CHARACTERIZATION OF DIOECY AND STANDARDIZATION OF PROPAGATION THROUGH CUTTINGS IN ALLSPICE (*Pimenta dioeca* (L.) Merr.)

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "**Characterization of dioecy and standardization of propagation through cuttings in allspice [*Pimenta dioeca* (L.) Merr.]**" 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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CERTIFICATE

Certified that the thesis entitled "**Characterization of dioecy and standardization of propagation through cuttings in allspice [*Pimenta dioeca* (L.) Merr.]**" is a record of research work done independently by **Ms.V.S.Sreeja,** 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 her.

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V.S. Sreeja

Affectionately dedicated to

my

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CONTENTS

Introduction

INTRODUCTION

Allspice [*Pimenta dioeca* (L.) Merr.] is a small evergreen spice which still remains as an underexploited crop plant in India. Albeit a native of West Indies, allspice is extensively grown in Jamaica, Mexico, Honduras, Guatemala and Costa Rica. The world annual production of allspice is estimated to be about 4000 tonnes.

The spice takes its popular name "allspice" from its resemblance in flavour and taste to a mixture of other tree spices such as cinnamon, clove and nutmeg. Allspice is also known as "Jamaican pepper" because of its popularity in Jamaica and the mature fruits resemble the green berries of black pepper.

Pimento of commerce is the dry unripe fruits of *Pimenta dioeca*. Pimento is marketed in the form of dry berries and after extraction as oil and oleoresin. The fresh leaves are also used for extraction of oil. The yield of oil ranges from 0.7 to 2.9 per cent from dry leaves whereas from berries it varies from 3 to 5 per cent. There are selections which give oil as high as 16 per cent.

Pimento is widely used in flavouring food products like fish, beef, chicken, pork, soup and puddings. Flavouring fish preparations with pimento is popular in USA. Though the spice is popular in American cuisine, it is yet to find a prominent place in Indian food preparations.

The commercial cultivation of the crop in India is handicapped due to various reasons. Dioecy, long juvenile phase and lack of viable vegetative propagation method are highlighted as the major problems in taking up commercial cultivation of allspice.

Allspice is polygamodioecious in nature i.e., the plants are structurally hermaphrodite but functionally dioecious. The sex of the plant cannot be determined until flowering, which normally takes 8 to 10 years. Hence, this phenomenon causes considerable loss in time and resources to the farmer. Presently, the only option to overcome dioecy is the use of vegetatively propagated material or top working the excess male plants. Eventhough approach grafting in allspice has been reported to be successful in Jamaica (Chapman, 1965), this method could not yield any successful result in India. Seedlings continue to be the major propagating material of allspice in India. However, preliminary observations at the Regional Agricultural Research Station, Ambalavayal give encouraging results with respect to stem cuttings in allspice.

The need of the hour is a definite methodology for identifying the sex of the plant in the early stage of growth and a successful method of vegetative propagation for the large scale multiplication of superior types, which may pave way to overcome the problems due to dioecy. Considerable work has been done in this respect in other dioecious crops but not much work has been reported in allspice. The investigations reported herein envisaged a comprehensive study to characterize the dioecious nature of allspice through morphological and biochemical means. Another objective of the study was to standardize the vegetative propagation technique through stem cuttings.

Review of Literature

2. REVIEW OF LITERATURE

Allspice (*Pimenta dioica* L.), a small evergreen tree spice is a member of the family Myrtaceae to which another important tree spice clove also belongs. The crop is commonly known as allspice as its fruits and leaves represent a mixture of flavour of cinnamon, clove and nutmeg. Pimento is also known as Jamaican pepper. The world annual production of allspice is about 4000 tonnes (Rema and Krishnamoorthy, 1991).

The dried unripe fruit is the spice of commerce, either ground, solvent extracted or distilled to give pimento berry powder, oleoresin and oil respectively. Albeit leaves also yield essential oil, they are seldom distilled since the recovery of oil is very low often below economic level. The major use of pimento in the ground state is as a flavouring and curing agent in processed meats, bakery products and in domestic culinary. It is also mixed with other ground spices for culinary uses.

The synonyms are *Myrtus pimenta* L., *M. dioica* L., *Eugenia pimenta* DC and *Pimenta officinalis* Lindl.

2.1 Description

Allspice usually attains a height of 7-10 m, sometimes reaching up to 15 m (CSIR, 1956). The trunk is slender and upright with primary branches formed 1-3 m above the ground. The secondary branches are profusely branched at their extremities (Purseglove *et al.*, 1981).

The leaves borne in clusters at the end of the branches are simple, opposite, entire thinly coriaceous, punctuate with pellucid glands and are aromatic when crushed (Purseglove *et al.*, 1981). The leaves contain an essential oil. Rodriquez (1969) have reported yields of 0.35 to 1.25 per cent from fresh leaves by steam distillation with phenol content of oil ranging from 80 to 86 per cent in high grade oils and as low as 52 per cent in low grade oils.

The flowers are structurally hermaphrodite but functionally dioecious in which fruiting trees are functionally female (Chapman, 1964, 1965). The whitish aromatic flowers are borne in stalked many flowered cymes and branches trichotomously. The flowers measure 5-15 cm in length with 8-10 mm diameter. The pedicel is about 1 cm long, pale green and pubescent with small brownish pubescent bracteoles. The calyx is formed on the receptacle which is persistent on the fruit. Petals are white 3-4 mm long and are quickly deciduous. The number of stamens are about 100 in male and 50 in bearing trees. The style is white, shortly pubescent with a small terminal yellow stigma.

The fruit is a sub-globose berry, 4-6 mm in diameter. It matures 3-4 months after flowering. The fruits normally contain two kidney shaped seeds surrounded by a pericarp and separated by a thin membrane. The male barren trees occasionally produce single seeded fruits.

2.2 Dioecy in allspice

Allspice is a typical polygamodioecious tree ie. the plants are structurally hermaphrodite but functionally dioecious in which, there are barren trees which do not produce fruits and are functionally male and fruiting trees which are functionally female (Purseglove *et al.*, 1981).

This character makes it difficult for the identification of functional male and female till it comes to bearing. Generally the tree flowers in 6-7 years after which the males are thinned to have a proportion of 1 male : 10 females which is adequate for pollination (Nel, 1994). It is also noticed that the male trees flower earlier than females (Krishnamoorthy and Rema, 1991). Males continue to flower longer than females (Kanthaswamy and Pillai, 1995). Chapman (1964, 1965) recognised two different sexes, functionally male and a functionally female sexes in allspice. Chapman (1965) has shown that both these forms are visited by domestic honey bees and small wild bees and suggests that floral synchrony must occur with simultaneous flowering of male and female trees to ensure pollination. Mayer and Charlesworth (1992) have reported that males and females have large and presumably resource - costly opposite sex structure that are sterile which is termed as cryptic dioecy. Most of the androdioecious plants with staminate flowers co-exist in a population with hermaphrodite plants having non-functional anthers.

2.2.1 Dioecy in other crops

Dienum (1931) has shown that in dioecious crop like nutmeg, on an average 55 per cent of the seeds from a female plant will give rise to female, 40 per cent male and 5 per cent bisexual. Studies on flowering pattern in nutmeg by Nazeem *et al.* (1981) revealed that the male and female trees showed variation in flowering. In females, flowering was confined to seven months whereas in males flowering was observed throughout the year. Maximum flowering in both cases occurred in July followed by October.

Flach (1996) has observed that female trees of nutmeg had a significantly higher stem diameter during the pre-bearing stage while no significant difference in terms of plant height was observed. Thomas (1997) has reported that the average leaf width was found to be larger in female plants compared to male plants of nutmeg tree. The length by width ratio of leaves was found to be larger in male plants than in female plants. In the case of leaf characters, elliptic and oblanceolate leaves were observed in the male and female plants. No significant differences were noticed between male and female plants for the morphological characters such as plant height spread, collar girth, height at branching and number of main branches in one metre height.

Longo *et al.* (1996) have reported that male and female flowers of *Asparagus officinalis* at early stages of development posses both carpels and stamens. The transition from the pathway starts at an earlier stage in male flowers than in female flowers.

Knapp *et al.* (1998) have recently shown that in genus *Solanum*, functionally male flowers have normal anther, normal pollen and reduced stigma whilst functionally female flowers have stigma and anthers that appear normal but contain nonfunctional, usually imperturate pollen.

2.3 Determination of sex in allspice

Eventhough many works have been done, no method has so far been standardised for identifying the sex of allspice plants. At the same time a number of workers have devised different methods in other dioecious plants.

Janse (1898) stated that male trees of nutmeg had smaller leaves and less horizontal branches. Prestoe (1948) had reported that leaves of female trees of nutmeg which were less than 30 cm high would be nearly elliptical with more or less straight veins, whereas male trees possessed nearly obovate leaves with their veins running to the more pronounced point of the leaf. Flach (1966) observed a slight difference in the tree size between female and male trees of nutmeg.

Methods used by Flach (1966) to determine sex linked chromosomes did not reveal a heteromorphic chromosome pair in nutmeg. No difference in the number, shape and size of chromosome was noticed in mitosis of trees of different sexes studied by him.

Phadnis and Chaudhari (1971) reported difference in the colour reaction of leaf extracts of male and female plants of nutmeg with ammonium molybdate reagent. They observed the development of a faint green colour for male plant and sea green colour for females.

Nair *et al.* (1976) postulated a method for distinguishing the sex of nutmeg by the shape of calcium oxalate crystals in the lower epidermal cells of leaves of plants of at least two year old plants. Male plants contain a single larger rhomboidal or prismatic crystal with rectangular or square flat faces. Female plants had a cluster of small crystals. They have also reported that the number of flowers produced per unit area in males is three times more than that of female plants. Thomas (1997) is of the opinion that the crystal pattern was not found to be a reliable indicator of sex because both in male and female plants, rhomboidal and clustered crystal pattern was observed.

Krishnamoorthy *et al.* (1992) have studied the reliability of some of the earlier reported methods to identify the sex in nutmeg at seedling stage. They observed that the crystal pattern of calcium oxalate was well differentiated in 70 per cent males and 78 per cent females. In seedlings, the crystal pattern was not definite. Thomas (1997) has revealed that on colour reaction of ammonium molybdate with leaf extracts of male and female plants both male and female plants showed dark green and light green colour.

Bojappa and Singh (1974) have conducted chemical tests based on pH of leaf extracts in papaya seedlings and obtain 47 and 95 per cent accuracy for male and female plants respectively. Studies by Singh *et al.* (1977) have revealed that sex identification is possible based on the seed colour. Dark seed colour is associated with a high proportion of female plants and most seeds of female groups germinated within 20 days of sowing.

Rao *et al.* (1985) have shown through colorimetric tests in papaya that Remodified Almen Reagent could correctly identify 92.5 per cent female plants and

70 per cent of males. Rojast *et al.* (1985) have stated that there is significant differences between the male and female plants of papaya only during flowering. The female plants are taller with more leaves and flower later than males.

Studies by Lesham and Ophir (1977) in carob and datepalm have shown that a clear correlation exists between female growth and high GA activity.

Studies by Gauda *et al.* (1991) indicated that the average number of days taken for flower bud development from visible initiation to full bloom was 7 and 9.5 days respectively for male and female plants of *Bursea delpichiana.*

Sherly (1994) opined that in *Garcinia cambogia* the colour of emerging leaves exhibit marked difference among male and hermaphrodite trees. In male trees the emerging leaves will be light green in colour while it will be pinkish red in hermaphrodite trees.

Mathew *et al.* (1996) has stated that there was significant variation in length and breadth of the leaves of male and female garcina trees. Difference in leaf breadth was more conspicuous than leaf length and hence leaf breadth can be taken as one of the identifying characters for distinguishing the female trees from the male.

2.4 Influence of sex on morphological characters

A number of studies have been done to confirm the differences in the morphological traits of male and female plants of different dioecious species. These morphological characters can be used as sex markers and have been investigated by many.

Significant differences were observed in the internodal length, growth rate and plastochron of male and females of hemp, *Cannabis sativa* (Lacombe, 1980). He has also reported that sex of the plant could be determined at the age of 15 days after germination based on the early vegetative characters.

In both *Morus alba* and *Ficus carica* the females had larger leaves and denser spreading crowns. Leaf fall began earlier in males than in females (Kotaeva *et al.*, 1982). Chen *et al.* (1985) reported 100 per cent correlation between sex and length by width (LIW) ratio of leaves of jojaba. According to him, length by width ratio of all females will be more than average. The males register only a smaller L/W ratio. Oyama (1990) has shown that in the dioecious palm *Chamaedorea tepijelote*, the male plants showed spatial variation in growth rate but not the females. Both sexes had different rate of production of leaves among years and males produced significantly more inflorescence than females. Kohorm (1994) has reported that the females of jojaba, *Simmondsia chinensis* a dioecious desert shrub were found to have larger leaves and more open canopies.

A study of the male and female plants of a forest tree species odum (*Milicia excelsa*) by Nyong *et al.* (1994) revealed that the females possess more spreading crown and thicker stem than the males.

In the dioecious perennial *Asparagus officinalis* the male plants produced more thinner stems than the females (Machon *et al.*, 1995). Earlier studies in the same plant showed that staminate plants had more whorls per unit of branch than pistillate plants while the latter had a greater leaf area per cladophyll (Benson, 1982). Mathew *et al.* (1996) have shown in *Garcinia cambogia* that the female trees had a higher leaf area value than males.

2.5 Influence of sex on biochemical characters

2.5.1 Carbohydrates and sex expression

Choudhri *et al.* (1957) have reported that the leaves of male papaya plants were richer in total carbohydrate than the females. Another study by Prasad and Iyengar (1982) showed that the leaves of female plants of jojoba, *Simmondsia chinensis* had a higher carbohydrate than the males.

2.5.2 Amino acids and Proteins

McDaniel *et al.* (1979) analysed the leaf protein from individual plants of jojoba by PAGE and reported that the male and female plants were readily distinguishable due to quantitative and qualitative differences in fast migrating proteins. Meige (1981) has reported that the tubers from male and female plants of *Dioscorea opposita* could be differentiated by electrophoresis of albumin present in it.

Khan *et al.* (1982) have shown that in papaya, the level of amino acids was similar in both sexes except for proline which was present in female plants only and cysteine which was absent in female plants. The level of tryptophan in female plants was twice as high as in male plants.

Studies on the protein content of male and female papaya by Dutta and Majumdar (1989) revealed a higher protein content in female plants regardless of plant part examined.

Kovaleva *et al.* (1980) analysed proteins in the stem apices of dioecious hemp by immuno - electrophoretic method and reported a protein unique to female plants. Talyshinskii (1982) reported greater electrophoretic mobility for proteins in the leaves of polyploid male forms of mulberry than in females.

2.5.3 Phenols and sex expression

The phenolics have a pioneer role to play in giving hormonal balance, disease resistance and protection of injured tissue from infection (Crompton and Preice, 1986). Phenolics in high concentrations are toxic to plant cells themselves

(Tepper and Anderson, 1984). Hence phenolics are present in plant in small quantities.

In general the number of phenolic compounds present and the content are found to differ in male and female plants of some dioecious species. Thomas (1997) has reported that the male plants of nutmeg were found to have slightly higher content of phenols than that of females. Studies by Singh *et al.* (1974) indicated that when the leaf extracts of *Carica papaya* were subjected to ten colourimetric tests specific to phenolics, the sex of the plant could be determined with an accuracy of 86 per cent using Folins Ciocaltean reagent.

She has also revealed that the phenol content was found to be lower in one year old plants than in two year old plants.

Jindal and Singh (1975) have reported that the amount of free, acid-hydrolysable and alkali-hydrolysable phenolic compounds were considerably higher in male plants of papaya. Another study by Bhattacharya and Rao (1982) indicated that by the application of growth regulators the males continued to have a higher phenol content except for TIBA (50-200 ppm). It was suggested that any treatment that reduces the phenolic content would enhance femaleness.

Billau *et al.* (1987) conducted High Performance Liquid Chromotography (HPLC) of phenolic compounds in storage roots of asparagus during vegetative period and found that male plants contained less caffeic acid and chelidonic acid and more coniferin than the female plants. Another study by Packiyasothy *et al.* (1991) revealed that the thin layer chromatographic profile of phenolics in leaves of adult male plants showed two additional phenolic compounds compared with that of female plants.

In the dioecious plant rosell, *Rumex acetosella* the content of hydroxy cinnamic acid (p-caumaric acid and ferrulic acid) and hydroxy benzoic acid (vanillic acid) were found to be more in the leaves and reproductive organs of male plants than the female plants (Dyurdevich *et al.*, 1992). Variyar and Bandyopadhyay (1995) have reported the presence of phenolic acids like protocatechnic, gallic and caffeic and vanillic acids in cinnamon, clove and nutmeg respectively.

2.5.4 Total nitrogen

Choudhari *et al.* (1957) have reported that the leaves of female plants of papaya were richer in total nitrogen. Basu *et al.* (1967) recorded that the total nitrogen in the bark and wood decreased during root formation.

2.5.5 Essential oil content of leaves and oleoresin

Studies by Thomas (1997) indicated that the essential oil content was higher in the leaves of female plants of nutmeg.

Veek and Russell (1973) established a firm identification for the presence of 23 different components in the essential oil of pimento leaf oil. Another study by Green *et al.* (1988) revealed that volatile oil content is lower in larger berries but its composition remains constant. In a related species of allspice *Pimenta jamaicensis* the foliar oil was found to be predominated by eugenol (61.79%) and limonene (10.33%) (Tucker *et al.*, 1992).

