf (1.84 g) and root (0.60 g). Besides, softwood cuttings had the maximum driage percentage of stem (28.92%), leaf (25.46%) and root (33.13%). These results were in conformity with the works of Rao and Selvarajan (1982) and Pal *et al.* (1993a and b).

The semi-hardwood and hardwood cuttings have ample supply of stored food whereas the softwood cuttings are usually low in stored food reserve. This may have caused slightly delayed sprouting and rooting in softwood cuttings, compared to hardwood and semi-hardwood cuttings.

High adventitious rooting potential is a juvenile character. Besides, the softwood cuttings of *Gymnema* comprise of younger and tender tissues, which are possibly unsaturated in latex and its content of metabolites like tannin, lignin etc. Presence of these materials may adversely interfere with sprouting and root development (Hegde, 1988).

The hardwood cuttings recorded a rooting success of only 6.38 per cent in the control (Table 6). This, on the other hand represents a physiologically inactive region, highly saturated with inhibitory substances like tannins, phenols etc. This may be the reason for better rooting success of softwood cuttings compared to hard and semi-hardwood cuttings.

Effect of mist

The present investigation conclusively showed that cuttings kept under intermittent misting environment had a significantly higher rooting succes (23.42%) compared to cuttings kept in open (14.25%) (Table 6). This was especially significant for softwood cuttings, which recorded 47.50 per cent of rooting under misting environment compared to open, non-misting environment (27.25%). Beneficial effect of mist on rooting in a wide variety of difficult-to-root species has been recorded by Erickson and Bitters (1953). Similarly Mitra and Kushari (1985) reported that mist propagation under plastic had resulted in high percentage of rooting in *Solanum khasianum*.

It is well known that misting maintains a film of water on the leaves which not only result in high humidity surrounding the leaf but also maintains turgidity and optimum temperature. Intermittent misting also prevents desiccation of the cuttings. At the same time cuttings kept in open environment showed markedly low rooting percentage. Under relatively dry conditions, desiccation and death should have occurred by excessive water loss for these cuttings, before the roots were formed.

Effect of growth regulators

The use of growth regulators is now in practice for enhancing the rooting efficiency in cuttings. In the present study, the cuttings without any growth regulator treatment recorded the least rooting success (Table 6) showing that commercial propagation through cuttings in *Gymnema* is possible only with hormonal treatment.

Among the growth regulators tried, IAA was found to be more effective than NAA and IBA. Softwood cuttings kept under misting environment recorded 81 per cent rooting when treated with IAA 500 mg l^{-1} (Table 5). The quality of roots and shoots were also the best with the same concentration. In conformity to this result, Basak *et al.* (1995) had reported that in *Thespesia populnea* stem cuttings responded better to IAA than to NAA or IBA.

Different species respond differently to the growth regulators. Even with the same growth regulator, response of a species to adventitious root formation will vary with applied concentrations of the chemical. Pal *et al.* (1993b) reported that in *Tylophora indica*, cuttings prepared from lateral shoots showed maximum rooting response when treated with IBA at 1000 mg l^{-1} concentration. Similarly in neem (*Azadirachta indica*) leafy stem cuttings treated with 0.2-0.4 per cent IBA gave the best results for root development (Kamaluddin and Ali, 1996).

Indole acetic acid (IAA) is a natural plant hormone and is primarily responsible for adventitious root formation in cuttings. IAA is synthesised in shoot tips and terminal buds and undergoes polar transport to reach the basal portion of cuttings, where it induces formation of root initials, when cuttings are placed under favourable conditions for rooting. As a result the content of IAA will be more in softwood cuttings made from terminal ends and lateral shoots of *Gymnema* than in semi hardwood and hardwood cuttings. Hence the softwood cuttings may have an inherant ability to produce adventitious roots.

Besides this, the difficult-to-root nature of *Gymnema* may be attributed to the competitive inhibitory effect exerted by the different secondary metabolites present in it, viz., latex, tannin, lignin etc. which may have adversely interfered with root development. The softwood cuttings comprising of younger and tender tissues may be possibly unsaturated in latex and other metabolites when compared to semi-hardwood and hardwood cuttings, which represents a physiologically inactive region, active in inhibitory substances like tannins, phenols etc.

In this context, external application of IAA might have easily nullified the effect of negligibly small amounts of inhibitory metabolities present in softwood cuttings and this inturn may have triggered better rooting response of softwood cuttings especially towards IAA treatment. At the same time, application of IAA could not overcome the inhibitory effect exerted by relatively large amounts of tannins, phenols and latex present in hardwood cuttings, resulting in their poor rooting response even after treatment with growth regulator substances.

Leafy Vs leafless cuttings

The investigation proved beyond doubt that the presence of lamina on the cutting of *Gymnema* greatly enhance rooting efficiency and quality of roots (Table 17). This result can be substantiated from three angles. Firstly, it is a known fact that photosynthesis during rooting by leafy cuttings provides carbohydrates to the base of the cuttings and they accumulate in the base during rooting period (Haissig, 1982). Secondly, under decreased photosynthetic activity, auxin synthesis and its polar transport may also be greatly reduced (Heide, 1968 and Vardar, 1968) thus indirectly influencing rooting. Thirdly, rooting cofactors normally originate in leaves and bud and may be transported to the base of the cutting where they promote rooting (Sachs, 1882).

Field survival rate

The study shows that neither the environment nor the type of wood had significant influence on the field establishment rate of rooted cuttings. Once rooted all the cuttings showed more or less similar field establishment rates, irrespective of the environment provided or the type of wood kept for rooting.

Cuttings kept in mist showed a field survival rate of 88.17 per cent, whereas those in open environment recorded 95 per cent establishment rate. Field survival rate of softwood, semi-hardwood and hardwood cuttings were 90.04, 92.21 and 92.50 per cent respectively (Table 18).