2.6 Propagation through stem cuttings

Allspice is propagated by seeds selected from ripe berries freed from the pulp and sown in nursery. The need of the hour is a definite methodology for a successful vegetative propagation for the large scale multiplication of superior types, which may pave the way to overcome dioecy. Very little work has been carried out on the vegetative propagation aspects of this valuable tree spice.

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

Purseglove *et al.* (1991) have reported that more investigations have to be done on rooting of cuttings in allspice and also on root stock effect of other related members of Myrtaceae as stock-scion incompatibility has been observed. Another study by Chapman (1965) revealed that vegetative propagation is possible by chip budding (30% success) and approach grafting (90% success). Semi-hardwood cuttings rooted in a period of 6-7 months. Bottle grafting technique did not show any success.

Studies by Halkandar *et al.* (1995) indicated that air layers on 11 year old allspice trees in each month during 1991 showed highest rate of success in January.

In *Rauwolfia serpentina* Chandra (1950) reported that the hardwood cuttings of size five to eight inches length produced roots within 15 days after planting with hormonal treatment. Sahu (1979) also has shown that 7.5 cm long stem cuttings with two buds was the best material for vegetative propagation and observed 60 per cent germination.

Gauniyal *et al.* (1988) suggested stem cuttings of 6-7 cm length with two buds as the best suited material for propagation of *R. serpentina* and the hardwood cuttings performed better than soft wood cuttings.

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

Nambiar *et al.* (1977) showed that for rapid multiplication of the hybrid pepper. 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.

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. The higher the number of nodes, the greater will be the rooting percentage and survival rate.

Sudhadevi (1992) compared the rooting behaviour of softwood and semi hardwood cuttings in *Coscinium fenestratum*. Semi hardwood cuttings recorded maximum sprouting compared to hardwood cuttings. She has also reported that in *Coscinium fenestratum*, the percentage of sprouting was only 40 in stem cuttings on 60^{th} 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.

2.6.1 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).

Vadivel *et al.* (1981) had reported that hardwood cuttings from four year old trees of cinnamon, *Cinnamomum zeylanicum* gave the best rooting percentage (45%) when treated with IBA at 2500 ppm. Higher concentration of these substances reduced rooting. Epicotyl grafting in nutmeg was standardised by Mathew and Joseph (1982).

Studies by Hegde (1989) on the effect of growth regulator and pre-girdling treatments on rooting of cinnamon air-layers revealed that girdling had no marked effect but NAA increased the number of primary roots and root length. Bhat *et al.* (1989) obtained highest average number of primary roots and the greatest cumulative length of primary roots (99 cm) with 1000 ppm IBA + 2500 ppm NAA treatment in *Cinnamomum zeylanicum.*

Rema and Krishnamoorthy (1993) have standardized a method of propagation by cuttings with the highest rooting percentage with IBA at 2000 ppm.

Another study by Rema and Krishnamoorthy (1994) has revealed that the best month for air-layering in cassia, *Cinnamomum aromaticum* is July.

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 percentage 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 medium 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 20^{th} and 90^{th} 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).

Founda 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).

2.6.2 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) had 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.

2.6.3 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 could be used for large scale propagation. Ivanova and Gladun (1986) had 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) had revealed that retention or removal of leaves had no influence on the rooting of cuttings in *Alstonia venenata.*

2.6.4 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) had recorded that in *Shorea parvifolia*, rooted terminal cuttings survived best in the field whereas the rooted basal cuttings showed the lowest survival rate.

2.7 Biochemical changes during rooting

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

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

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 lately 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 the protein synthesis was a prerequsite for root formation. New proteins were synthesised during root intiation.

A decrease in the total carbohydrate, polyphenols and an increase in total nitrogen were recorded in the gridded tissues of *Thespesia populnia* cuttings at the time of root initiation and development (Basak *et al.*, 1995).

3. MATERIALS AND METHODS

The investigations reported herein were conducted at the College of Horticulture, Vellanikkara and the Regional Agricultural Research Station (R.A.R.S.), Ambalavayal during 1997-1999.

Experimental material

The proposed studies were taken up as two separate experiments. 1. The characterisation of dioecy for which sex differentiated male and female allspice plants and seedlings at two different stages of maturity were selected at RARS, Ambalavayal. Twenty five plants each belonging to one and four year old seedlings were subjected to detailed biochemical analyses. The morphological observations relating to the pattern of growth as well as leaf characters were recorded for ten male and female trees under the age group of eight years (Plates 1 and 2). The biochemical status of the male and female trees were also studied. 2. The standardization of propagation for which stem cuttings from female plants were collected at monthly interval for a period of twelve months from April 1998 to March 1999 and induction of rooting was tried in the rooting medium consisting of sand, soil and farmyard manure (fym) in 1:1:1 proportion using different growth regulators under mist chamber.

Experiment I

A. Morphological characterisation

3.1 Mature trees

3.1.1 Plant characters

The following biometric observations on mature sex differentiated male and female trees were recorded:

Materials and Methods

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The proposed studies were taken up as two separate experiments. 1. The characterisation of dioecy for which sex differentiated male and female allspice plants and seedlings at two different stages of maturity were selected at RARS, Ambalavayal. Twenty five plants each belonging to one and four year old seedlings were subjected to detailed biochemical analyses. The morphological observations relating to the pattern of growth as well as leaf characters were recorded for ten male and female trees under the age group of eight years (Plates 1 and 2). The biochemical status of the male and female trees were also studied. 2. The standardization of propagation for which stem cuttings from female plants were collected at monthly interval for a period of twelve months from April 1998 to March 1999 and induction of rooting was tried in the rooting medium consisting of sand, soil and farmyard manure (fym) in 1:1:1 proportion using different growth regulators under mist chamber.

Experiment I

A. Morphological characterisation

3.1 Mature trees

3.1.1 Plant characters

The following biometric observations on mature sex differentiated male and female trees were recorded:

3.1.1.1 Height and spread of the plant

The height of the tree from ground level to the tip of the orthotropic growth was measured and expressed in metre. The maximum horizontal extension of branches in North-South and East-West directions was measured and their product gave a measure of the spread of the tree.

3.1.1.2 Canopy shape

The difference in the shape of the trees were observed visually and recorded as canopy shape.

3.1.1.3 Height at first branching

The height of the plant from ground level to the first branching point was measured in metre.

3.1.1.4 Number of main and secondary branches

The number of main and secondary branches starting from the first branching point of the plant was observed and recorded separately.

3.1.1.5 Leaf characters

From the selected male and female plants, twenty leaves each were selected and following leaf characters were recorded.

a. Number of leaves per flush

The number of leaves that developed at each flushing was recorded for twenty flushes per plant from all the four sides of the plant and average was worked out.

Plate 1. General view of experimental plot

Plate 2. Female tree in bloom

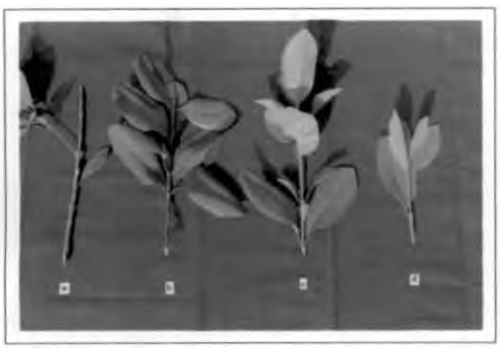


Plate 3. Cuttings with leaf

a. Hardwood b. Semihardwood
c. Softwood d. Seedling tip

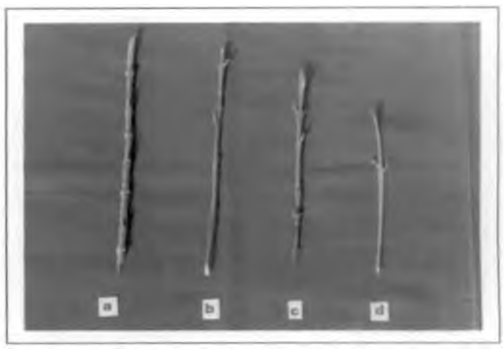


Plate 4. Cuttings without leaf

a. Hardwood b. Semihardwood
c. Softwood d. Seedling tip

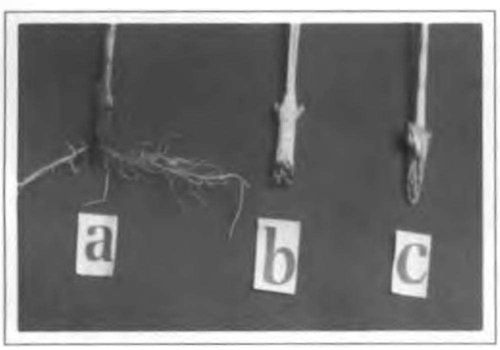


Plate 5. Rooted seedling tips

a. 4 MAP b. 2 MAP c. 1 MAP

Plate 6. Rooted seedling tips transplanted in polybags

b. Size, shape and colour of leaves

The comparative differences in size, shape and colour of leaves of male and female trees were observed visually and recorded. Accordingly, based on size, the leaves were classified as small, medium and large. The shape of mature leaves and colour of the flush were recorded as per the standard procedure.

c. Venation

The difference in the pattern of venation was recorded for male and female trees.

d. Petiole length

The petiole length was measured in centimetre and mean of 20 leaves was worked out.

e. Leaf area

The leaf area was worked out by multiplying the product of maximum length and width of the leaf with a correction factor. For assesing the leaf area five randomly selected leaves each from twenty male and female trees were used.

B. Biochemical characterization

Biochemical analyses were carried out at the Biochemistry laboratory of the College of Horticulture, Vellanikkara.

3.2 Mature plants

The following biochemical analyses with respect to the mature plants were done:

3.2.1 Estimation of total soluble carbohydrate

The total carbohydrate in leaf was estimated by the Phenol Sulphuric Acid method (Sadasivam and Manikam, 1992).

Hundred milligram of powdered leaf sample was taken into a boiling test tube. The content was hydrolysed by keeping the tube in a boiling water bath for three hours with 5 ml of 2.5 N hydrochloric acid and was cooled to room temperature. This was neutralised with solid sodium carbonate. The volume was made up to 100 ml and centrifuged. From this, 0.8 ml of the solution was taken and 1 ml of 5 per cent phenol and 5 ml of 96 per cent H_2SO_4 were added to the test tube and the contents were shaken well. After 10 minutes, the contents were mixed well by shaking once more and the test tube was placed in the water bath at 25-30°C for 20 minutes. Then the colour developed was read at 490 nm in a colorimeter.

Total soluble carbohydrate was calculated from a standard curve of glucose (100 mg in 100 ml) and was expressed in percentage.

3.2.2 Estimation of total phenols

Total phenols in the leaf was estimated by Folin Ciocalteau method (Sadasivam and Manikam, 1992).

Two grams of freshly collected leaf sample was crushed in a mortar and pestle in ten times volume of 80 per cent ethanol. The homogenate was centrifuged for 20 minutes at 10,000 rpm. The residue was again centrifuged twice with five times the volume of 80 per cent ethanol and the supernatent was pooled. This was evaporated to dryness and made up to 100ml with distilled water. From the made up solution, 1 ml was taken in a test tube and 0.5 ml of Folin-Ciocalteau reagent was added followed by 2 ml of Na_2CO_3 20 per cent after three minutes. The contents were mixed thoroughly by shaking and kept in a water bath for one minute. Then it was cooled and the absorbance was read at 650 nm, against a reagent blank in a spectrophotometer.

3.2.3 Estimation of total free amino acids

Total free amino acid was estimated by following the method suggested by Sadasivam and Manikam (1992).

One gram of leaf sample was ground in a mortar and pestle with a small quantity of acid washed sand and 10 ml of 10 per cent isopropanol. The homogenate was centrifuged and the filtrate was saved. The residue was once again centrifuged with 5 ml of 10 per cent isopropanol. The volume was made up to 50 ml and from this 0.8 ml of the extract was taken in a test tube. One ml of ninhydrin reagent solution was added to this and the volume was made up to 2 ml with distilled water. The tube was kept in a boiling water bath for 20 minutes. Five ml of diluent was added immediately and the contents were mixed well. After about 15 minutes, the intensity of the purple colour was read against a reagent blank in a colorimeter at 570 nm.

3.2.4 Estimation of total nitrogen

The total nitrogen content was estimated by Nesslers method as suggested by Snell and Snell (1967). Dried, powdered leaf sample (0.1g) was taken in a 100 ml standard flask and five ml of concentrated H_2SO_4 was added. It was heated for 10 minutes and digested with two ml of hydrogen peroxide. Excess of hydrogen peroxide was added to get a clear solution. This was made up to 100 ml. To five ml of sample solution one ml of 10 per cent sodium silicate and two ml of 10 per cent NaOH were added in a sequential manner and this was made up to 50 ml. Finally 1.6 ml Nesslers reagent was added to develop an unstable orange colour which was read in a spectronic 20 spectrophotometer at 410 nm.

3.2.5 C/N ratio

From the total soluble carbohydrates and total nitrogen content the C/N ratio was worked out.

3.2.6 Essential oil content of the leaf

Essential oil was estimated by clevenger apparatus. Leaf samples of 50 g each were collected from mature sex differentiated trees and seedlings of one and four year old seedlings for distillation. The oil was collected in the clevenger trap (for oils lighter than water) for four hour and expressed as percentage.

3.2.7 Oleoresin content of the leaves

Soxhlet extraction was carried out for the estimation of foliar oleoresin content. Methanol was used as the solvent.

Five grams of the finely powdered sample was taken in a filter paper thimble and soxhletted till the solvent became colourless. After extraction, the solvent was removed by vacuum drying and percentage recovery of oleoresin was calculated.

3.3 Chromatographic studies in mature plants and seedlings

3.3.1 Thin layer chromatography (TLC) of carbohydrates phenols and free amino acids

Thin layer chromatography was carried out to separate the biochemical constituents (Sadasivam and Manikam, 1992). Silica gel was used as the adsorbant. Leaf samples from sex differentiated male and female plants (8 year old) as well as from one and four year old seedlings were used for chromatographic studies.

a) Preparation of TLC plates

A slurry of silica gel was prepared by adding 60 ml of distilled water to 30 g of the absorbant. The slurry was stirred for 25 minutes and air bubbles were removed by vacuum system. Plates of 0.25 mm thickness were prepared by TLC plate preparation system. The plates were activated at 120°C for half an hour just before use. The plates were cooled at room temperature and used for sample analysis.

b) Screening for carbohydrate

Hundred mg of powdered leaf sample was extracted with five ml of distilled water. It was centrifuged and collect the supernatent. From the supernatent, 5 µl was spotted on the plate and eluted with the following solvent system and spray reagent. Solvent system - ethylacetate:isopropanol:water:pyridine in the ratio 26:14:7:2. Spray reagent containing five volumes each of 1 per cent aniline and 1 per cent diphenylamine in acetone and one volume of 85 per cent phosphoric acid were used for this experiment. The plate was air dried and placed in a chromatogram drying oven after spraying the reagent for development of spots.

c) Total phenols

Five ml each of ethanol extract of phenolics of one, four and eight year old male and female plants were taken separately. This was centrifuged and the supernatent was used for the analysis. From the sample, 5 µl was spotted on this plate and eluted with 10 per cent acetic acid in chloroform as the solvent system. The plate was sprayed with 20 per cent Folin Ciocalteau reagent and kept in a chromatogram drying oven for the development of the spots.

d) Total free amino acids

Separation was not clear by TLC and therefore paper chromatography was tried.

3.3.2 Paper chromatography

Free amino acids were detected by paper chromatography (Harborne, 1973). For sample analysis, Whatman No.1 filter paper was used. Vertical strips of 2.5 cm width was taken. The sample was spotted at 2.5 cm from the base and the strip was hung on a copper wire in such a way that the strips were straight without folds and the paper was in contact with following solvent system: Solvent system - Butanol:acetic acid:water in the ratio 40:10:50. Spray reagent - 0.3 per cent solution of ninhydrin in 97 ml butanol and 3 ml acetic acid.

The Rf values for all the above constituents were calculated as

$$\frac{\text{Distance moved by the spot}}{\text{Distance moved by the solvent}}$$

Experiment II

3.4 Propagation

The second part of the experiment was on standardization of propagation by using stem cuttings. The present studies were carried out at the Regional Agricultural Research Station, Ambalavayal during the period from April 1998 to March 1999.

Ambalavayal is situated at an elevation of 974 m above MSL at geographic location of 11° 37' N latitude and 76° 12' E longitude. Soil is loamy in nature and rich in humus.

The meteorological data for the experimental period as recorded at the Agrometeorological observatory of RARS, Ambalavayal are presented in Appendix I.

3.4.1 Type of cutting and its preparation

Stem cuttings from female plants were collected at monthly interval for a period of twelve months and induction of rooting was tried in the rooting medium consisting of sand, soil and fym in 1:1:1 proportion using different growth regulators under mist chamber.

3.4.2 Selection of trees and type of cuttings

High yielding female trees were selected for taking the cuttings. Softwood, semihardwood and hardwood cuttings having 4-5 nodes and 15 cm length were prepared (Plates 3 and 4). Both leafy and leafless cuttings were prepared under each type. Four leaves were normally retained for all the leafy cuttings. In addition to the above, based on the encouraging results obtained with seedling tips at RARS, Ambalavayal, one year old seedling tips were also used for rooting studies.

3.4.3 Preparation of the cutting

Softwood cuttings were prepared from the top succulent portion of the branches. The lateral shoots arising from the nodes and the axillary sprouts were also used for the purpose.

Semihardwood cuttings were prepared from the medium matured middle portion of the branches, leaving a few cm from the tip and the hardwood portion of the branch.

Hardwood cuttings were prepared from the mature and woody basal portion of the branch.

Tips of one year old seedlings having 10 cm length and 3 nodes were prepared with the leaves intact.