Interaction effect

The study also revealed that there is strong synergistic effect for the different combination of factors which influenced rooting of cuttings. Among the two-factor interactions, those between the environment and type of wood and those between type of wood and growth regulator treatments were the most prominent interactions. In the former case, softwood cuttings kept under mist showed maximum synergistic effect with a rooting success of 47.50 per cent while the hardwood cuttings kept in open condition showed the least amount of synergistic effect with a rooting succes as low as 5 per cent (Table 6).

Similarly softwood cuttings treated with IAA recorded the most significant interaction with a rooting success of 62 per cent, followed by softwood cuttings treated with NAA (49%), whereas untreated hardwood cuttings recorded merely 4 per cent rooting (Table 4).

Among the three factor interactions, synergistic effect was maximum for softwood cuttings treated with IAA and kept under intermittent mist, which recorded a rooting success of 81 per cent (Table 5). Other root and shoot characters also showed similar synergistic effects.

Anatomical studies

The anatomical studies revealed that the root initials originated from the secondary phloem cells in *Gymnema sylvestre* irrespective of the type of wood. In conformity with this result, Nanda and Anand (1970) reported that in woody perennials where one or more layers of secondary xylem and phloem are present, root initials originated from young secondary phloem which includes the dividing phloem cells, phloem mother cells and radially enlarging phloem cells.

The time at which the root initials develop after the cuttings are made, differs widely with the plant species (Corbett, 1897). In the present investigation the different stages of rooting especially the root emergence of *Gymnema* were almost in conformity with the findings of White and Lovell (1984) in *Agathis australis* Don. They found root initiation in 17-18 days, primordia formation within 20-21 days and root emergence on 27th day after planting.

The study also revealed that there was no anatomical differences among the different types of cuttings and also no anatomical barriers could be located.

Biochemical studies

In general cuttings with a high carbohydrate content root better than those with low carbohydrate content (Nanda and Kochhar, 1991). In the present study the better rooting efficiency of softwood cuttings may be attributed to their increased levels of carbohydrate content at all stages of root development. Hardwood cuttings on the other hand showed lower level of carbohydrate and hence a poor rooting efficiency. The carbohydrates and other such primary metabolites in the hardwood cuttings may have transformed into secondary metabolites like tannins, phenols, latex etc. which in turn have an inhibitory effect on rooting. The difference in the rooting ability of juvenile and adult cuttings had been ascribed to the difference in their nutritional status, especially of their carbohydrate content (Ali and Westwood, 1966).

The present study clearly showed an increase in the carbohydrate content in the stem as well as leaf during root initiation upto primordia formation and a sudden decline in the levels of carbohydrate at root emergence. This was in confirmation with the studies of Davis and Potter (1981) and Spellengberg (1985). They found that a net accumulation of carbohydrates normally occurred until root emergence in cuttings. The cuttings use a portion of carbohydrates during root regeneration and emergence. This may be the reason for the decrease in carbohydrate levels at root emergence.

The nitrogen content showed a similar trend as that of the carbohydrates during different stages of rooting. Identical results were obtained by Basu *et al.* (1967) showing that total nitrogen in stem and bark decreased during root formation. The nitrogen content rose during initial stages, but fell before root emergence. Hambrick *et al.* (1985) had reported a negative correlation between nitrogen content and root emergence in stem cuttings of *Rosa multiflora*. Hartmann and Kester (1976) reported that root formation on cuttings is influenced by the nitrogen level in stock plants and a low level of nitrogen favoured rooting.

C/N ratio also revealed a similar variation as in the carbohydrates and N content during the different stages of rooting. The wide C/N ratio observed during the early stages of rooting narrowed down at the final stages of root emergence. Sen *et al.* (1965) had shown that a high C/N ratio was beneficial for rooting of cuttings. A positive correlation between C/N ratio and rooting is generally observed.

As in the case of N, the protein content also showed a gradual rise upto root primordia formation and then there was a fall at root emergence. It is likely that auxins initiate synthesis of structural proteins or enzymes in the process of adventitious root formation. Similar results have been reported by Sereena (1996). The results supported the observations of Knypl (1966) that protein synthesis is a pre-requisite for root formation.

Summary

SUMMARY

The investigations were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara from May 1997 to January 1998 to standardise the propagation through cuttings in *Gymnema sylvestre* R.Br. The salient findings of the investigations are summarised below:

Among the type of cuttings softwood cuttings performed better than semi-hardwood and hardwood cuttings.

Treatment with growth regulators in general resulted in better rooting efficiency when compared to untreated cuttings kept as control.

Among the growth regulators, IAA was superior to NAA and IBA.

Intermittent mist was found to significantly increase the rooting percentage of cutting and was a necessity for commercial propagation through cuttings in *Gymnema*.

The different factors of propagation under investigation viz., environment provided, treatment with hormones and types of wood selected were found to significantly interact with one another.

Among the interaction involving environment and type of wood, the softwood cuttings kept in mist chamber showed maximum rooting efficiency.

With respect to interaction involving type of cuttings and nature of hormone treatment, the highest rooting efficiency and other related root and shoot

characters were exhibited by softwood cuttings treated with IAA 500 mg l^{-1}, followed by softwood cuttings treated with NAA 500 mg l^{-1}.

Among the interactions involving environment and the type of hormone treatment, IAA treated cuttings kept in mist showed the maximum rooting efficiency.

When interactions involving all the three factors under study were taken together, softwood cuttings treated with IAA 500 mg l^{-1} and kept under mist showed the maximum rooting success (81%). Other qualitative characters related to root and shoot development also showed a similar result.

Presence of leaves was found to be a must for enhancing rooting efficiency and quality of roots in *Gymnema* cuttings.

Field establishment rate of rooted *Gymnema* cuttings were not influenced by the environment provided for rooting or the type of cuttings selected for rooting.