Number of cuttings per treatment were 75, of which twenty five were intended for destructive sampling.

3.4.4 Growth regulator treatment

3.4.4.1 Type of growth regulator

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

3.4.4.2 Preparation of different growth regulator solution

Two grams each of the growth regulators were taken and dissolved separately in one litre each of water to get a stock solution of 2000 ppm. From this 500 ml was taken and made up to 1000 ml to get a concentration of 1000 ppm. From the stock 300 ml was taken and was made up to 400 ml to get 1500 ppm solution.

The different treatment combinations were:				
	Softwood (ppm)	Semihardwood (ppm)	Hardwood (ppm)	Seedling tip (ppm)
IAA	1000	1500	2000	1000
IBA	1000	1500	2000	1000
NAA	1000	1500	2000	1000

Control with distilled water

3.4.5 Potting

Polythene bags of 15 cm x 8 cm size were provided with 20 punch holes for the excess water to drain out. Potting mixture consisting of sand, soil and fym in 1:1:1 proportion was prepared well in advance and the bags were filled with the potting mixture tightly without leaving any air space inside.

3.4.6 Method of growth regulator dip and planting of the treated cuttings

The prepared softwood, semihardwood, hardwood cuttings and seedling tips were given a slanting cut at the lower end and the cut end was dipped in the required concentration of growth regulator for 45 seconds, i.e., a quick dip treatment was given.

The growth regulator treated cuttings were planted immediately in the filled polythene bags. Planting was repeated on 14th of every month for a period of one year.

3.4.7 Environment provided

The cuttings were kept for rooting after labelling in the mist chamber provided with automatic misting facility. The misting was done at the rate of two minutes for every 15 minutes interval.

3.4.8 Layout of the experiment

The experiment was laid out in a Completely Randomized Design (CRD) with two replications and 13 treatments. Number of cuttings per treatment was 75, of which twenty five were intended for destructive sampling in order to record biometric and biochemical observations related with root development.

3.4.9 Observations

3.4.9.1 Biometric

Three cuttings were selected from each treatment for taking biometric observations at monthly interval.

a) Number of days for sprouting

Days taken for sprouting was noted separately for each treatment. The number of cuttings which produced viable sprouts was also recorded.

b) Number of days for rooting

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

c) Percentage of rooting

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

d) Number of roots per cutting

The number of roots produced on successfully established cuttings was counted for each treatment.

e) 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.

3.5 Statistical analysis

The data collected were subjected to statistical analysis by applying `Analysis of Variance' technique as described by Panse and Sukhatme (1978).

Results

4. RESULTS

The results of the investigations on "Characterization of dioecy and standardization of propagation through cuttings in allspice (*Pimenta dioeca* [L.] Merr.)" conducted in the Department of Plantation Crops and Spices and Biochemistry lab of College of Horticulture, Vellanikkara and RARS, Ambalavayal during 1997-1999 are presented in this chapter.

I Characterization of dioecy

A Morphological characterization

4.1 Plant characters

The data with regard to plant characters of male and female plants of allspice are presented in Table 1, 2a and 2b. The results of the analysis of variance for all the plant characters studied are furnished in Appendix-I.

4.1.1 Height and spread of the plant

The height of male plants was found to range from 1.80 m to 2.93 m whereas in female plants it varied from 2.50 m to 3.70 m. The average height of male and female plants were 2.42 m and 3.21 m respectively (Table 1). The analysis of variance indicated significant difference for this character between male and female trees.

The mean spread of the male plants was observed to 3.45 m^2 which in turn was lower than that in female which registered 3.51 m^2. Wide variation was noticed in the spreading pattern of both male and female plants. The spread of male plants varied from 1.52 m^2 to 5.70 m^2 whereas in females it ranged from 1.98 m^2 to 4.50 m^2 (Table 1). The difference in this character was not significant enough to discriminate between male and female plants.

4.1.2 Canopy shape

In females, the canopy shape was found to be conical and the males had a rhomboidal shape. Male and female plants did not show any difference in these characters.

4.1.3 Height at first branching

There was not much variation in the branching heights of male and female plants, no significant difference existed between males and females with respect to this character. The branching height varied from 9.2 cm to 15.0 cm in males and 5.0 cm to 45.0 cm in females. The mean branching heights of male and female plants were 12.35 cm and 19.70 cm respectively (Table 1).

Table 1. Vegetative characters as influenced by sex in allspice

Sl. No.	Character	Male		Female	
		Mean	Range	Mean	Range
1	Plant height (m)	2.420± (0.214)	1.800-2.930	3.210± (0.109)	2.500-3.700
2	Spread of the plant (m²)	3.450± (0.413)	1.523-5.700	3.513± (0.278)	1.980-4.500
3	Height at first branching (cm)	12.345± (0.674)	9.200-15.000	19.700± (3.856)	5.000-45.000
4	No. of primary branches	15.300± (1.922)	6.000-33.000	9.900± (1.251)	6.000-18.000
5	No. of secondary branches	24.500± (1:922)	13.000-33.000	19.600± (1.293)	13.000-28.000
6	No. of leaves per flushes	2	-	2	-
7	Petiole length (cm)	0.780± (0.039)	0.600-1.000	0.760± (0.022)	0.700-0.900
8	Leaf area (m²)	43.713± (1.600)	36.720-53.370	50.499± (1.521)	43.990-58.280

Values in parenthesis indicate standard error

4.1.4 Number of main and secondary branches

The number of main branches observed along the length of the tree was slightly different for male and female plants. The number of primary branches varied from 6 to 25 in males and 6 to 18 in females (Table 1). The mean number of branches in males was 15.3 and in females it was 9.9. There was significant difference between male and female trees for this character. The number of secondary branches varied from 13 to 33 in males and 13 to 28 in females. The mean number of secondary branches in males was 24.5 and in females it was 19.6 (Table 1). This character also showed significant difference between males and females.

4.2 Leaf characters

The observations in respect of leaf characters are presented in Table 1 and 2a & 2b.

4.2.1 Number of leaves per flush

Both male and female plants produced the same number of leaves per flush with two leaves (Table 1). No sex linked difference was evident in respect of this character.

4.2.2 Size, shape and colour of leaves

The observations recorded with respect to size, shape and colour of leaves are furnished in Table 2a and 2b. It can be seen that in both males and females, the leaf size varied from small to large. Elliptic leaf shape was observed for both male and female plants. The colour of the leaf varied with maturity from light green to dull green for male and light to dark green for female plants.

4.2.3 Venation

Both male and female plants showed parallel venation. No sex linked difference was evident in respect of this character (Table 2a and 2b).

Table 2a. Size shape, colour and venation of leaves in male plants				
Sl. No.	Size	Shape	Colour	Venation
1	Large	Elliptic	Dull green	Parallel
2	Large	Elliptic	Dull green	Parallel
3	Large	Elliptic	Dull green	Parallel
4	Medium	Elliptic	Olive green	Parallel
5	Medium	Elliptic	Olive green	Parallel
6	Medium	Elliptic	Olive green	Parallel
7	Medium	Elliptic	Olive green	Parallel
8	Medium	Elliptic	Olive green	Parallel
9	Small	Elliptic	Pale green	Parallel
10	Small	Elliptic	Pale green	Parallel

Table 2b. Size shape, colour and venation of leaves in female plants				
Sl. No.	Size	Shape	Colour	Venation
1	Large	Elliptic	Dark green	Parallel
2	Large	Elliptic	Dark green	Parallel
3	Large	Elliptic	Dark green	Parallel
4	Large	Elliptic	Dark green	Parallel
5	Medium	Elliptic	Light green	Parallel
6	Medium	Elliptic	Light green	Parallel
7	Medium	Elliptic	Light green	Parallel
8	Small	Elliptic	Pale green	Parallel
9	Small	Elliptic	Pale green	Parallel
10	Small	Elliptic	Pale green	Parallel

4.2.4 Petiole length

No pronounced difference in the petiole length was observed between male (0.78 cm) and female (0.76 cm) plants (Table 1).

4.2.5 Leaf area

The leaf area recorded in male plants ranged from 36.72 cm^2 to 53.37 cm^2 (Table 1). The corresponding values for females were 43.99 cm^2 to 59.78 cm^2. The mean leaf area of female plants (50.50 cm^2) was found to be larger than that of the males (43.71 cm^2).

B Biochemical characterization

4.3 Mature plants

4.3.1 Total carbohydrate content of leaf

The data on total carbohydrate content of leaves given in Table 3, have revealed that the carbohydrate content in the leaves of female plants ranged from 2.19 to 5.78 per cent whereas in males the range observed was 4.17 to 9.34 per cent. Out of the twenty five male plants analysed, six plants had carbohydrate content of six per cent and above. On the other hand majority of the female plants showed a carbohydrate content less than four per cent. The mean value for female plants was 4.10 per cent and male plants had a higher value of 6.02 per cent.

4.3.2 Total phenols

The total phenol content in male plants ranged from 0.17 per cent to 0.33 per cent with an average of 0.26 per cent. In female plants, the total phenol content varied from 0.07 per cent to 0.20 per cent with a mean of 0.12 per cent. Of the 25 male plants analysed, 17 plants had a phenol content of 0.25 per cent and above. Among the female plants, none had a phenol content of above 0.19 per cent. However, 14 plants had a phenol content of 0.12 per cent and above. The rest showed lower phenol content (Table 3).

In the present study, male plants showed a high phenol content whereas female plants registered a low phenol content.

Table 3. Variation in carbohydrate, nitrogen and C/N ratio in male and female plants of allspice						
Sl. No	Total soluble carbohydrate(%)		Total nitrogen (%)		C/N ratio	
	Male	Female	Male	Female	Male	Female
1	5.19	4.94	0.073	0.113	71.09	44.15
2	4.84	5.24	0.070	0.158	69.85	33.16
3	9.23	3.20	0.090	0.098	102.55	32.05
4	4.89	5.14	0.071	0.119	68.87	43.19
5	5.25	2.93	0.074	0.090	70.94	32.55
6	5.88	4.32	0.079	0.108	74.43	40.00
7	6.30	3.26	0.082	0.099	76.82	32.92
8	8.15	5.27	0.083	0.165	98.12	31.93
9	5.87	3.06	0.079	0.094	74.30	32.55
10	6.29	4.04	0.080	0.104	77.62	38.84
11	9.34	5.06	0.092	0.114	101.52	32.98
12	4.71	3.20	0.068	0.099	69.26	39.81
13	5.65	4.26	0.076	0.107	74.34	33.36
14	8.32	3.17	0.088	0.095	94.54	44.60
15	6.48	5.04	0.083	0.113	78.07	30.97
16	5.93	2.54	0.080	0.082	74.12	43.96
17	4.17	5.10	0.062	0.116	67.25	34.01
18	5.12	3.47	0.072	0.102	71.11	34.69
19	4.29	2.88	0.064	0.083	66.00	43.44
20	4.63	5.17	0.066	0.119	70.15	43.27
21	6.29	4.76	0.081	0.110	78.62	35.48
22	8.19	3.69	0.088	0.104	93.06	34.81
23	5.36	5.78	0.074	0.166	72.43	34.81
24	5.93	4.85	0.080	0.111	74.12	43.69
25	4.29	2.19	0.064	0.080	66.00	27.37
Mean	6.02	4.10	0.110	0.105	71.51	37.12

4.3.3 Total free aminoacids

The statistical analysis of the values of the total free aminoacids showed that the male plants had a lower value which ranged from 0.016 to 0.020 per cent whereas females had a higher value of 0.035 to 0.068 per cent. The male and female plants had a mean value of 0.018 per cent and 0.054 per cent respectively (Table 4).

4.3.4 Total nitrogen content

The total nitrogen content in male plants varied from 0.062 per cent to 0.092 per cent. On the other hand the females had a value ranging from 0.080 to 0.166 per cent. However, there was no difference in the mean foliar nitrogen content of both males and females (0.11%) (Table 3).

4.3.5 C/N ratio

The C/N ratio ranged from 27.37 to 44.60 in the case of female plants and 66.00 to 102.55 for the male plants with the mean of 37.12 and 71.51 respectively (Table 3).

4.3.6 Essential oil

The results of the statistical analysis of essential oil content showed that the essential oil content ranged from 1.2 to 1.6 per cent in males and 1.4 to 1.8 per cent in females with a mean of 1.38 per cent and 1.61 per cent respectively (Table 4).

Table 4. Variation in phenol, amino acids, essential oil and oleoresin in male and female plants of allspice

Sl.No.	Phenols (%)		Total free amino acids (%)		Essential oil (%)		Oleoresin (%)	
	Male	Female	Male	Female	Male	Female	Male	Female
1	0.269	0.145	0.0165	0.0676	1.2	1.4	10.2	16.8
2	0.257	0.066	0.0189	0.0637	1.6	1.6	10.3	17.4
3	0.186	0.150	0.0157	0.0347	1.2	1.8	10.2	16.2
4	0.275	0.117	0.0193	0.0591	1.4	1.8	10.3	19.1
5	0.265	0.127	0.0177	0.0424	1.3	1.8	10.4	18.6
6	0.270	0.136	0.0185	0.0558	1.6	1.8	10.5	19.0
7	0.166	0.097	0.0167	0.0415	1.6	1.8	10.3	19.4
8	0.208	0.130	0.0170	0.0576	1.2	1.8	10.5	19.4
9	0.273	0.070	0.0172	0.0489	1.4	1.6	10.6	17.8
10	0.175	0.155	0.0190	0.0672	1.2	1.4	10.3	16.3
11	0.246	0.106	0.0176	0.0623	1.4	1.4	10.6	18.8
12	0.270	0.195	0.0181	0.0389	1.6	1.4	10.2	17.9
13	0.264	0.098	0.0196	0.0597	1.4	1.4	10.3	18.5
14	0.309	0.139	0.0179	0.0417	1.4	1.4	10.2	19.2
15	0.294	0.121	0.0183	0.0573	1.6	1.4	10.5	17.6
16	0.277	0.088	0.0183	0.0573	1.3	1.6	10.2	19.2
17	0.306	0.073	0.0170	0.0395	1.4	1.6	10.3	16.4
18	0.248	0.117	0.0191	0.0576	1.3	1.6	10.5	18.2
19	0.281	0.154	0.0175	0.0419	1.6	1.6	10.6	18.0
20	0.183	0.126	0.0156	0.0652	1.2	1.8	10.2	19.4
21	0.315	0.080	0.0170	0.0661	1.4	1.8	10.4	16.2
22	0.251	0.147	0.0163	0.0563	1.6	1.8	10.6	18.3
23	0.327	0.163	0.0159	0.0570	1.2	1.8	10.3	16.8
24	0.291	0.141	0.0174	0.0439	1.4	1.4	10.4	17.3
25	0.214	0.101	0.0199	0.0671	1.2	1.6	10.2	18.6
Mean	0.260	0.120	0.0180	0.0540	1.38	1.61	10.36	18.03

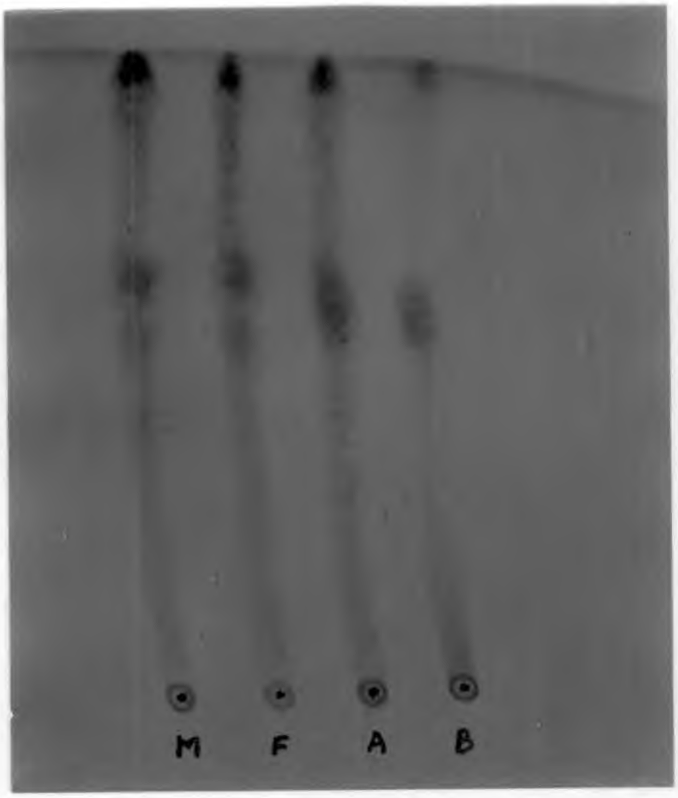


Plate 7. TLC studies of total soluble carbohydrate of male, female, four year and one year old seedlings