The anatomical studies of the cuttings showed that the root initials originated from the secondary phloem cells in *Gymnema sylvestre*.

The root initials developed 20 days after planting, the primordia formed 24 days after planting and the root emerged on the 27th day of planting.

There was hardly any anatomical difference among the different type of cuttings and also no anatomical barriers could be located.

Biochemical studies revealed that the content of carbohydrate, nitrogen and protein in the leaf as well as base of the stem of *Gymnema* at different stages of root development were influenced by environment, hormone treatment and type of wood selected for rooting.

Maximum content of these materials in leaf as well as stem at all stages of rooting were observed in softwood cuttings treated with IAA and kept under intermittent mist. Thus it is established that the rooting efficiency of the cuttings in *Gymnema* are positively correlated with the content of carbohydrate, nitrogen and protein in leaf as well as stem.

C/N ratio worked out showed that high ratio was positively correlated with treatments having maximum rooting efficiency.

The study also revealed that the carbohydrate content in all the three type of cuttings increased from root initiation to root primordia formation but reduced during root emergence. The N content, protein content and the C/N ratio also showed a similar trend.

References

REFERENCES

* Ali, C.N. and Westwood, M.N. 1966. Rooting of pear cuttings as related to carbohydrates, nitrogen and rest period. *Proc. Am. Soc. hort. Sci.*, **88**:145-150

Anu, K.I. 1993. Standardisation of *in vitro* propagation technique in *Gymnema sylvestre* R.Br. M.Sc.(Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur

Arumugam, R. and Kumar, N. 1980. Effect of leaves on rooting of stem cutting of bergamot mint (*Mentha citrata* Ehrh). *Indian Perfumer*. **24**(3):166-167

Basak, U.C., Das, A.B. and Das, P. 1995. Metabolic changes during rooting in stem cuttings of five mangrove species. *Plant Growth Regulation* **17**(2):141-148

Basu, R.N., Lahiri, B. and Sen, P.K. 1967. Biochemical changes during regeneration of roots in air layers of mango (*Mangifera indica* L.). *Curr. Sci.* **15**:413-415

Bavappa, K.V.A. and Gurusinghe, P. 1980. New technique for the rapid multiplication of pepper planting material. *Indian Cocoa Arecanut Spices J.* **3**(3):53-55

Chakrabarti, D. and Debnath, N.B. 1981. Isolation of gymnemagenin the sapogenin from *Gymnema sylvestre*. *J. Inst. Chemists (India)*. **53**(iv):155-158

Chandra, V. 1956. Studies on root development of *Rauvolfia serpentina*. *J. Sci. indus. Res.* **14**C:36

Chattopadhyay, R.R., Medda, C., Das, S., Basu, T.K. and Poddar, G. 1993. Hypoglycaemic and antihyperglycemic effect of *Gymnema sylvestre* leaf extract in rats. *Fitoterapia*. **64**(5):450-454

Chaturvedi, H.C., Misra, P. and Jain, M. 1984. Proliferation of shoot tips and clonal multiplication of *Costus speciosus* in long term culture. *Pl. Sci. Lett.* **35**:67-71

Chopra, R.N., Bose, J.P. and Chatterjee, N.R. 1928. A comment on the medicinal properties of *Gymnema sylvestre. Indian J. med. Res.* **16**(1):115

* Cooper, W.C. 1935. Hormones in relation to root formation on stem cuttings. *Pl. Physiology* **10**:789-794

* Corbett, L.C. 1897. *W. va. agric. Exp. Stat. Ann. Rept.* **9**:196-199

Davis, T.D. and Potter, J.R. 1981. Current photosynthate as a limiting factor in adventitious root formation in leafy pea cuttings. *J. Am. Soc. hort. Sci.* **106**:278-282

Dubois, M., Gilles, K., Hamilton, J.K., Rebers, P.A. and Smith, F. 1951. A colorimetric method for the determination of sugars. *Nature* **167**:167

Duraiswamy, P.I. and Arumugam, R. 1980. Preliminary studies on the rooting of geranium cuttings as affected by soil reaction. *Indian Perfumer.* **24**(3):168-169

El-Keltawi, N.E. and Croteau, R. 1985. Single node cuttings as a new method of mint propagation. *Scientia Hort.* **29**(1/2):101-105

* Erickson, L.C. and Bitters, W.P. 1953. Effect of various plant growth regulators and mist on rooting of cuttings of citrus and related species. *Proc. Am. Soc. hort. Sci.* **11**:84-86

Federer, W.T. 1977. Experimental Design: Theory and Application. Oxford and IBH Publishing Co., New Delhi

Fouda, R.A. and Schmidt, G. 1994. Rooting ability of Rosa hybrid cv. Red Success. *Kerteszeti Tudomany.* **26**(1):62-66

*Garner, R.J. 1944. Propagation by cuttings and layers. Recent work and its application with special reference to pome and stone fruits. *Tech. Commun. Imp. Bur. Hort. Plant. Crops* **14**:40

Gauniyal, A.K., Kumar, A. and Virmani, O.P. 1988. *Rauvolfia serpentina* - A review. *Curr. Res. med. arom. Pl.* **10**(3):113-137

Gautam, D.R. and Chauhan, J.S. 1990. Standardisation of rooting of wild olive cuttings under intermittent mist. *Indian J. Hort.* **47**(3):278-285

Haissig, B.E. 1982. Carbohydrate and amino acid concentrations during adventitious root primordium development in *Pinus banksiana* Lamb. cuttings. *For. Sci.* **28**:813-821

Haissig, B.E., Davis, T.D. and Sankhla, N. 1988. Adventitious root formation in cuttings. *Advances in Plant Sciences Series*. Dioscorides Press, Port land, Oregon, **2**:29-43