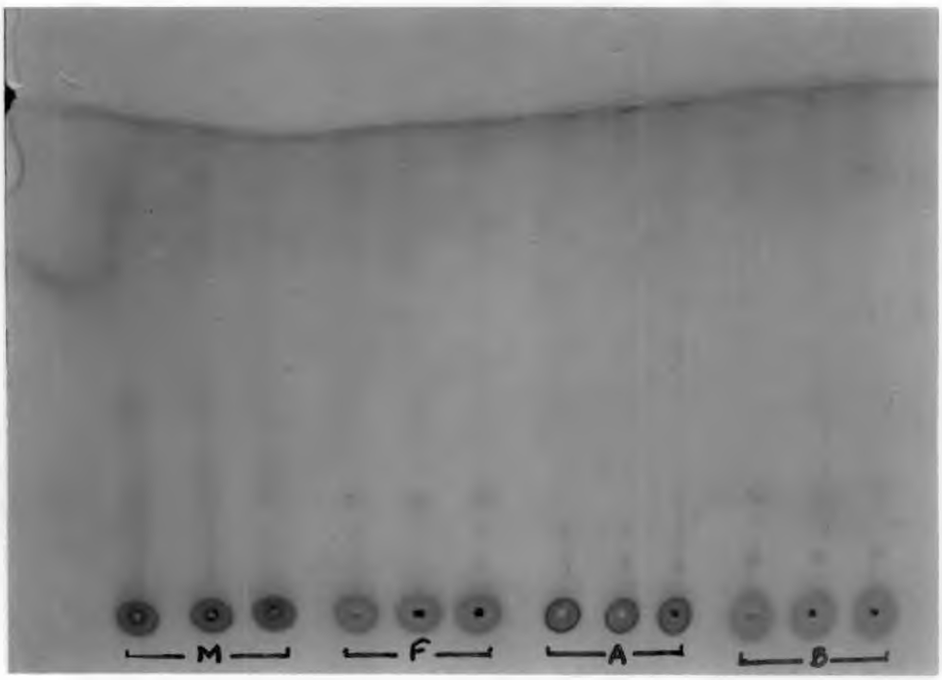


Plate 8. TLC studies of total phenols of male, female, one year and four year old seedlings

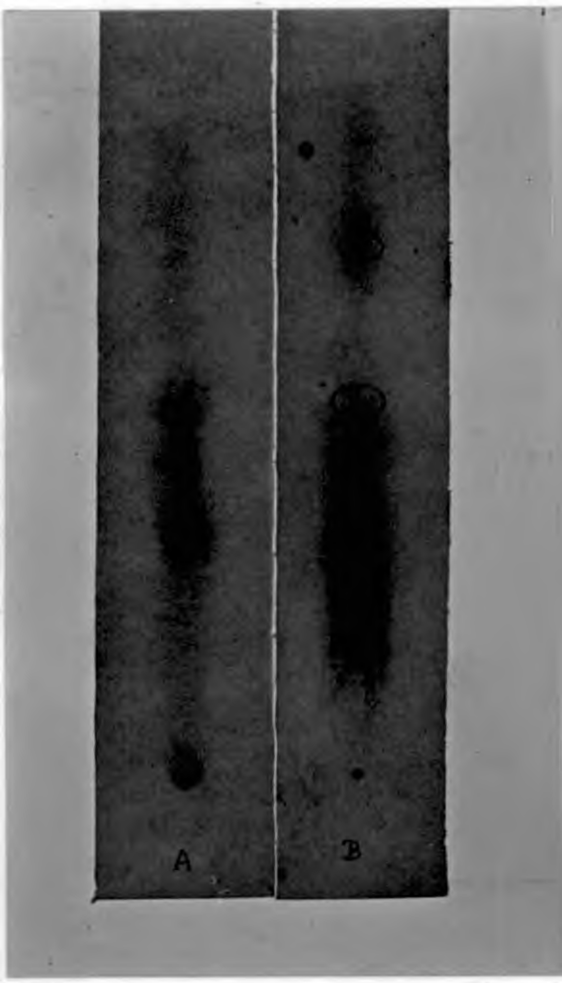


Plate 9.

Paper chromatography of total free amino acids of one year and four year old seedlings

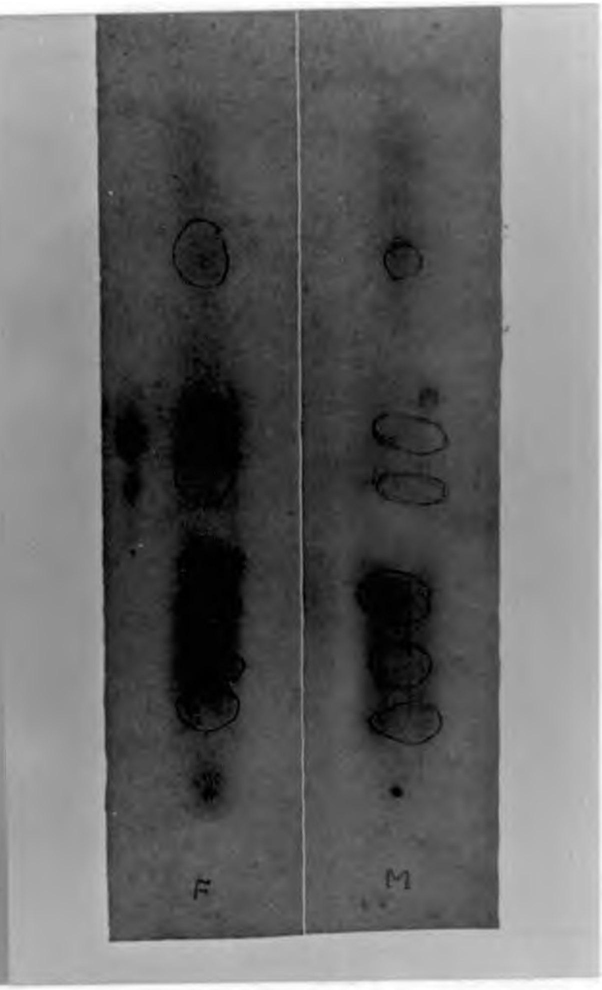


Plate 10.

Paper chromatography of total free amino acids of male and female plants

4.3.7 Oleoresin

The oleoresin content in male plants varied from 10.2 to 10.7 per cent while in females the content ranged from 16.2 to 19.4 per cent with an average of 10.36 per cent and 18.03 per cent respectively (Table 4).

4.3.8 Thin layer chromatography studies of mature plants and seedlings

4.3.8.1 Total soluble carbohydrate

The profile of carbohydrates for male plants showed two distinct spots with Rf values 0.541 and 0.958 and females also showed a similar spotting pattern with Rf 0.591 and 0.979.

One year old plants gave a single spot with an Rf value of 0.421. On the other hand, four year old plants showed two distinct spots with Rf values of 0.531 and 0.957 (Plate 7).

4.3.8.2 Total phenols

The TLC profile of phenolic extract after acid hydrolysis showed that the males had four distinct spots with Rf values 0.103, 0.365, 0.500 and 0.730. However, there were only two spots with Rf values 0.110 and 0.356 in female plants. The one year plants had a single spot with an Rf value of 0.107 and four year old seedlings showed two spots with Rf values 0.110 and 0.362 (Plate 8).

4.3.9 Paper chromatography of total free amino acids of mature plants and seedlings

The profile showed seven spots with Rf values 0.143, 0.179, 0.277, 0.321, 0.491, 0.554 and 0.768 for female plants and six spots with Rf values 0.103, 0.181, 0.276, 0.431, 0.500 and 0.741 for males (Plate 9). One year old plants showed three spots with Rf values 0.365, 0.478 and 0.539. On the other hand four year old plants showed six distinct spots with Rf values 0.187, 0.285, 0.358, 0.472, 0.537 and 0.756 (Plate 10).

4.4 Seedlings

4.4.1 Biochemical characterization

The biochemical characters of one year and four year old seedlings were studied and summarised in Table 5.

Table 5. Biochemical characters as influenced by age of the plants in allspice

Sl. No.	Characters	One year old seedlings			Four year old seedlings		
		Mean	Range	Coefficient of variation	Mean	Range	Coefficient of variation
1	Carbohydrates (%)	2.786	1.773-4.770	2.03	4.102	2.630-6.110	3.17
2	Phenol (%)	0.142	0.065-0.272	7.21	0.153	0.091-0.207	2.34
3	Total free amino acids (%)	0.026	0.014-0.039	1.26	0.038	0.013-0.067	2.85
4	Total nitrogen (%)	0.120	0.065-0.199	3.36	0.126	0.071-0.178	2.67
5	C/N ratio	23.32	21.27-26.81	4.23	32.18	26.45-36.57	3.46
6	Essential oil (%)	0.70	0.60-0.80	2.13	1.10	1.00-1.20	1.35
7	Oleoresin (%)	3.5	3.10-4.20	1.42	10.2	6.20-12.50	2.93

4.4.1.1 Carbohydrate

The carbohydrate content in leaves showed a range of 1.773 to 4.770 per cent in one year old seedlings (Mean 2.786%) and 2.630 to 6.110 per cent in four year old seedlings with a mean of 4.012 per cent.

4.4.1.2 Total phenol

Total phenol content in one year old seedlings ranged from 0.065 to 0.272 per cent with a mean of 0.142 per cent and 0.091 to 0.207 per cent in four year old plants with a mean of 0.153 per cent.

4.4.1.3 Total free amino acids

The statistical analysis of foliar amino acid content gave values ranging from 0.014 to 0.039 per cent for one year old seedlings and 0.013 to 0.067 per cent in four year old plants with a mean of 0.026 per cent and 0.038 per cent respectively.

4.4.1.4 Total nitrogen content

The total nitrogen content varied from 0.065 to 0.199 per cent with a mean of 0.120 per cent in one year old seedlings. On the other hand the four year old plants showed a range of 0.071 to 0.178 per cent with a mean of 0.126 per cent.

4.4.1.5 C/N ratio

The C/N ratio varied from 21.27 to 26.81 with a mean of 23.32 in old year old seedlings and 26.45 to 36.57 in four year old plants with the mean of 32.18.

4.4.1.6 Essential oil

The essential oil content varied from 0.60 to 0.80 per cent with a mean of 0.70 per cent for one year old seedlings and 1.00 to 1.20 per cent with a mean of 1.10 per cent for four year old seedlings.

4.4.1.7 Oleoresin

The oleoresin content varied from 6.2 to 12.5 per cent with an average of 10.2 per cent for four year old seedlings and 3.1 to 4.2 per cent with an average of 3.5 per cent for one year old seedlings.

II Propagation through stem cuttings

4.5 Biometric observations

4.5.1 Number of days for sprouting

The number of days for sprouting varied with the type of cuttings and growth regulator treatments given. It was observed that the treatment with growth regulators (IAA, IBA and NAA) in general induced early sprouting of cuttings. Effect of these growth regulators differed significantly from untreated cuttings, which took about 25 days for sprouting.

Type of cuttings differed significantly with one another with respect to days taken for sprouting. Hardwood cuttings took lesser number of days to sprout (14 days) when compared to semi-hardwood (26 days) and softwood (37 days). It was observed that after three to four months, all types of cuttings showed cent per cent mortality except for seedling tips.

4.5.2 Number of days for rooting

The observation related to the number of days for rooting was taken at monthly interval by destructive sampling method. It was observed that none of the cuttings under any treatments showed any sign of rooting except for the August and December plantings.

4.5.3 Percentage of rooting

In the case of August planting, initiation of procambium could be observed in leafy and leafless softwood (IBA 1000 ppm) and semi hardwood cuttings (IBA 1500 ppm). For December planting, there was initiation of rootlets for leafy softwood cuttings but further development was arrested in both the above cases.

In the case of seedling tips, good rooting was observed during all the months irrespective of the seasonal variation (Plates 5 and 6). It was observed that when there was flushes it took less days for rooting. In this case, rooting was obtained for control also.

4.5.4 Number of roots per cutting

The data could not be recorded as there was no root initiation in any of the cuttings under any treatment in any of the month except for December planting. The maximum number of rootlet initiation was obtained in softwood leafy cuttings (IBA 1000 ppm) and in some cuttings of same category root development was restricted to procambium stage with small protuberance of root.

The seedling tips gave the maximum number of roots per cutting with well developed primary, secondary and tertiary roots (Plate 5).

4.5.5 Length of the longest root

With respect to December planting, the length of the longest root in the case of softwood was 0.03 cm whereas for the seedling tip it was as long as 28.2 cm (Plate 5).

4.6 Biochemical observations

4.6.1 Total carbohydrate content

The data related to the total carbohydrate content in the case of softwood, semi hardwood, hardwood and seedlings taken at monthly interval as influenced by the treatments are presented in Table 6. In general, the total carbohydrate content was less in all the types of planting material.

a) Softwood cutting

The softwood cutting in general showed a range varying from 2.02 to 3.91 per cent. The lowest content was recorded during the month of April and the highest in December. The trend which could be seen was that the content showed a slight increase from April (2.02%) to August (2.94%) following which there was a decrease in the month of September and October when compared to August. Then again slight rise in the content was observed for the month of November and the highest content was recorded in the month of December following a continuous fall for the months of January, February and March (Fig.1).

b) Semihardwood cutting

These cuttings also showed a similar picture as that of softwood cuttings (Fig.1). The total carbohydrate content ranged from 1.70 to 2.86 per cent. The lowest content of 1.7 per cent was recorded in the month of April which was followed by an increasing trend with a peak during the month of August. During September and October, there was a decrease in the total carbohydrate content and thereafter an increase in November with the highest content of 2.86 per cent for the month of December. Another steep fall was noticed during the months of January, February and March.

Table 6. Monthly variation in carbohydrate content in different types of cuttings (%)

	Softwood	Semihardwood	Hardwood	Seedling tip
April 98	2.02	1.70	1.12	2.49
May 98	2.47	2.04	1.42	2.70
June 98	2.67	2.25	1.62	2.78
July 98	2.76	2.49	1.93	2.81
August 98	2.94	2.68	2.07	3.56
September 98	2.40	2.32	1.62	4.64
October 98	2.13	2.07	1.47	2.43
November 98	2.57	2.29	1.28	2.69
December 98	3.91	2.86	1.98	5.14
January 99	2.72	2.57	1.79	3.92
February 99	2.51	2.39	1.42	3.79
March 99	2.17	2.08	1.28	2.63

c) Hardwood cutting

Hardwood cuttings also showed a similar graphical representation with the lowest content of 1.12 per cent in the month of April which was followed by a steep rise till the month of August with a carbohydrate content of 2.07 per cent. The months of September, October and November showed a decreasing trend with a second peak in the month of December (1.98%) following which a fall was picturised during the months of January, February and March (Fig.1).

d) Seedling tip

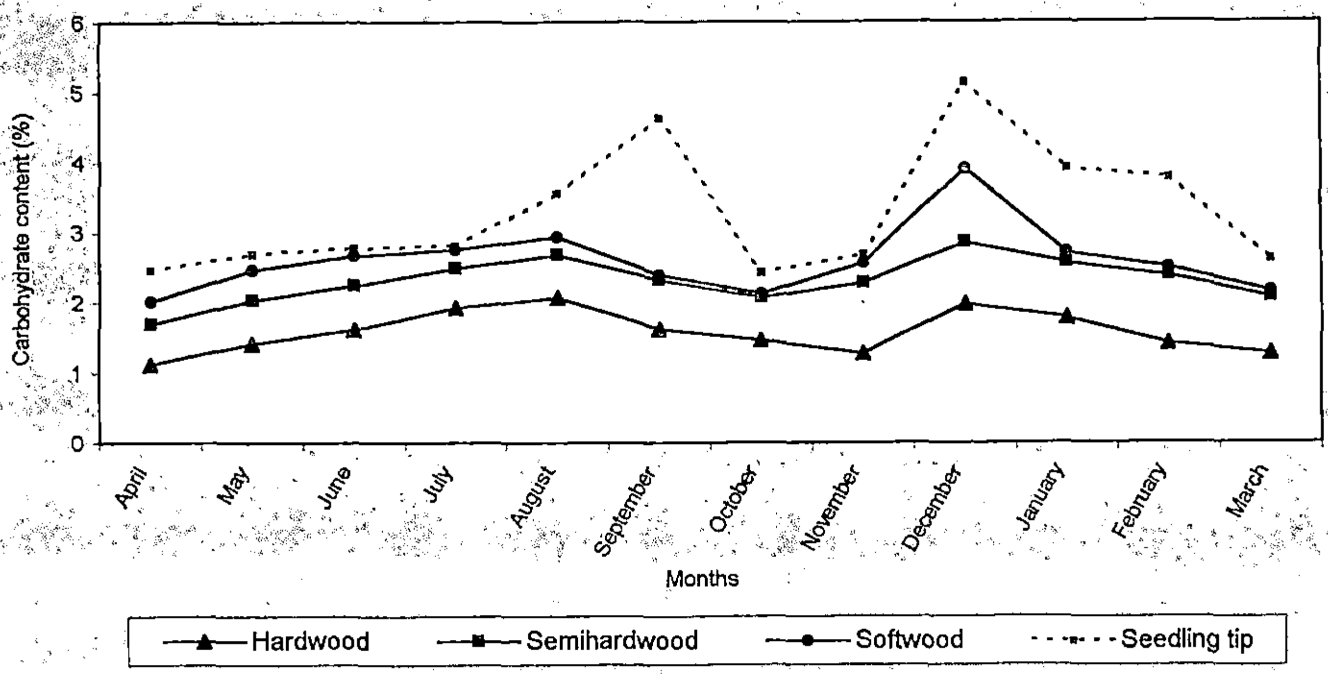
Seedling tips in general had a higher content of carbohydrate with the lowest content the month of October (2.43%) after which an increasing trend was observed. The highest content was obtained in the month of December 5.14. During October and November total carbohydrate content showed a declining trend (Fig.1).

The comparison of different types of cuttings in general showed that among the softwood, semihardwood and hardwood, the softwood had the highest content of carbohydrate (3.91%) with two peaks, a lower peak during the month of August (2.94%) and the highest peak during the month of December (3.91%). This was followed by the semihardwood cuttings which showed a slightly lesser amount in general. The two peaks for the month of August (2.68%) and December (2.86%) showed a similar pattern as in the case of softwood. The hardwood had the lowest content of carbohydrates in comparison to the other two types. Two peaks were obtained in a similar fashion as in the case of softwood and semihardwood with a lower maxima in December (1.98%) and a higher maxima in August (2.07%). The seedling tips had the highest carbohydrate content of 5.14 per cent during December and a lower peak was obtained in august (3.56%). But in general, it was higher in carbohydrate content when compared to other types of cutting.