Hambrick, C.E., Davis, F.T. and Pemberton, H.B. 1985. Effect of cutting position and carbohydrate/nitrogen ratio on seasonal rooting of *Rosa multiflora. Hort. Sci.* **20**:570

*Hartmann, H.T. 1956. Rooting of cuttings under mist. *Calif. Agric.* **10**(5):7

Hartmann, H.T. and Kester, D.E. 1976. Plant Propagation - Principle and Practices. Prentice-Hall of India Pvt. Ltd., New Delhi

Hartmann, H.T., Kester, D.E. and Davis. F.T. 1993. Plant Propagation - Principles and Practices. Prentice Hall of India Pvt. Ltd. (Ed. 5). p.199-304

Hegde, G.S. 1983. Effect of number of nodes on cuttings and growth regulators on rooting of Panniyur-1 pepper (*Piper nigrum* L.) vine. Thesis Abstracts. Haryana Agriculture University, **9**(1);64

Hegde, S.S. 1988. Propagation studies in some ornamental shrubs by cuttings. M.Sc.(Agri.) thesis, University of Agricultural Science, Dharwad

* Heide, O.M. 1968. Auxin level and regeneration of Begonia leaves. *Planta* **81**:153-159

* Hitchcock, A.E. and Zimmermann, P.W. 1940. Effects obtained with mixtures of root inducing other substances. *Contr. Boyce Thompson Inst.* **2**:145-160

Hurov, R. 1967. The polythene bag method of rooting cuttings. *Plant Propagator* **13**(1):79

* Ivanova, Z.Y.Z. and Gladun, M.I. 1986. Propagation of essential oil rose by softwood cuttings. *Byulletin' Gosudarstvenogo Nikitskogo Botanicheskogo* Sada, **60**:72-76

Jackson, M.L. 1973. Soil Chemical Analysis. 2nd ed. Prentice Hall of India (Pvt.) Ltd., New Delhi, p.1-498

Kamaluddin, M. and Ali, M. 1996. Effects of leaf area and auxin on rooting and growth of rooted stem cuttings of neem. *New Forests* **12**(1):11-18

Kamei, K., Takano, R., Miyasaka, A., Imoto, T. and Hara, S. 1992. Amino acid sequence of sweet-taste - suppressing peptide (gurmarin) from leaves of *Gymnema sylvestre*. *J. Biochem.* **111**(1):109-112

* Kamineck, M. 1968. Dynamics of amino acids in pea stem sections during root formation and its inhibition by Kinetin and Methionine. *Biol. Pl.* **10**:462-471

Kirtikar, K.R., Basu, B.D. and An, I.C.S. 1975. Indian Medicinal Plants Phanerogamia. In: Blaster, K., Caius, J.F. and Mhaskar, K.S. (Ed.). Indian Medicinal Plants p.1624-1627

* Knypl, J.S. 1966. *Acta Soc. Bot. Pol.* **35**:357-373

Linder, R.C. 1939. Effects of indole acetic acid and naphthalene acetic acid on development of buds and roots in horse radish. *Bot. Gaz.* **100**:500-527

Manni, P.E. and Sinsheimer, J.E. 1965. Constituents from *Gymnema sylvestre* leaves. *Pharm. Sci.* **54**:1541-1544

Mhaskar, K.S. and Caius, J.F. 1930. A study of Indian medicinal plants. II. *Gymenma sylvestre* R.Br. *Indian med. Res. Memoris.* **16**:75

Mitra, S. and Kushari, D.P. 1985. Rooting *Solanum khasianum* stem cutting in relation to season, light and cutting age. *Plant Propagator* **31**(3):3-6

Miyatake, K., Takenaka, S., Fujimoto, T., Kensho, G. and Nakano, Y. 1993. Isolation of conduritol A from *Gymnema sylvestre* and its effects against intestinal glucose absorption in rats. *Bioscience-Biotechnology and Biochemistry* **57**(12):2184-2185

Modak, S.B., Hati, A.P., Biswas, N. and Basu, P.K. 1990. Effect of exogenously applied sucrose on rooting of *Adhatoda vasica* Nees. shoot cuttings. *Indian J. Forestry* **13**(1):43-45

Nambiar, P.K.V., Pillai, V.S. and Sasikumar, S. 1977. A note on rapid multiplication of Panniyur-1 pepper. *Arecanut Spices Bull.* **8**(3):57-58

Nambiar, P.K.V., Pillai, V.S., Sasikumar, S. and Chandy, K.S. 1978. Pepper Research at Panniyur - A Resume. *J. Plant. Crops.* **6**(1):4-11

Nanda, K.K. 1975. Anatomical changes during rooting. *Indian J. Pl. Physiol.* **18**:80-89

Nanda, K.K. and Anand, V.K. 1970. *Physiologia Pl.* **123**:99-107

Nanda, K.K. and Kochhar, V.K. 1991. Vegetative Propagation of plants. Kalyani Publishers, New Delhi, p.1-226

Nazeem, P.A., Sudhadevi, P.K., Joseph, L. and Geetha, C.K. 1991. *In vitro* callus production from leaves of *Gymnema sylvestre* R.Br. *Ancient Sci. Life* **11**(182):43-45

Noor-Aini, A.B. and Ling, T.T. 1993. A note on the rooting of *Shorea acuminata* and *Shorea parvifolia* leafy stem cuttings. *J. trop. Forest Sci.* **6**(2):206-208

Ota, M. and Ariyoshi, Y. 1995. Location of the disulphide bonds of the sweetness - suppressing polypeptide gurmarin. *Bioscience-Biotech. and Biochem.* **59**(10):1956-1957

Pal, M., Mishra and Bhandari, H.C.S. 1993a. Promotion of rooting of shoot cuttings of *Datura stramonium* by IBA. *Indian J. Forestry.* **80**(11):36

Pal, M., Goel, C.L. and Bhandari, H.C.S. 1993b. Effect of auxins on rooting branch cuttings of antamul (*Tylophora indica*). *Indian J. Forestry.* **16**(3):243-245

Panse, V.G. and Sukhatme, P.V. 1995. Statistical Methods for Agricultural Workers (4th ed.). Indian Council of Agricultural Research, New Delhi

Philip, J., Nair, G.S., Premalatha and Sudhadevi, P.K. 1991. Standardisation of vegetative propagation techniques in some of the medicinal plants grown in Kerala. *Indian Cocoa Arecanut Spices J.* **15**(1):12-14

Pillai, V.S., Ali, A.B.M. and Chandy, K.C. 1982. Effect of 3-indole butyric acid on root initiation and development of roots in stem cuttings of pepper. *Indian Cocoa Arecanut Spices J.* **6**(1):79

Rao, V.N. and Selvarajan, M. 1982. Studies on rooting of patchouli (*Pogostemon cablin*) cuttings under different environments. *South Indian Hort.* **30**(2):121

Roychoudhary, N. 1971. Amino acid metabolism during regeneration of roots in mango seedling cuttings. *Indian Agric.* **15**(2):95-102

* Sachs, J. 1882. *Arb. Bot. Inst. Warzburg,* **2**:452-488

Sahu, B.N. 1979. Rauvolfia's: Botany and Agronomy. Vol.I. Today and Tomarrow Printers and Publishers, New Delhi, p.595

Sahu, N.P., Mahato, S.B., Sarkar, S.K., Gurudas, P. and Poddar, G. 1996. Triterpenoid saponins from *Gymnema sylvestre* R.Br. Phytochemistry **41**(4):1181-1185

Sen, P.K., Sen, S. and Basu, R.N. 1965. Studies on rooting of cuttings. II effect of auxin. *Indian J. Pl. Physiol.* **8**:36-50

Sereena, J. 1996. Standardisation of propagation techniques in Baduvapuli (*Citrus pennivessiculata* Tan.). M.Sc.(Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur

Shanmugasundaram, E.R.B., Gopinath, K.L. and Rajendran, V.M. 1990. Possible regeneration of the islets of Langerhans in streptosotocin diabetic rats given *Gymnema sylvestre* leaf extracts. *J. Ethenopharmacol* **30**(3):265-279

Shanthamalliah, M.R., Sulladmath, U.V. and Krishnamurthy, K. 1974. Influence of maturity and time of planting on the rooting of stem cuttings of pepper (*Piper nigrum* L.). *Indian J. Hort.* **31**(3):250-254

Simpson, J.E., Adair, C.R., Kohler, G.O., Dawson, E.H., Debald, H.A., Kester, E.B. and Klick, J.T. 1965. Quality evaluation studies of foreign and domestic rices. *Tech. Bull.* **1331**:140-142

* Spellenburg, B. 1985. Verbesserung des Vermehrungserfolges beischwer vermchrbaren Laubgeholzen. Gartenbau-wissenschaft. **50**:71-77

Srivastava, Y., Jhala, C.I., Nigam, S.K., Kumar, A. and Bhatt, V.H. 1981. Experimental evaluation of hypoglycaemic effect of *Gymnema sylvestre* R.Br. in alloxan diabetic Charles Foster rats. *Diabetic. Assoc. India.* **21**(4):139-142

Srivastava, Y., Raval, B.H. and Verma, Y. 1988. *Gymnema sylvestre* R.Br. in diabetes mellitus. *Arogya J. Health Sci.* **16**:62-64

Sudhadevi, P.K. 1992. Ethnobotany of medicinal plants used by Tribes of Kerala. Ph.D. thesis, Kerala Agricultural University, Vellanikkara, Thrissur

Swamy, A.Y. and Kalyanasundaram, S. 1960. Propagation of *Pelargonium graveolens* Ait. by root suckers, single node stem cuttings and root cuttings. *South Indian Hort.* **11**(3&4):34-35

* Thimann, K.V. and Went, F.W. 1934. On the chemical nature of the root forming hormone. *Proc. Kon. Ned. Akad.* **37**:456-459

* Vardar, Y. 1968. Agents modifying longitudinal transport of auxin. Transport of Plant Hormones. North-Holland Pub. Amsterdam, Netherlands, p.156-191

White, J. and Lovell, P.H. 1984. Anatomical changes which occur in cuttings of *Agathis australis* Don. Lindl 2. The initiation of root primordia and early root development. *Ann. Bot.* **54**:633-645

Yoshikawa, K., Amimoto, K., Arihara, S. and Matsura, K. 1989. Gymnemic acid V, VI, VII from gur-mar, the leaves of *Gymnema sylvestre* R.Br. *Chem. pharm. Bull.* V.**37**(3):952-854

Yoshikawa, K., Nakagawa, M., Yamamoto, R., Arishara, S. and Matsuura, K. 1992. Antisweet natural products from *Gymnema sylvestre* R.Br. *Chem. pharm. Bull.* **40**(7):1779-1782

*Originals not seen

Appendices

APPENDIX-I

Meteorological data (monthly average) for the crop period

Month	Air temperature mean (°C)	Relative humidity (%)	Total rainfall (mm)	Mean sunshine hours
1997				
May	29.5	72.0	63.0	6.7
June	27.1	8.20	720.5	5.9
July	25.2	89.5	979.2	1.9
August	25.9	86.5	636.8	3.4
September	27.0	82.0	164.0	6.8
October	27.9	76.5	194.7	7.3
November	27.4	77.5	209.7	5.3
December	27.8	72.0	66.7	7.5
1998				
January	28.0	63.5	0	9.3

APPENDIX-II

Abstract of ANOVA

Influence of environment, hormone treatments, type of wood and their interaction effects on sprouting and rooting of cuttings