4.6.2 Total nitrogen content

Data presented in Table 7 reveal that there was drastic variation in the total nitrogen content with respect to different planting materials and months.

Fig. 1. Monthly variation in carbohydrate content in different types of cuttings



a) Softwood cutting

Results on the total nitrogen content show that the content varies from 0.057 to 0.094 per cent. In the month of April, the content was 0.094 per cent followed by a drop reaching as low as 0.064% in August. The values showed an increase in the following two months i.e., September and October thereafter a drop in the month of November and the lowest minima was observed in December. The month of January witnessed an increase in the nitrogen content which continued till March (Fig.2.).

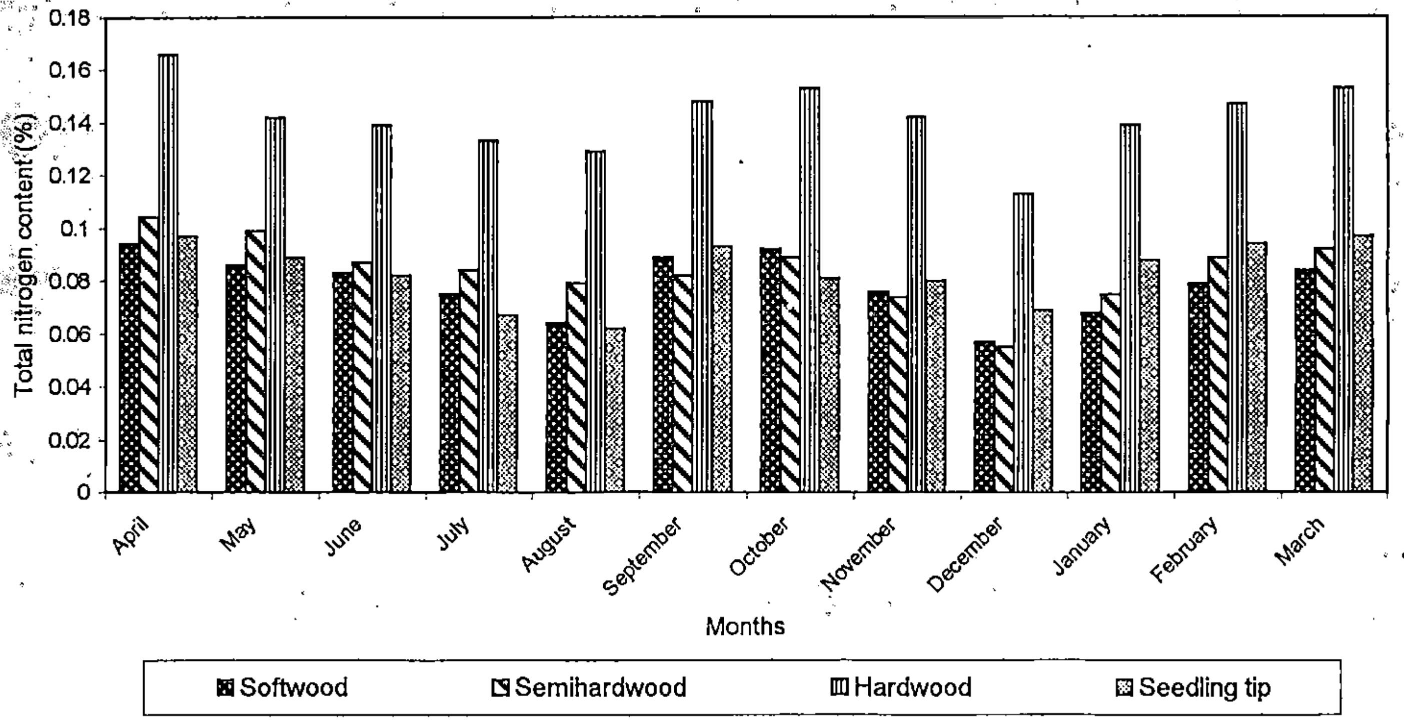
b) Semihardwood cutting

From the data furnished in Table 7 could be seen that semihardwood cuttings in general had a higher nitrogen content in comparison to softwood cuttings. The month of April showed the highest content (0.104%) following which there was a fall till the month of August (Fig.2). The month of September and October witnessed an increase when compared to the month of August and again a fall in November. December recorded the minimum value (0.055%) and thereafter an increase from January to March. The nitrogen content in general varied from 0.055 to 0.104 per cent.

Table 7. Monthly variation in total nitrogen content in different types of cuttings (%)

	Softwood	Semihardwood	Hardwood	Seedling tip
April 98	0.094	0.104	0.166	0.097
May 98	0.086	0.099	0.142	0.089
June 98	0.083	0.087	0.139	0.082
July 98	0.075	0.084	0.133	0.067
August 98	0.064	0.079	0.129	0.062
September 98	0.089	0.082	0.148	0.093
October 98	0.092	0.089	0.153	0.108
November 98	0.076	0.074	0.142	0.080
December 98	0.057	0.055	0.113	0.069
January 99	0.068	0.075	0.139	0.088
February 99	0.079	0.089	0.015	0.094
March 99	0.084	0.092	0.015	0.097

Fig. 2. Monthly variation in total nitrogen content in different types of cuttings



c) Hardwood cutting

The hardwood cuttings had the highest amount of nitrogen among all the three types of cuttings with a range of 0.113 to 0.166 per cent. The trend was same as that of softwood and semihardwood cuttings with the highest content of 0.166 per cent in April followed by a fall up to August (0.129). A slow increase was observed for the next two months i.e., September and October and a fall for November and December (0.113%). The next three months showed an increasing trend in the total nitrogen content (Fig.2).

d) Seedling tip

The seedling top showed a trend somewhat similar to that of softwood with a content varying from 0.062 to 0.108 per cent. The month of April showed a content of 0.097 per cent which decreased to as low as 0.062 per cent in the month of August. After that there was a drastic rise during September and another fall in December (0.069%) which showed a value slightly higher than August unlike in other types of cuttings which showed the lowest content in December. This was followed by an increasing trend till the month of March (Fig.2.).

The comparison of all these cuttings give a general conclusion that the softwood and seedling tip have lower nitrogen content followed by semihardwood and hardwood. All these categories showed the minimum nitrogen content during December except seedling tips which registered the minimum value in August.

4.6.3 C/N ratio

Table 8 explain the results pertaining to C/N ratio.

a) Softwood cutting

The data reveal that the softwood in general had a higher C/N ratio than the semihardwood and hardwood cuttings. The range observed was 21.40 to 56.31 in softwood. The lowest ratio was recorded for the month of April following which there was a steady increase in the ratio to 45.93 in August. The month of September and October witnessed a steep fall and then a slight increase in November and reached a maximum ratio of 56.31 in December and thereafter a steady fall was observed (Fig.3.).

b) Semihardwood cutting

The ratio varied from 16.34 to 52.00. The month of April recorded the lowest ratio in this category following which there was an increase in the ratio till August which recorded a higher ratio of 33.92. The month of September and October showed a decreasing trend and by November slight increase in the ratio was observed. The month of December recorded the maximum ratio of 55.00 and thereafter the C/N ratio decreased upto March (Fig.3.).

c) Hardwood cutting

Hardwood cuttings showed the lowest C/N ratio. The ratio varied to as low as 6.74 showing a very similar graphical representation as softwood and semihardwood. The lowest content was recorded in April followed by an increase which reached a maximum of 16.04 in the month of August and thereafter a decrease was noticed upto the month of November and a sudden rise in the ratio (17.52) during December (Fig.3).

Table 8. Monthly variation in C/N ratio in different types of cuttings

	Softwood	Semihardwood	Hardwood	Seedling tip
April 98	21.40	16.34	6.74	25.15
May 98	28.70	20.60	10.00	30.33
June 98	32.40	25.86	12.01	33.90
July 98	36.80	29.64	14.51	41.94
August 98	45.93	33.92	16.04	57.41
September 98	26.96	28.29	10.94	49.89
October 98	23.15	23.25	9.60	22.50
November 98	33.81	30.94	9.01	30.56
December 98	56.31	52.00	17.52	74.49
January 99	40.00	33.86	12.87	44.04
February 99	31.77	30.25	9.65	40.31
March 99	25.83	22.60	8.36	27.68

d) Seedling tip

These showed a ratio somewhat similar to that of softwood. The range observed was 25.15 to 74.49. April recorded the lowest ratio of 25.15 followed by a slight increase in the ratio till the value reached 57.41 in August. During September and October lower ratios were recorded and by November the C/N ratio increased and reached the maximum in the month of December (74.49). There was a fall in the ratio during the following three months (Fig.3.).

The comparison of all the types of cuttings in general showed that there are two maxima, a lower maxima in the month of August and the higher maxima for December. The lower C/N ratio in all types of cuttings was recorded in April except in seedling tips which registered the lowest value in October.

4.6.4 Total phenols

Data given in Table 9 in general indicate a higher content of phenols in all types of planting material.

a) Softwood cutting

The range varied from 0.095 to 0.063 per cent. The highest content was recorded in April while the lowest content was noticed in November. There was a decreasing trend from April to August. Then the phenol content increased during September and October (Fig.4.).

b) Semihardwood cutting

From the data it is seen that in the month of April the content was as high as 0.130 per cent after which there was a progressive decrease in the total phenols till August (0.093%). This was followed by an increase in the content to 0.113 per cent in September following which a steady fall was observed till the lowest content of 0.090 per cent was obtained in December (Fig.4.). The following months showed a steady increase in the content.

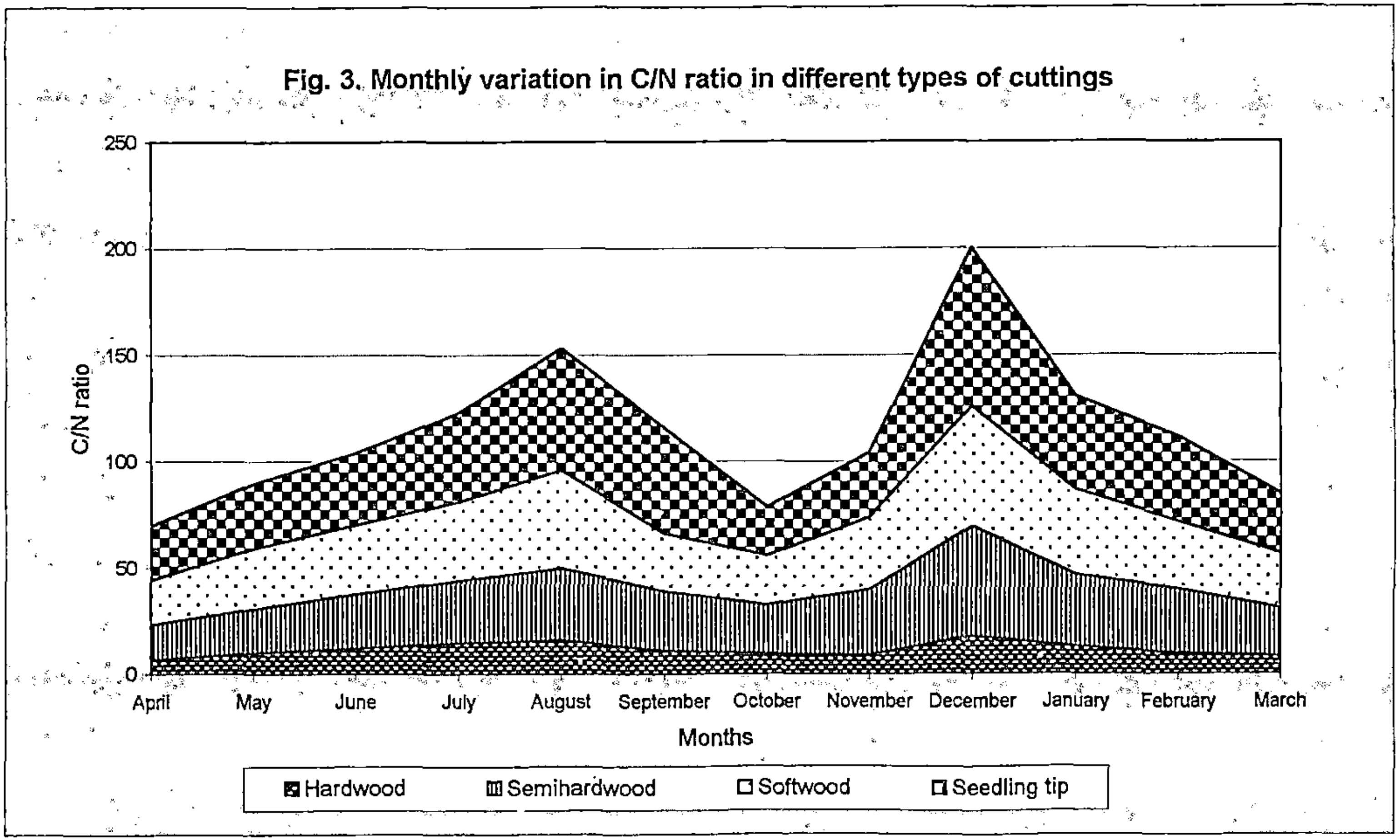


Fig. 3. Monthly variation in C/N ratio in different types of cuttings
250
200
150
100
50
0
C/N ratio
April
May
June
July
August
September
October
November
December
January
February
March
Months
Hardwood
Semihardwood
Softwood
Seedling tip

Fig. 4. Monthly variation in phenols in different types of cuttings

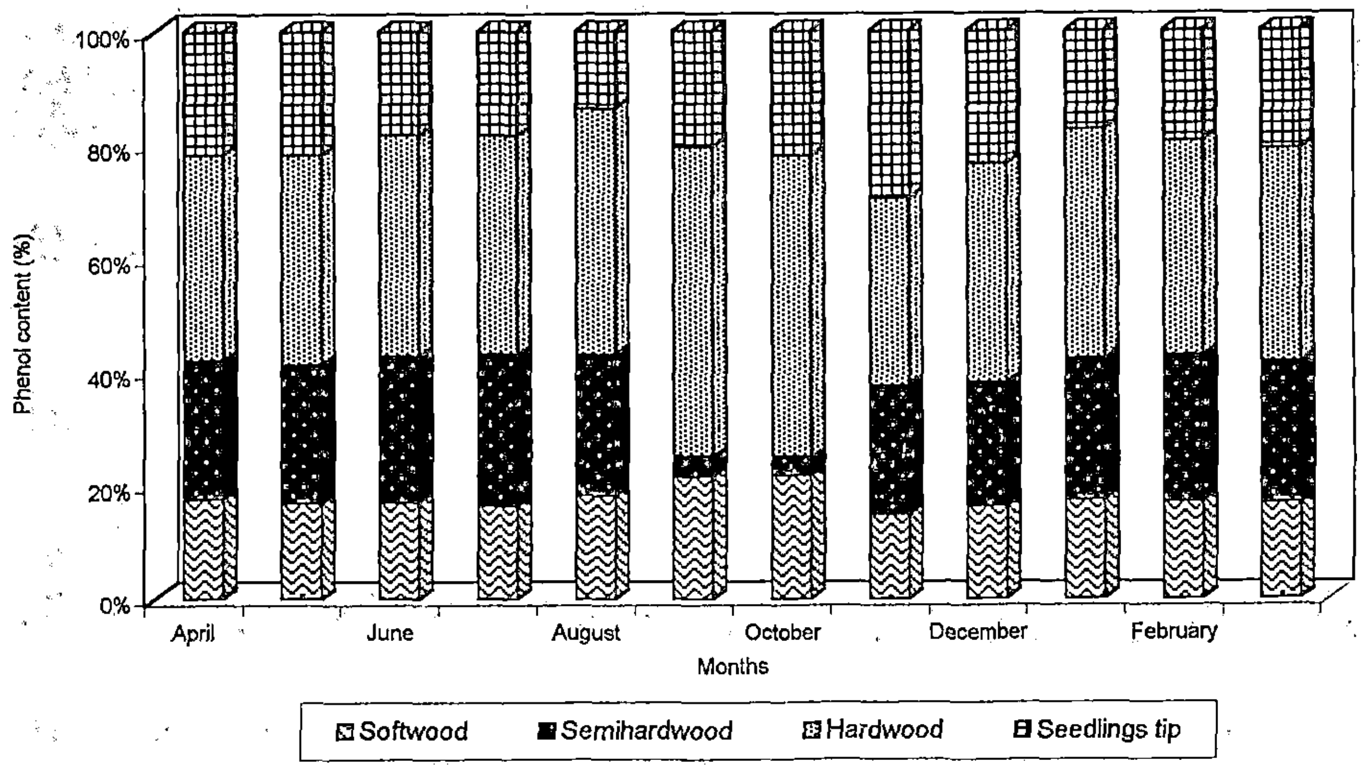


Table 9. Monthly variation in phenols in different types of cuttings (%)				
	Softwood	Semihardwood	Hardwood	Seedling tip
April 98	0.095	0.130	0.195	0.116
May 98	0.086	0.123	0.187	0.110
June 98	0.080	0.119	0.182	0.085
July 98	0.073	0.117	0.171	0.081
August 98	0.069	0.093	0.163	0.051
September 98	0.071	0.113	0.179	0.067
October 98	0.075	0.102	0.182	0.075
November 98	0.063	0.098	0.143	0.128
December 98	0.068	0.090	0.159	0.097
January 99	0.073	0.103	0.168	0.072
February 99	0.079	0.117	0.173	0.089
March 99	0.086	0.125	0.189	0.105

c) Hardwood cutting

The month of April recorded the highest content of 0.195 per cent following which there was a decrease till the month of August (0.163). An increase was noticed in September and October and again fall with lowest value in November (0.143). Thereafter an increase was observed up to March. The range of phenol content for hardwood was 0.195 to 0.143 per cent (Fig.4.).

d) Seedling tip

These showed somewhat erratic pattern with respect to the total phenols. The range varied from 0.051 to 0.128 per cent. The month of April recorded 0.116 per cent following which there was a fall in the content with the lowest value in August (0.051%) (Fig.4.). During September to November there was rise in the content and once again a decrease was recorded up to January.