Source	Degrees of freedom	Mean squares				F values			
		No.of days to sprout	% sprouting	No.of days to root	% rooting	No.of days to sprout	% sprouting	No.of days to root	% rooting
Factor A	1	45.708	0.206	34.003	0.165	73.661**	120.146**	114.618**	122.620**
Factor B	3	8.328	0.198	2.610	0.219	13.422**	115.783**	8.798**	162.895**
A x B	3	1.256	0.008	1.366	0.007	2.024	4.709*	4.603*	5.263**
Factor C	2	78.797	0.730	2.453	0.679	126.986**	367.564**	8.267**	504.600**
A x C	2	0.982	0.031	0.401	0.037	1.583	17.987**	1.351	27.762**
B x C	6	1.040	0.042	0.289	0.047	1.676	24.728**	0.975	34.960**
A x B x C	6	1.476	0.006	0.273	0.005	2.378	3.367*	0.920	3.910**
Error	24	0.621	0.002	0.297	0.001				

A - Environment

B - Hormone treatments

C - Type of cuttings

* Significant at 5% level

** Significant at 1% level

APPENDIX-III

Abstract of ANOVA

Influence of different factors and their interactions on length of the new shoot

Source	Degrees of freedom	Mean squares			F values		
		Length of the new shoot			Length of the new shoot		
		1 MAP	2 MAP	3 MAP	1 MAP	2 MAP	3 MAP
Factor A	1	0.347	320.333	781.660	13.001**	97.712**	323.976**
Factor B	3	0.337	71.442	233.684	12.626**	21.792**	96.856**
A x B	3	0.021	14.367	1.171	0.188	4.382*	0.486
Factor C	2	15.805	1012.757	1482.788	592.514**	308.925**	614.574**
A x C	2	0.097	108.101	24.951	3.648*	32.974**	10.342**
B x C	6	0.123	19.240	58.100	4.597	5.869**	24.081**
A x B x C	6	0.013	6.894	6.166	0.482	2.103	2.556*
Error	24	0.027	3.278	2.413	0.001		

MAP - Months after planting

* Significant at 5% level

** Significant at 1% level

APPENDIX-IV

Abstract of ANOVA

Effect of different factors and their interactions on number of leaves

Source	Degrees of freedom	Mean squares			F values		
		Number of leaves produced			Number of leaves produced		
		1 MAP	2 MAP	3 MAP	1 MAP	2 MAP	3 MAP
Factor A	1	5.950	81.120	203.363	112.889**	90.301**	125.533**
Factor B	3	0.274	26.883	52.420	5.200**	29.926**	32.358**
A x B	3	0.018	2.000	0.574	0.341	2.226	0.355
Factor C	2	1.821	145.852	408.413	34.557**	162.359**	252.107**
A x C	2	0.414	14.342	27.563	7.854**	15.966**	17.014**
B x C	6	0.062	3.739	15.580	1.177	4.162**	9.617**
A x B x C	6	0.037	2.342	0.974	0.697	2.608*	0.602
Error	24	0.053	0.898	1.620			

MAP - Months after planting

* Significant at 5% level

** Significant at 1% level

APPENDIX-V

Abstract of ANOVA

Effect of different factors and their interaction on biometric characters of rooted cuttings

Source	Degrees of freedom	Mean squares				F values			
		No.of shoots	Diameter of vine	Root length	Root volume	No. of shoots	Diameter of vine	Root length	Root volume
Factor A	1	0.068	8.250	10.735	0.579	1.209	58.495**	13.848**	12.960**
Factor B	3	0.516	2.357	40.410	0.318	9.249**	16.714**	52.128**	7.114**
A x B	3	0.028	0.277	2.050	0.069	0.493	1.963	2.644	1.538
Factor C	2	0.843	6.385	145.994	1.561	15.090**	45.272**	188.329**	34.970**
A x C	2	0.018	0.815	0.640	0.128	0.313	5.780**	0.826	2.874
B x C	6	0.308	0.857	6.938	0.058	5.517**	6.079**	8.950**	1.303
A x B x C	6	0.027	0.282	0.385	0.005	0.493	1.999	0.496	0.110
Error	24	0.056	0.141	0.775	0.045				

A - Environment

B - Hormone treatments

C - Type of cuttings

** Significant at 1% level

APPENDIX-VI

Abstract of ANOVA

Effect of different factors and their interaction on stem characters of rooted cuttings

Source	Degrees of freedom	Mean squares			F values		
		Stem fresh weight	Stem dry weight	Driage percentage	Stem fresh weight	Stem dry weight	Driage percentage
Factor A	1	0.209	0.027	0.000	2.668	3.974	6.636*
Factor B	3	0.652	0.112	0.002	8.303**	16.740**	56.424**
A x B	3	0.037	0.004	0.000	0.469	0.529	0.453
Factor C	2	4.438	0.533	0.003	56.552**	79.593**	91.536**
A x C	2	0.114	0.008	0.000	1.446	1.122	2.092
B x C	6	0.146	0.021	0.000	1.862	3.139*	5.021**
A x B x C	6	0.005	0.002	0.000	0.063	0.289	5.487**
Error	24	0.078	0.007	0.000			

A - Environment
B - Hormone treatments
C - Type of cuttings

* Significant at 5% level
** Significant at 1% level

APPENDIX-VII

Abstract of ANOVA

Effect of different factors and their interaction on leaf characters of rooted cuttings

Source	Degrees of freedom	Mean squares			F values		
		Leaf fresh weight	Leaf dry weight	Driage percentage	Leaf fresh weight	Leaf dry weight	Driage percentage
Factor A	1	0.209	0.013	0.001	0.134	0.950	75.277**
Factor B	3	1.838	0.223	0.001	8.653**	16.895**	71.866**
A x B	3	0.021	0.002	0.000	0.097	0.129	8.719**
Factor C	2	14.835	1.272	0.001	69.825**	90.581**	90.642**
A x C	2	0.688	0.071	0.000	3.237	5.036*	8.538**
B x C	6	0.201	0.031	0.000	9.947	2.186	15.890**
A x B x C	6	0.102	0.004	0.000	0.481	0.269	4.256**
Error	24	0.212	0.014	0.000			

A - Environment

B - Hormone treatments

C - Type of cuttings

* Significant at 5% level

** Significant at 1% level

APPENDIX-VIII

Abstract of ANOVA

Effect of different factors and their interaction on root characters

Source	Degrees of freedom	Mean squares			F values		
		Root fresh weight	Root dry weight	Driage percentage	Root fresh weight	Root dry weight	Driage percentage
Factor A	1	0.279	0.037	0.000	3.827	5.078*	0.081*
Factor B	3	0.415	0.075	0.004	5.691**	10.223**	58.016**
A x B	3	0.017	0.005	0.001	0.232	0.696	15.361**
Factor C	2	3.575	0.471	0.004	49.025**	63.906**	61.053**
A x C	2	0.077	0.014	0.001	1.050	1.956	19.339**
B x C	6	0.033	0.005	0.001	0.450	0.668	9.926**
A x B x C	6	0.014	0.002	0.001	0.192	0.333	8.456**
Error	24	0.073	0.007	0.000			

A - Environment

B - Hormone treatments

C - Type of cuttings

* Significant at 5% level

** Significant at 1% level

APPENDIX-IX

Abstract of ANOVA

Effect of different factors and their interaction on carbohydrate content of leaf and stem at different stages of rooting

Source	Degrees of freedom	Mean squares						F values					
		R.I.		R.P.F.		R.E.		R.I.		R.P.F.		R.E.	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Factor A	1	0.000	0.001	0.000	0.002	0.000	0.000	13809.967**	20341.556**	2111.024**	45186.036**	84.762**	4547.272**
Factor B	3	0.000	0.000	0.003	0.003	0.001	0.001	20599.886**	8776.970**	86710.878**	82429.797**	19419.311**	16145.065**
A x B	3	0.000	0.000	0.000	0.000	0.000	0.000	359.132**	1584.048**	5118.289**	4442.207**	3644.891**	989.425**
Factor C	2	0.001	0.001	0.002	0.001	0.000	0.000	28323.543**	15915.602**	51679.565**	32218.680**	2991.147**	11395.755**
A x C	2	0.000	0.000	0.000	0.001	0.000	0.000	8695.806**	1995.747**	6679.279**	16671.792**	8161.939**	8190.194**
B x C	6	0.000	0.000	0.000	0.000	0.000	0.000	4082.530**	1874.496**	9086.113**	8564.434**	2469.607**	3605.086**
A x B x C	6	0.000	0.000	0.000	0.000	0.000	0.000	2313.106**	2862.903**	6274.609**	6204.661**	3305.619**	2017.272**
Error	24	0.000	0.000	0.000	0.000	0.000	0.000						

A - Environment

B - Hormone treatments

C - Type of cuttings

** Significant at 1% level

APPENDIX-X

Abstract of ANOVA

Effect of different factors and their interactions on protein content of leaf and stem at different stages of root development

Source	Degrees of freedom	Mean squares						F values					
		R.I.		R.P.F.		R.E.		R.I.		R.P.F.		R.E.	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Factor A	1	0.000	0.000	0.000	0.000	0.001	0.002	14.384**	1564.054**	206.721**	4156.412**	4686.819**	9139.816**
Factor B	3	0.000	0.000	0.000	0.000	0.002	0.000	70.894**	1461.766**	634.674**	1156.766**	8605.686**	954.016**
A x B	3	0.000	0.000	0.000	0.000	0.000	0.000	45.139**	941.212**	215.043**	1657.861**	102.135**	328.575**
Factor C	2	0.001	0.009	0.002	0.011	0.002	0.007	645.428**	66770.052**	13777.783**	115189.295**	12830.377**	27171.159**
A x C	2	0.000	0.001	0.000	0.001	0.000	0.001	88.446**	3848.500**	1079.022**	6588.056**	640.694**	2913.951**
B x C	6	0.000	0.000	0.000	0.000	0.000	0.000	6.832**	228.945**	628.135**	730.843**	641.446**	356.448**
A x B x C	6	0.000	0.000	0.000	0.000	0.000	0.000	51.958**	1273.438**	958.210**	1754.731**	755.385**	312.170**
Error	24	0.000	0.000	0.000	0.000	0.000	0.000						

A - Environment

B - Hormone treatments

C - Type of cuttings

** Significant at 1% level

APPENDIX-XI

Abstract of ANOVA

Effect of different factors and their interaction on the nitrogen content of leaf and stem at different stages of rooting

Source	Degrees of freedom	Mean squares						F values					
		R.I.		R.P.F		R.E.		R.I.		R.P.F.		R.E.	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Factor A	1	0.000	0.000	0.000	0.000	0.000	0.000	14.375**	1551.076**	210.668**	4130.778**	4727.058**	9121.962**
Factor B	3	0.000	0.000	0.000	0.000	0.000	0.000	71.853**	1458.978**	638.306**	1152.383**	8618.035**	954.097**
A x B	3	0.000	0.000	0.000	0.000	0.000	0.000	45.757**	940.631**	215.798**	1655.918**	1029.237**	328.256**
Factor C	2	0.000	0.001	0.000	0.002	0.000	0.001	654.623**	66801.772**	13775.145**	115211.216**	12905.469**	27168.824**
A x C	2	0.000	0.000	0.000	0.000	0.000	0.000	89.398**	3828.991**	1085.437**	6541.185**	652.338**	2897.687**
B x C	6	0.000	0.000	0.000	0.000	0.000	0.000	7.013**	227.343**	628.303**	725.870**	642.295**	357.871**
A x B x C	6	0.000	0.000	0.000	0.000	0.000	0.000	52.408**	1274.241**	957.412**	1756.071**	760.059**	312.447**
Error	24	0.000	0.000	0.000	0.000	0.000	0.000						