The comparison of all the cuttings show that in general there was a higher phenol content in all types of cuttings and it remained somewhat same with very little variation in the case of softwood. The semihardwood had a higher content in comparison to softwood but lesser than hardwood. In the case of softwood minima was obtained for November but for semihardwood minima was obtained for the month of December. The seedling tips had the lowest content in August.

4.6.5 Total free amino acids

Information from the Table 10 indicate that the total free amino acid content ranged from medium to high level.

Table 10. Monthly variation in total free amino acids in different types of cuttings (%)

	Softwood	Semihardwood	Hardwood	Seedling tip
April 98	0.0364	0.0164	0.0127	0.0168
May 98	0.0389	0.0171	0.0139	0.0179
June 98	0.0392	0.0175	0.0148	0.0162
July 98	0.0398	0.0183	0.0163	0.0173
August 98	0.0413	0.0191	0.0181	0.0216
September 98	0.0387	0.0183	0.0175	0.0165
October 98	0.0373	0.0175	0.0162	0.0162
November 98	0.0386	0.0181	0.0179	0.0185
December 98	0.0625	0.0203	0.0193	0.0298
January 99	0.0392	0.0178	0.0181	0.0166
February 99	0.0376	0.0174	0.0165	0.0158
March 99	0.0371	0.0166	0.0139	0.0142

a) Softwood cutting

This category in general showed a high percentage of amino acids with a range of 0.0364 to 0.0625 per cent. April was the month which recorded the least amount following which an increment was seen till the month of August (0.0413%). From September to October a slight drop and again an increase in November with

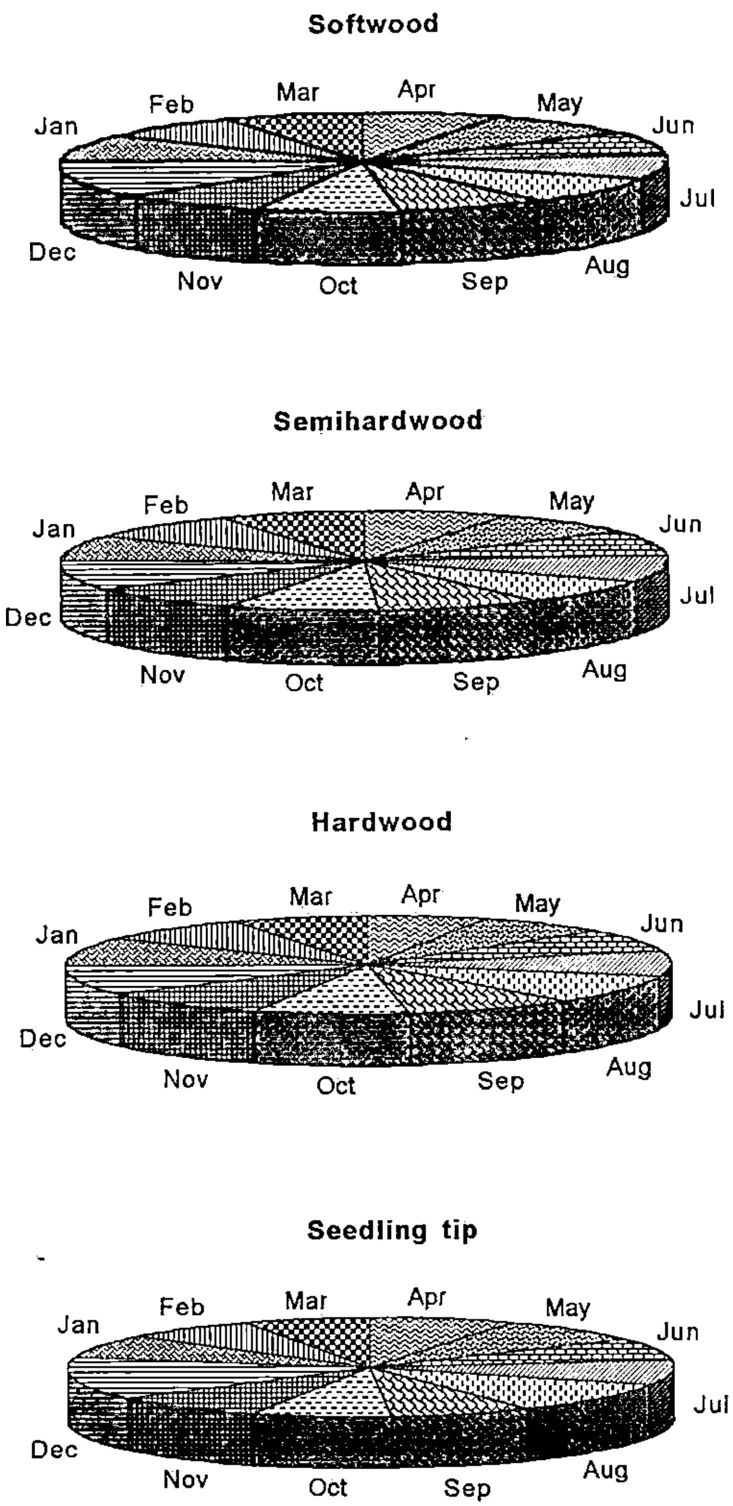


Fig. 5. Monthly variation in amino acid content in different types of cuttings

the maximum level in December (0.0625%) was observed. After December, a sudden fall in the content was noticed till the month of March (Fig.5).

b) Semihardwood cutting

The general trend observed in these cuttings is that they had a lower content in comparison to the softwood cuttings. The content varied from 0.0164 to 0.0203 per cent. The lowest content of 0.0164 per cent was recorded during the month of April after which somewhat linear increase was recorded till a higher value of 0.0191 per cent was attained in August. Then a slight drop was witnessed during the month of September and October and another increase was recorded for the month of November and the highest peak was obtained for the month of December. Thereafter a decrease was noticed for the month of January, February and March (Fig.5).

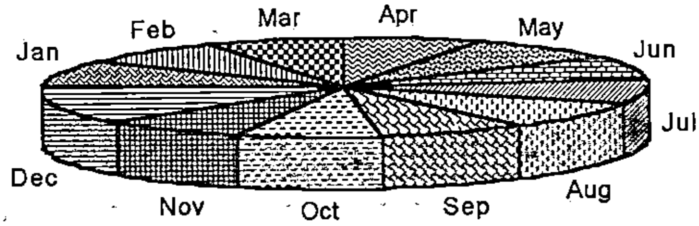
c) Hardwood cutting

The hardwood cuttings showed the lowest amount of amino acid with a range of 0.0127 per cent (April) to 0.0193 per cent (December). After April, the trend was very similar as in other two types of cuttings moving towards a peak of 0.0181 per cent in August, following which a fall was noticed during September and October. From November, there was an increase in the content and the highest peak was obtained in December thereafter a fall was observed (Fig.5).

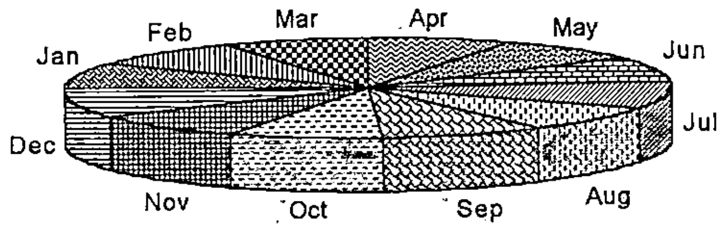
d) Seedling tip

This category showed an erratic behaviour. An increase in amino acid content was observed from April (0.0168%) to May (0.0179%) then decreased during June and a rise in July and August (0.0276%). The month of September and October witnessed somewhat similar values following which an increase was seen with a peak in December (0.0298%) and again a linear fall for the next three months. The range varied from 0.0142 per cent to 0.0298 per cent (Fig.5).

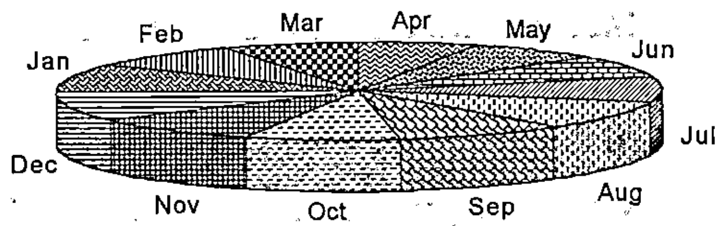
Softwood



Semihardwood



Hardwood



Seedling tip

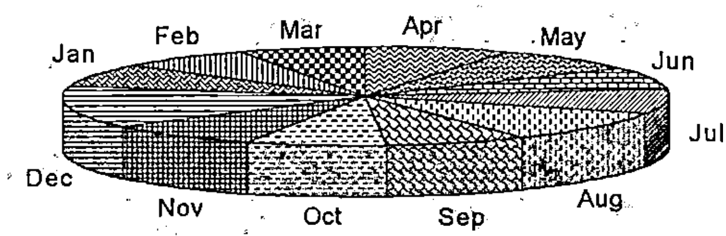


Fig. 5. Monthly variation in amino acid content in different types of cuttings

A comparison of all the types of planting materials show that in general the softwood had a higher content of total free amino acids than the semihardwood and the lowest content was recorded in hardwood. The seedling tip did not register a wide variation with regard to the content except for the month of August and December which showed slightly higher amounts.

Discussion

5. DISCUSSION

Allspice is a polygamodioecious tree spice, the cultivation of which is getting popularized in India. However, lack of a fool proof technique for the identification of sex before flowering is one of the limiting factors for the commercial cultivation of allspice. Therefore, the thrust area of research in this crop should be the standardization of a commercially viable method of vegetative propagation which may pave way to overcome the problems due to dioecy.

The results of the investigations on "Characterization of dioecy and standardization of propogation through cuttings in allspice [*Pimenta dioeca* (L.) Merr.]" are discussed in this chapter as two separate experiments.

I. Characterization of dioecy

A. Morphological characterization

5.1 Height, spread and shape of the plant

Information from the observations recorded reveal that the female plants are taller than the males (Table 1). Rojast *et al.* (1985) have observed that the female plants of papaya are taller than the male plants and showed late flowering. However, a contradictory result has been reported by Flach (1966) in nutmeg wherein the males and females did not show any marked difference in tree size.

With respect to the spread of the tree though not conspicuous, males had a better spread than the females (Table 1). Usually in dioecious plants, the general observation is that the females are more spreading in nature than males. The females of *Ficus carica* and *Morus alba* had denser spreading crowns (Kotaeva *et al.*, 1982). The females of dioecious shrub jojoba also had more open canopies (Kohorn, 1994). Results of the studies conducted by Nyong *et al.* (1994) in a forest plant Odum (*Milicia excelsa*) revealed that females possess more spreading crown than males. However in nutmeg, Thomas (1997) has observed no significant difference in canopy spread between male and female plants.

The canopy shape was observed to be conical in females and rhomboidal in males. Since females are less spreading types conical shape is quite expected.

5.2 Height at first branching

The female plants branched at a higher level as compared to males (Table 1). Thomas (1997) has reported no difference in height at first branching between male and female nutmeg plants.

5.3 Number of primary and secondary branches

The number of primary and secondary branches were less for the female trees as compared to males. It may be due to this reason that the males have a rhomboidal canopy shape. No significant difference could be observed between males and females in nutmeg by Thomas (1997).

5.4 Leaf characters

There was exactly the same number of leaves per flush for male and female trees (Table 1). No difference could be made out between male and female trees for the petiole length also (Table 1). With respect to size, shape and colour of leaves (Table 2a & 2b), it was observed that they were not influenced by sex in allspice. In male trees of nutmeg, Janse (1898) has observed comparatively smaller leaves.Prestoe (1948) observed elliptical leaves with straight veins in female nutmeg trees whereas the males had obovate leaves with veins running to the more pronounced point of the leaf. However, Thomas (1997) has reported elliptic and oblanceolate leaves in male and female plants of nutmeg respectively. In *Garcinia* *cambogia*, the colour of emerging leaves of males was found to be light green and that of hermaphordite trees pinkish red (Sherly, 1994).

The leaf area was found to be larger for female trees than the males in allspice (Table 1). Kotaeva *et al.* (1982) studied the male and female plants of *Morus alba* and *Ficus carica* and reported that the females of both the species had larger leaves than the males. In nutmeg also most of the females studied were found to have larger leaves than the males (Janse, 1898 and Thomas, 1997). The length by width ratio of leaves was found to be larger in male plants of nutmeg (Thomas, 1997). In garcinia, Mathew *et al.* (1996) had reported significant variation in length and breadth of leaves of male and female trees.

B. Biochemical characterisation

5.5 Total carbohydrate content of leaf

The males recorded a higher foliar carbohydrate content than the females (Table 3). This finding is in agreement with the observations made by Choudhari *et al.* (1957) in papaya, wherein the male leaves were richer in total carbohydrate than the females. However, the leaves of female trees of jojaba showed a higher carbohydrate content than the males which is in contradiction to the above result (Prasad and Iyengar, 1982).

The one year old seedlings recorded a lower content of carbohydrate than the four year old seedlings. The four year old seedlings registered a mean of 4.10 per cent similar to that of the female plants indicating that sex would be differentiated at the age of four. Among the selected twenty five plants some showed a content similar to male and others towards females (Table 5). However, to obtain a conclusive result on the influence of carbohydrate on sex expression in allspice the study has to be continued till the flowering stage.

5.6 Total phenols

The males had a higher phenol content in comparison to the female trees (Table 3). In general, the content of phenolic compounds present in leaves is found to differ in male and female plants of dioecious species. Studies by Thomas (1997) revealed that the male plants of nutmeg had a higher phenol content as compared to females. She had also observed that the phenol content was lower in one year old plants than in two year old plants. Bhattacharya and Rao (1982) were of the opinion that any treatment that reduces the phenolic content would enhance femaleness in papaya.

The phenol content was less for the one year old seedlings but the four year old seedlings had a slightly higher content than that of one year old seedlings (Table 5). The four year old seedlings had a range of 0.091 to 0.207 per cent. However, no pronounced difference could be obtained between the two sexes so as to consider this as a sex determining character.

5.7 Total free amino acids

The females had a higher amino acid content than the males with a mean value of 0.054 and 0.018 per cent respectively (Table 3). The level of amino acid was similar in both sexes except for proline which was present in female plants only and cysteine which was absent in female plants in the case of papaya (Khan *et al.*, 1982). The average of total free amino acids was higher for the four year old seedlings as compared to one year old seedlings (Table 5). Differentiation of the 25 plants studied into males and females based on the content of total free amino acids may not be a fool proof method at this stage. This has to be confirmed at the stage of flowering.

5.8 Total nitrogen content

The total nitrogen content in general showed no conspicuous difference in both males and females, but the values ranged from 0.06 to 0.76 per cent in the case of females and 0.08 to 0.17 per cent in the case of males (Table 5). The leaves of female plants of papaya are richer in total nitrogen content (Choudhari *et al.*, 1957).

The average content was almost same for both one year and four year old seedlings.

5.9 C/N ratio

The male plants of allspice showed a higher C/N ratio as compared to females (Table 3). This is quite expected since the carbohydrate content is higher and nitrogen content lower in the case of male. So also translocation of more stored food reserves to the developing sink takes place in the case of females which results in the depletion of carbohydrates in the leaves of females.

The C/N ratio was lower for one year old seedlings and slightly higher for four year old seedlings (Table 5).

5.10 Essential oil

The mean content of essential oil was slightly higher in females with a range from 1.2 to 1.6 per cent in males and 1.4 to 1.8 per cent in females (Table 4). According to Thomas (1997), the essential oil content is higher in the leaves of females than in males of nutmeg.

The one year old seedlings had a lower essential oil content while the four year old seedlings had a content somewhat similar to the matured trees (Table 5).

5.11 Oleoresin content

The females showed a higher oleoresin content than the males (Table 4). Males showed an average of 10.36 per cent while the females recorded as high as 18.03 per cent oleoresin. The higher oleoresin content of the females can be partially attributed to the higher essential oil content also.

The oleoresin content was very low for one year old seedlings while the four year old seedlings had a content overlapping that of the male and female trees (Table 5).