A - Environment

B - Hormone treatments

C - Type of cuttings

** Significant at 1% level

APPENDIX-XII

Abstract of ANOVA

Effect of different factors and their interaction on carbohydrate content of leaf and stem at different stages of rooting

Source	Degrees of freedom	Mean squares						F values					
		R.I.		R.P.F.		R.E.		R.I.		R.P.F.		R.E.	
		Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem
Factor A	1	0.272	6.335	0.336	0.131	2.991	532.053	103.297**	84.424**	904.393**	2.769**	5290.581**	5070.977**
Factor B	3	1.789	130.479	5.375	217.293	2.451	112.764	680.221**	1738.954**	12603.869**	4581.605**	4335.042**	1074.751**
A x B	3	0.174	51.855	0.069	35.821	0.643	53.919	66.195**	691.099**	160.963**	755.282**	1138.006**	513.903**
Factor C	2	6.810	5227.985	13.247	6104.681	9.887	3115.068	2589.497**	69675.545**	31065.153**	128716.678**	17487.428**	29689.580**
A x C	2	0.517	145.173	0.321	150.108	1.121	86.679	196.754**	1934.786**	752.844**	3165.003**	182.149**	826.130**
B x C	6	0.269	13.647	0.522	34.541	0.189	85.895	102.184**	181.876**	1223.740**	728.301**	334.957**	818.660**
A x B x C	6	0.336	83.884	0.719	58.724	0.243	51.058	127.656**	1117.959**	1687.075**	1238.192**	430.294**	486.632**
Error	24	0.003	0.075	0.000	0.047	0.001	0.105						

A - Environment

B - Hormone treatments

C - Type of cuttings

** Significant at 1% level

APPENDIX-XIII

Abstract of ANOVA

Effect of different factors and their interaction on the rootings of leafy and leafless cuttings

Source	Degrees of freedom	Mean squares						F values					
		R.I.		R.P.F.		R.E.		R.I.		R.P.F.		R.E.	
		Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless	Leafy	Leafless
Factor A	1	0.206	0.120	0.165	0.098	11.702	11.408	120.146**	87.264**	122.620**	90.153**	14.109**	39.621**
Factor B	3	0.198	0.103	0.219	0.092	39.746	11.108	115.783**	74.869**	162.895**	85.109**	47.923**	38.581**
A x B	3	0.008	0.006	0.007	0.005	1.811	0.396	4.709**	4.572*	5.263**	4.853**	2.183	1.375
Factor C	2	0.630	0.350	0.679	0.366	149.029	39.162	367.564**	255.008**	504.600**	337.781**	180.051**	136.018**
A x C	2	0.031	0.025	0.037	0.023	0.801	1.131	17.987**	18.023**	27.762**	21.024**	0.965	3.927*
B x C	6	0.042	0.010	0.047	0.013	6.527	1.951	24.728**	7.621**	34.960**	11.972**	7.869**	6.775**
A x B x C	6	0.006	0.002	0.005	0.001	0.331	0.289	3.367*	1.117	3.910**	1.302	0.399	1.004
Error	24	0.002	0.001	0.001	0.001	0.829	0.288						

A - Environment

B - Hormone treatments

C - Type of cuttings

** Significant at 1% level

APPENDIX-XIV
Abstract of ANOVA
Effect of leafiness on the rooting of cuttings

Source	Degree of freedom	Mean squares			F values		
		% sprouting	% rooting	Length of longest root	% sprouting	% rooting	Length of longest root
Leafiness	1	0.399	0.370	0.306	9.919**	8.905**	185.96**
Error	94	0.040	0.042	0.002			

** Significant at 1% level

APPENDIX-XV

Abstract of ANOVA

Effect of environment, type of wood and their interaction on field establishment rate of rooted cuttings

Source	Degree of freedom	Mean squares	F values
Factor A	1	0.107	4.1309
Factor B	2	0.002	0.0639
A x B	2	0.002	0.0639
Error	6	0.026	

Factor A - Environment

Factor B - Type of wood

STANDARDISATION OF PROPAGATION THROUGH CUTTINGS IN *Gymnema sylvestre* R. Br.

By
D. RAJESH

ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Science in Horticulture

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ABSTRACT

Investigations were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara to standardise the propagation through cuttings in *Gymnema sylvestre* R.Br., a much sought rare medicinal plant most valued for its hypoglycaemic property.

Being a difficult-to-root species through cuttings, the study included vegetative propagation measures with treatment combinations involving two different environments, four different growth regulator treatments and three different types of vines.

The investigation conclusively proved that softwood cutting comprising of terminal ends and young lateral shoots is the ideal propagule in *Gymnema*. Intermittent mist was found to significantly increase the rooting efficiency. Treatment with growth regulators in general gave higher rooting success and among the growth regulators IAA was found to be superior than NAA and IBA.

Significant interaction effect was noticed among different treatments. The softwood cuttings treated with IAA 500 mg l^{-1} and kept in mist showed maximum synergetic effect with regard to rooting success and other qualitative root characters. It was followed by softwood cuttings treated with NAA 500 mg l^{-1} and kept in mist.

The study also showed that presence of leaves was essential for enhancing rooting efficiency in *Gymnema* cuttings. The rooted cuttings did not significantly differ in their field establishment rate irrespective of the type of cuttings or the environment provided for rooting.

The root initials were found to be originating from secondary phloem cells. There was hardly any anatomical difference among the different type of cuttings. No anatomical barriers for rooting could be located. Biochemical analysis revealed that the carbohydrates, nitrogen, protein content and C/N ratio increased during root initiation and primordia formation but decreased during emergence of roots.