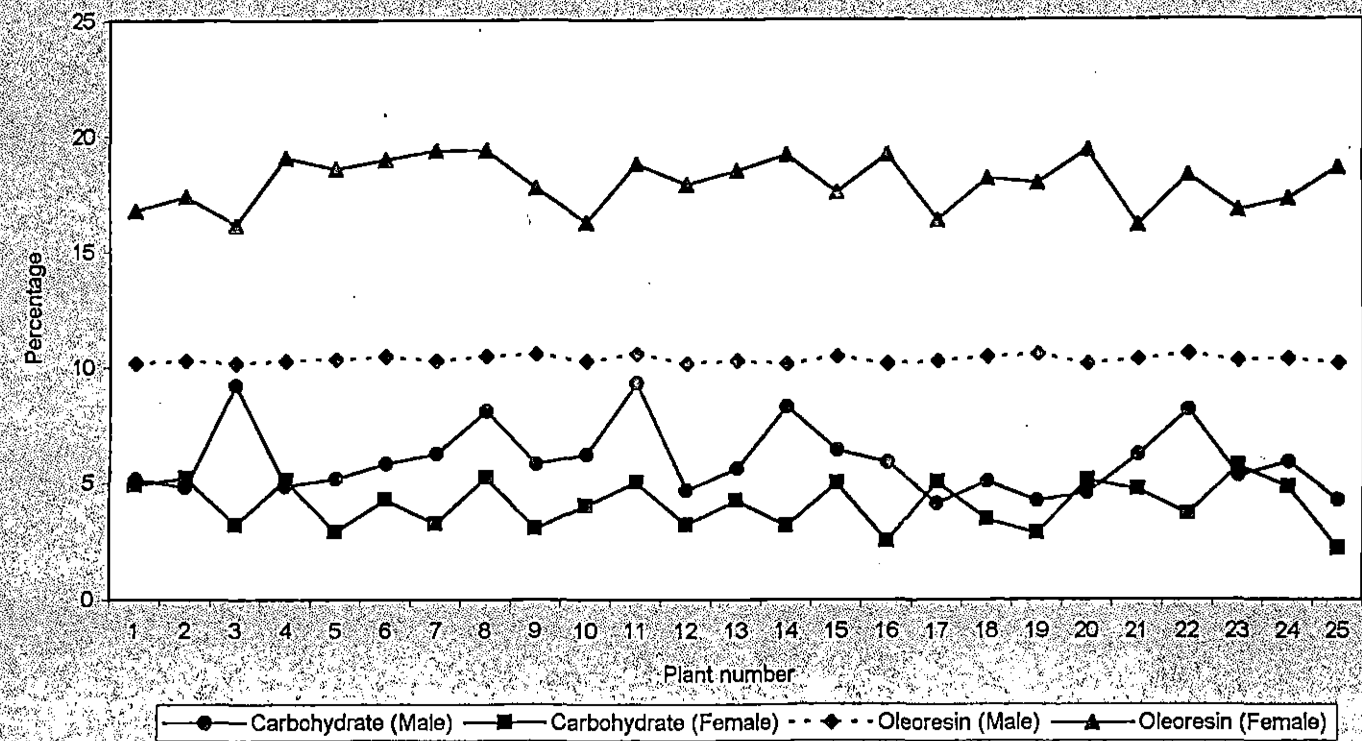
The comparison between carbohydrate content and oleoresin (Fig.6) of male and female trees shows that the males with a higher carbohydrate content had a lower oleoresin content and vice-versa. This may be due to the fact that the carbohydrates are not being converted to secondary metabolites in the case of males but on the other hand in females the conversion of carbohydrates to secondary metabolites may be taking place and hence a higher content of oleoresin. Similar is the trend in the case of essential oil also.

The soluble sugars are the initial products of photosynthesis. Plants always maintain an equilibrium of soluble sugars in the source and whenever the concentration exceeds it is either converted to the polysaccharide-starch or inter-converted to other primary products or translocated to other organs for the synthesis of secondary products. Once it is in the form of starch, further transformations are slow or limited.

The carbohydrate content when compared to the total free amino acids show a similar trend with respect to male and female trees (Fig.7).

Amino acids are the precursors of majority of the secondary products. Kudesia and Jetley (1995) have reported that precursors for the biosynthesis of

Fig. 6. Comparison of carbohydrate and oleoresin in male and female plants



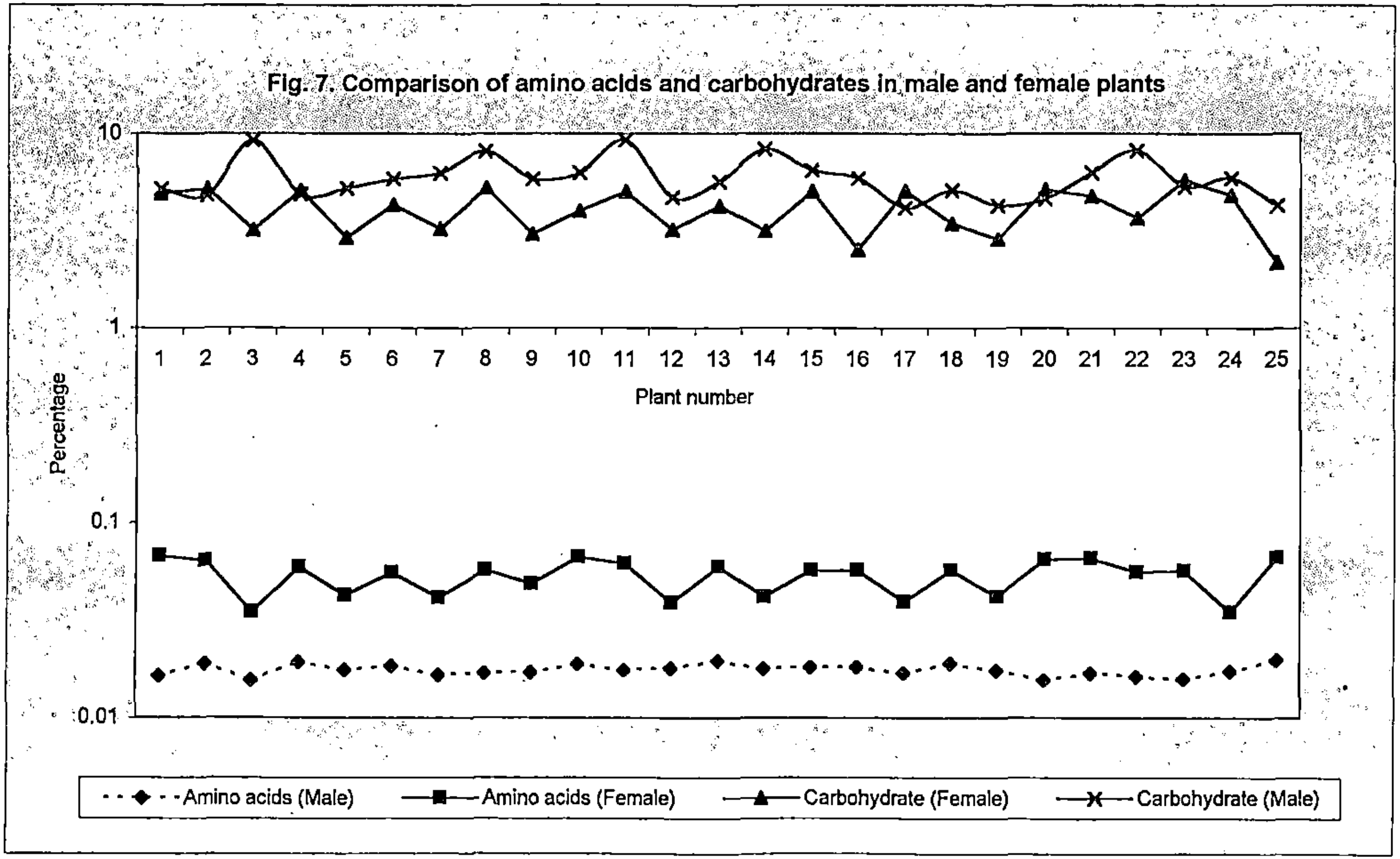


Fig. 7. Comparison of amino acids and carbohydrates in male and female plants
10
1
0.1
0.01
Percentage
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Plant number
Amino acids (Male)
Amino acids (Female)
Carbohydrate (Female)
Carbohydrate (Male)

alkaloids are amino acids such as ornithine, lysine, phenylalanine, tyrosine and tryptophan. Hence, high content of free amino acids is advantageous if they are utilised in the production of valuable secondary products.

The picture of comparison of the total free amino acids and phenols reveal that the amino acid content is higher for females and phenol content higher for males. So it can be assumed that the femaleness is due to the amino acids and the phenol content may be the biochemical constituent that points to the maleness (Fig.8).

The comparison between the phenols and oleoresin reveals that the males which have a higher phenol have a lower oleoresin content and vice-versa (Fig.9).

The general status of biochemical constituents in males and females of allspice can be summarized as follows:

Constituent	Male	Female
Total carbohydrates	H	L
Phenols	H	L
Total free amino acids	L	H
Total nitrogen content	L	H
C/N ratio	H	L
Essential oil	L	H
Oleoresin	L	H

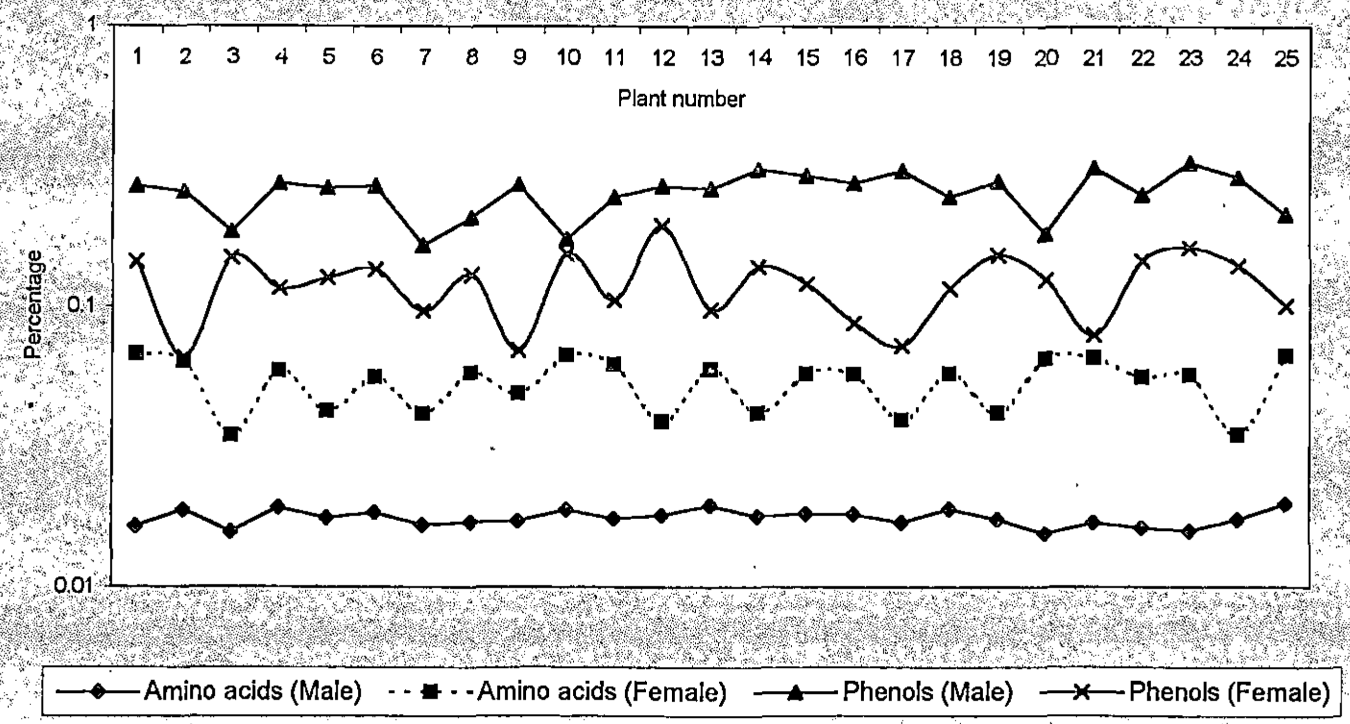
H - High, L - Low

5.12 Thin layer chromatography of mature plants and seedlings

5.12.1 Total carbohydrates

A similar spotting pattern with two distinct spots were obtained both for male and female plants. The one year old seedings had a single spot while the four year old seedling showed a pattern similar to the sex differentiated plants indicating

Fig. 8. Comparison of amino acids and phenols in male and female plants



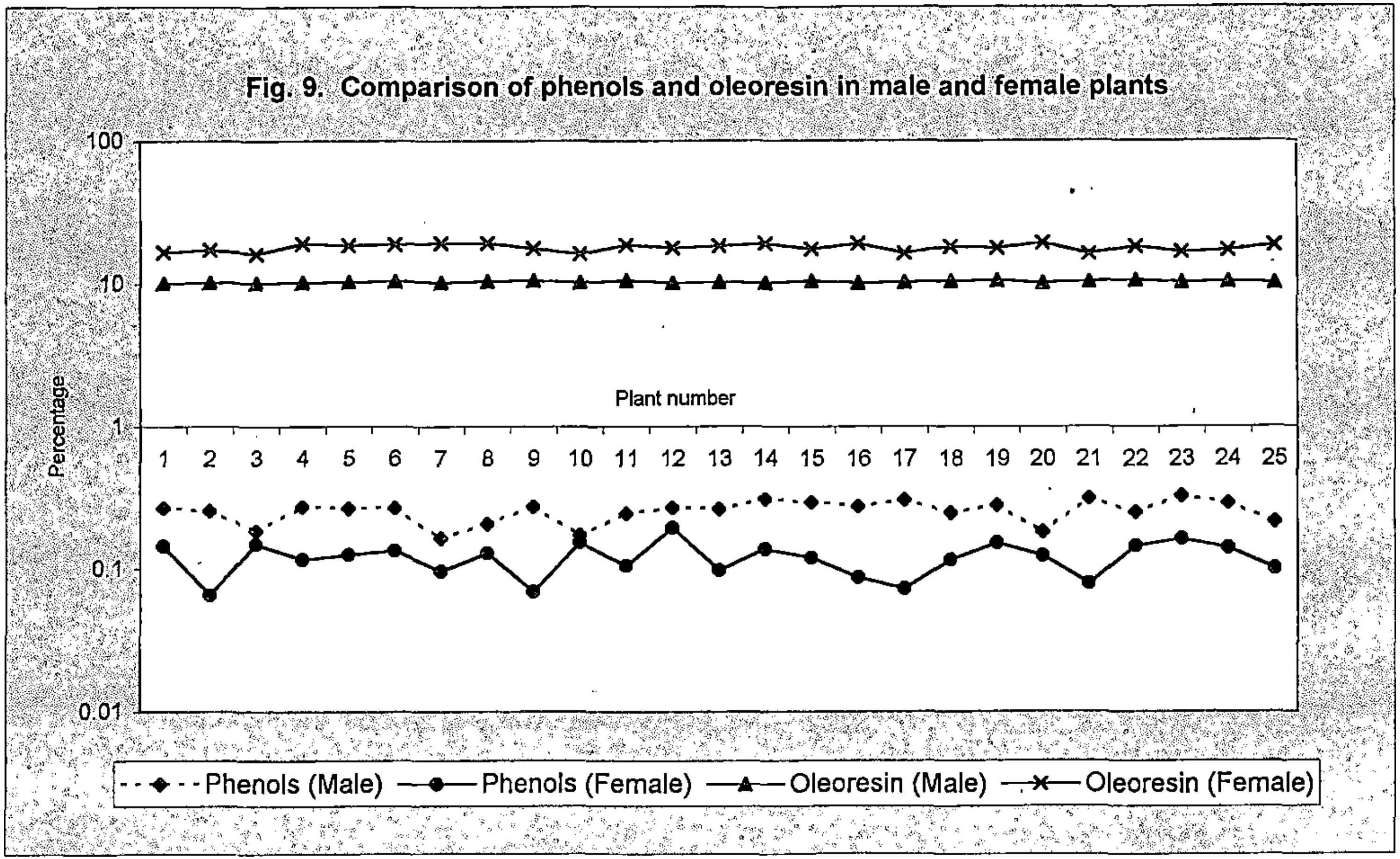


Fig. 9. Comparison of phenols and oleoresin in male and female plants
100
10
1
0.1
0.01
Percentage
Plant number
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Phenols (Male)
Phenols (Female)
Oleoresin (Male)
Oleoresin (Female)

that by attaining four years of growth all the carbohydrate constituents in the plant which determine a particular sex would have been decided. It was also seen that the second spot was more intense one and this could be the major component. The one year old seedling showed a carbohydrate content having Rf value (0.421) which is similar to that of mature plants (Plate 7).

5.12.2 Total phenols

The males had four distinct spots supporting the fact that the total phenol content and the number of components were higher (Plate 8). On the other hand the females had only two spots indicating that the third and fourth components were absent in females. The four year old seedlings also gave a similar spotting pattern as that of females while the one year old seedlings had only one component.

5.13 Paper chromatogrphy of total free amino acids of mature plants and seedlings

The profile of amino acids shows that the females have seven different amino acids while males have only six amino acids. The amino acids with the highest Rf value of 0.768 (female) and 0.741 (Male) exhibited an yellowish orange colour. All the other spots were purple in colour for females. The fourth and fifth spots were light lilac in colour in the case of males (Plate 9).

The four year old seedlings had a spotting pattern similar to the males with six different amino acids while the one year old seedlings have only three amino acids. The Rf values show that the third, fourth and fifth amino acids had been developed in the case of one year old seedlings (Plate 10).

5.14 Comparative evaluation of biochemical factors associated with sex differentiation in allspice

One year old seedlings expressed a single spot of carbohydrate which was observed in four year old seedlings as well as in mature male and female plants also. This can be considered as the basic carbohydrate necessary for the growth and development of the plant. At the same time, four year old seedlings showed two spots which were almost similar to that of mature plants. Eventhough the number of spots of carbohydrate was same in both four year old seedlings and mature plants (male and female), the quantity as well as quality of carbohydrate (type) may vary according to male and female sexes. This primary product has a definite role in the production process of other primary products such as amino acids and secondary products such as oleoresin and essential oils. The results of the analysis are in agreement to the above statement.

The TLC profile of mature plants expressed six amino acids in common for both male and female and one amino acid of Rf value 0.371 as additional in the female plants. The concentration as well as the number of amino acid, especially the presence of an additional amino acid in female can be utilized for screening and determination of sex in allspice.

One year old seedlings had only three spots with Rf values 0.365, 0.478 and 0.539 whereas the other three amino acids having Rf values 0.143, 0.179 and 0.277 were not recorded, which again supported the result of carbohydrate profile of TLC. The four year old seedlings had a TLC pattern of amino acid similar to that of the female plants in which the amino acid of Rf value 0.371 expressed in the female plants was absent which may be an indication to group the four year old seedlings as males. Since the carbohydrate content and secondary products of four year old seedling gave an indication of both male and female plants, the amino acid variation can be considered as a metabolic product at different stages. It may or may not have direct influence in the sex determination.

The secondary products such as oleoresin and essential oil are the byproducts of the precursors of amino acids. The effect of free amino acid concentration and carbohydrate are distinctly evident in the secondary products as well as in sex differentiation.

The studies with respect to phenols showed that the one year old seedlings had only one group of phenol, on the other hand four year old seedlings recorded two spots indicating the development of formation of the second group of phenols. The male had four distinct spots in TLC supporting the fact that both the total phenol content and the number of groups were higher. The females had only two spots which in agreement to the fact that the females had a lower content of phenolics.

Thus the biochemical screening showed that the females had a higher content of total free amino acids resulting in higher content of oleoresin and essential oils, on the other hand, phenols, nitrogen and carbohydrates were higher in males.

So the above results can be considered as a basic information for identifying a specific biochemical factor such as enzyme for sex determination in allspice.

II. Propagation through stem cuttings

Propagation using stem cuttings is preferred as a tool for large scale multiplication of elite genotype of a crop as it has some distinct advantage like overcoming dioecy which is not possible through conventional methods. The method is inexpensive, rapid, simple and free from any compatibility problems commonly encountered when plants are raised by grafting or budding.

5.15 Type of cuttings

The type of wood used for cutting is immaterial in the species of plants which are easy-to-root, but is a matter of great importance in the difficult-to-root material.

On comparison of the types of cuttings, it could be observed that although hardwood and semi hardwood cuttings were slightly early in sprouting and rooting, higher percentage of rooting and longer roots were observed in the case of softwood cuttings.

The semi-hardwood and hardwood cuttings have ample supply of stored food whereas the softwood is usually low in stored food reserve. This may have resulted in slightly delayed sprouting in softwood cutting, compared to hardwood and semihardwood cuttings. The hardwood cuttings in general dried faster. Hardwood represents a physiologically inactive stage highly staturated with inhibitory substances like tannins, phenols etc. This may be the reason for better rooting success of softwood cuttings compared to hardwood and semi-hardwood cuttings.

5.16 Effect of mist

The present investigations conclusively showed that cuttings kept under intermittent misting environment had significantly higher rooting. 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) had 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 results in high humidity surrounding the leaf but also maintains turgidity and optimum temperature. Intermittent misting also prevents dessication of the cuttings.

5.17 Effect of growth regulators

The use of growth regulators are now in practice for enhancing the rooting efficiency of cuttings in many crop plants and ornamentals.

Among the growth regulators tried IBA was found to be more effective than IAA and NAA. The maximum number of rootlet initiation was obtained in softwood leafy cuttings treated with IBA 1000 ppm.

The result of the present study is in confirmity with the result obtained by Pal *et al.* (1993a) in *Tylophora indica* cuttings prepared from lateral shoots wherein maximum rooting response was observed with IBA at 1000 mg^{-l} 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).

5.18 Leafy vs leafless cuttings

The results of the investigations suggest that presence of lamina on the cutting of allspice greatly enhance rooting efficiency. This result can be substantiated from the following 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 get reduced (Heide, 1968 and Vardar, 1968) thus indirectly influencing rooting. These may be the reason that the leafy cuttings could withstand drying when compared to the leafless cuttings.

5.19 Biochemical studies

In general, cuttings with a high carbohydrate content root better than those with a low carbohydrate content (Nanda and Kochar, 1991). In the present study, the better rooting efficiency of softwood cuttings may be attributed to their increased level of carbohydrates, especially in the seedling tips (Table 6). There were two peaks for all types of cuttings i.e., in August and December. This coincided with the flushing season. The month of December showed the highest content of carbohydrates and rooting was obtained in leafy softwood cuttings collected during December. The seedling tip irrespective of the season showed rooting. This may be because it contains a good amount of carbohydrates through out. This is supported by the results of the work by Ali and Westwood (1966) who showed that the difference in the rooting ability of juvenile and adult cuttings was due to the difference in their nutritional status, especially of their carbohydrate content.

The nitrogen content showed a different trend when compared to that of carbohydrates. The hardwood cuttings had the highest nitrogen content followed by semi hardwood and softwood cuttings (Table 7). There were two peaks in this case also and the lowest value was obtained in the month of December (Fig.2) during which positive signs of rooting was obtained in softwood. Hartmann and Kester (1976) had reported that root formation in cuttings was influenced by the nitrogen level in stock plants and a low level of nitrogen favoured rooting. However, Hambrich *et al.* (1988) had reported a positive correlation between nitrogen content and root emergence in stem cuttings of *Rosa multiflora*.

The C/N ratio also revealed a similar trend as that of carbohydrate content. The softwood had the highest C/N ratio followed by semihardwood and hardwood. As in the case of carbohydrate, two peaks i.e., during August and December were obtained in this case also (Fig.3). The month of December reported the highest C/N ratio. Sen *et al.* (1965) had shown that a higher C/N ratio was beneficial for rooting of cuttings. A positive correlation between C/N ratio and rooting is observed in this study also (Table 8).

The total phenol content revealed a picture similar to that of the nitrogen content. The lowest content favouring rooting was obtained in the month of December (Fig.4). The hardwood cuttings had the highest amount of phenols followed by semi hardwood and softwood cuttings (Table 9).

The total free amino acids had a positive correlation with rooting. There were two peaks (Fig.5) in the case of all types of cuttings. The softwood had the highest amount of amino acids followed by semi hardwood and least content was recorded in the hardwood cuttings (Table 10). The highest rooting percentage was obtained in the month of December when the total amino acid content was maximum.

Summary

6. SUMMARY

The present investigations entitled "Characterisation of dioecy and standardisation of propagation through cuttings in allspice [*Pimenta dioeca* (L.) Merr.]" were undertaken in two separate experiments at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara and the Regional Agricultural Research Station, Ambalavayal during the period 1997-1999. The results of the study are summarized below:

The first part of the study consisted of the morphological and biochemical variations between sexually differentiated male and female plants as well as one year and four year old seedlings.

Among the different morphological characters studied, spread of the tree, canopy shape, height at first branching, number of leaves per flush, venation and petiole length had no significant difference between the male and female plants and one year and four year old seedlings. On the other hand, height of the tree and number of primary and secondary branches showed significant differences. The female plants were taller with lesser number of primary and secondary branches as compared to males.

In the case of leaf characters, elliptic leaves were observed in both male and female plants. Female plants had a higher leaf area ($50.50m^2$) than the males ($43.71m^2$).

The biochemical studies in sex differentiated mature trees revealed that carbohydrate(6.02%), phenols (0.26%) and C/N ratio (71.51) were higher in the male trees whereas the total free amino acids (0.054%), essential oil (1.61%) and oleoresin (18.03%) were higher in female plants.

Results of the TLC studies with respect to carbohydrates, phenols and amino acids are in support to the above results. The TLC profile of carbohydrate was same for both male and female plants. There were seven different amino acids in females and only six in the case of males. The one year old seedlings showed no similarity to any of the sex but the profile of the four year old seedlings could be compared to that of sex differentiated plants.

None of the biochemical characters i.e total carbohydrates, nitrogen, phenol and total free amino acids of one year old seedlings did not confirm to a definite pattern and did not give any indication as far as sex is concerned, while the four year old seedlings had all the biochemical constituents comparable with that of the sex differentiated male or female plants. Out of the 25 seedlings (four year old) studied, 16 plants showed affinity towards maleness and others towards femaleness. However the results can be confirmed when these seedlings come to flowering.

The second part of the study on propagation included vegetative propagation with treatment combinations having three different growth regulators (NAA, IAA and IBA) at three different concentrations (1000, 1500 and 2000 ppm) tried on four different types of cuttings i.e, softwood, semihardwood, hardwood and seedling tip.

Among the different types of cuttings, softwood cuttings performed better than semi-hardwood and hardwood cuttings with respect to rooting.

Treatments with growth regulators in general resulted in better rooting efficiency when compared to control. Among the growth regulators tried IBA was found superior to NAA and IAA.

Intermittent mist was found to favourably influence the rooting percentage of cuttings. Among the interaction involving environment and types of cutting, the softwood cuttings kept in mist chamber showed maximum rooting efficiency.

The best month favouring rooting was December followed by August in leafy softwood cuttings treated with IBA 1000 ppm followed by semi hardwood cuttings (IBA 1000 ppm). Presence of leaves was found to be a beneficial for enhancing rooting efficiency.

Biochemical studies revealed that a higher content of carbohydrate and amino acids during the month of December and August favoured rooting. On the other hand, lower nitrogen and phenol showed a positive sign of rooting. The C/N ratio had a positive correlation with rooting percentage.

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

Arumugam, R. and Kumar, N. 1980. Effect of leaves on rooting of stem cutting of bergamot mit (*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

Benson, B.L. 1982. Sex influences on foliar trait morphology in asparagus. *Hort. Sci.* **17**(4):625-627

Bhat, V., Hegde, D. and Sulikeri, G.S. 1989. Effect of growth regulators on rooting of air layers of cinnamon. *Karnataka J. agric. Sci.* **2**(4):328-329

Bhattacharya, R.K. and Rao, V.N.M. 1982. Phenolics content of Co-2 papaya (*Carica papaya* L.) as effected by growth regulants. *J. Res. Assam agric. Unsty.* **3**(2):214-215

* Billau, W., Buchloh, G., Geiger, F. and Hartmann, D. 1987. HPLC – analysis of phenolic compounds in storage roots of white asparagus (*Asparagus officinalis*) during the vegetative period. *Asparagus Res. Newslett.* 4(1):18-19

Bojappa, K.M. and Singh, R.N. 1974. Sex identification in papaya in nursery. *Punjab hort. J.* **14**:14-20

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

* Chapman, G.P. 1964. Some aspects of dioecism in pimento (allspice). *Ann. Bot.* N. S. **28**:451-458

*Chapman, G.P. 1965. A new development in the agronomy of pimento, *Caribean Quarterly* **2**:1-12

* Chen, P.K., Fan, C.J., O'-Brien, W. and Venketeswaran, S. 1985. Preflowering sex determination : an aid to jojoba propagation. Proceedings of the sixth International conference on jojoba and its uses. Ben-Gurion University of the Neger: Beer Sheva, p.243-251

Choudhari, R.S., Garg, O.K. and Borah, P.C. 1957. Physiological changes in relation to sex in papaya. *Phyton* 9(15):137-141

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

Crompton, M.E. and Preice, J.E. 1986. Exudation and explant establishment. *Newslett.* IAPTC **50**:9-18

CSIR. 1956. *The Wealth of India (Raw Materials).* Vol.IV. Publications and Information Directorate, CSIR, New Delhi, p.116

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

*Dienum, H. 1931. De noot musaat cultur op de Banda, (Nutmeg culture in Banda Islands). *Landbow. J.* **1**:467-492

Dutta, P.K. and Majumdar, B.C. 1989. Studies on protein content of male and female papaya (*Carica papaya* L.) trees. *South Indian Hort.* 37(5):295

Dyurdevich, L., Chulafich, L., Kozhomara, B., Kof, E. and Kefeli, V. 1992. Content of phenolic compounds in relation to sex expression in the dioecious plant sorrel. Fiziologiya I *Biokhimiya Kul' Turmykh Rastenii* 24:64-68

* 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

* Flach, M. 1966. Nutmeg cultivars and sex problems. *Eng. Summ. Meded. Landb. Hogesh.* **66**:1

Founda, R.A. and Schmidt, G. 1994. Rooting ability of rose 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

Gauda, M.C., Thimmaraju, K.R. and Farooqui, A.A. 1991. Studies on floral biology and fruit set in bursea (*Bursera delpichiana*). *Indian perfumer* **35**(1):221-222

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

Green, C.L., Espinosa, F. and Lawrence, B.M. 1988. Flavors and fragrances : a world perspective. Proc. of the 10th International Congress of Essential Oils, Fragrances and Flavours. **18**:3-20

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.* Vol.II. Dioscorides Press. Port land, Oregon, p.29-43

Halkandar, P.M., Nagwekar, D.D., Desai, A.G., Patil, J.L. and Gunjate, R.T. 1995. Air layering in allspice. *Indian Cocoa Arecanut Spices J.* **19**(2):56-57

Hambrick, C.E., Davis, T.D. and Sankhla, N. 1988. Adventitious root formation in cuttings. *Advances in Plant Sciences Series.* Vol.II. Dioscorides Press, Port land, Oregon, p.29-43

Harborne, J.B. 1973. *Phytochemical Methods.* Chapman and Hall, London, p.416

Hartman, H.T. and Kester, D.E. 1976. Plant propagation – Principle and Practices. Prentice – Hall of India Pvt. Ltd., New Delhi, p.342

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

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

Hedge, S.S. 1989. 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 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 Botasnicheskogo* Sada **60**:72-76

* Janse, J.M. 1898. De mootmuskatcultuur inde Minahasa en op de Bande cilanden. Med's lands Plantent **28**:1-233

Jindal, K.K. and Singh, R.N. 1976. Modification of flowering pattern and sex expression in *Carica papaya* by morphactin, Ethephon and TIBA. *Zeitschrift fiir Pflanzenphysiologic* 5(78):403-410

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

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

Kanthaswamy, V. and Pillai, O.A. 1995. Studies on the floral biology of allspice. *Spice India* **6**(3):18

Khan, M.A.A., Rahman, M.A., Uddin, M.N. and Hossain, M.Z. 1982. Observations on amino acid contents in male and female papaya plants. *Bangladesh Hort.* **10**(2):27-29

Knapp, S., Person, V. and Blackmore, S. 1998. Pollen morphology and functional dioecy in *Solanum* (Solanaceae). *Plant – Systematics and Evolution* **210**(1-2):113-139

Kohorn, L.U. 1994. Shoot morphology and reproduction in jojoba : advantages of sexual dimorphism. Ecology **75**:2384-2394

*Kotaeva, D.V., Chkhubianishvili-El and Kezeli, T.A. 1982. Leaf mesophyll in dioecious representatives of the Moraceae in relation to questions of sex differentiation. *Bull. Acad. Sci. Georgian* SSR. **108**:149-152

* Kovaleva, L.V., Khryanin, V.N. and Chailakhyan, M.K.H. 1980. Specificity of proteins in the expression of sex in hemp plants. *Doklady Akademii Nauk SSR* **252**:1275-1276

Krishnamoorthy, B. and Rema, J. 1991. Air layering in cassia. *Spice India* 5(3):13-14

Krishnamoorthy, B., Zachariach, J.J., Ravindran, P.N. and Gopalan, A. 1992. Identification of sex of nutmeg seedlings based on morphological and chemical characters. *J. Pln. Crops* (Supplement) **20**:194-199

Kudesia, V.P. and Jetley, U.K. 1995. *Environmental and Biochemistry.* Pragati Prakasan, Meerut, p.574

Lacombe, J.P. 1980. Sex discrimination from early vegetative characters in dioecious hemp (*Cannabis sativa* L.) *Physiologic vegetable* **18**:419-430

Lesham, Y. and Ophir, D. 1977. Differences in endogenous levels of gibberellin activity in male and female partners of two dioecious tree species. *Ann. Bot.* **41**(172):375-379

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

Longo, G.P.H., Rossi, G., Scaglione, Longo, C. and Soave, C. 1996. Sexual differentiation in *Asparagus officinalis*. *Sexual Plant Reproduction* 3(4):236-243

Machon, N., Deletre, L.B.V. and Rarneau, C. 1995. Quantitative analysis of sexual dimorphism in Asparagus. *Can. J. Bot.* 73:1780-1786

Mathew, L., George, S.T. and Krishnan, S. 1996. Estimation of leaf area in *Garcinia cambogia* (Kodampuli) through linear measurements. *J. trop. Agric.* **34**:61-62

Mathew, P.A. and Joseph, J. 1982. Epicotyl grafting in nutmeg. *J. Pln. Crops* **10**(1):61-63

Mayer, S.S. and Charlesworth, D. 1991. Cryptic dioecy in flowering plants. *Trends in Ecology and Evolution* 6(10):320-325

* McDaniel, R.G., Barnett, J. and Anderson, J.O. 1979. Protein electrophoresis to determine sex of jojoba (*Simmondesia chinensis*) before flowering. *J. Arizona – Neveda Acad. Sci.* **14**:79-88

* Meige, J. 1981. Protein and enzyme differences between male and female tubers of *Dioscorea opposita* and *Dioscorea trifida* yams. International Seminar, Pointe – a Pitre, 28 July to 2 August 1980 (France INRA), p.279-280

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

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

Nair, B.K., Rai, R. and Vatsala, P. 1976. A simple morphological technique for distinguishing the sex of nutmeg seedlings. *Curr. Sci.* **46**:156-157

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. and Kochar, V.K. 1991. *Vegetative Propagation of Plants*. Kalyani Publishers, New Delhi, p.1-226

Nazeem, P.A., Nair, P.C.S. and Babu, L.C. 1981. Flower development, floral characters and pollination in nutmeg. *Indian Cocoa Arecanut Spices J.* **5**:2, 29-32

Nel, A. 1994. Allspice-the spice with three flavours. *Inligtings Bull.* **259**(10):25-28

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

Nyong 'O, R.N., Cobbinah, J.R. and Appiah, K.J. 1994. Flowering and fruiting patterns in *Milicia excelsa* and *Milicia regia*. *Ghana J. For.* **1**:19-29

Oyama, K. 1990. Variation in growth and reproduction in the neotropical dioecious palm, *Chamedorea tepejilote*. *J. Ecol.* **78**:648-663

Packiyasothy, E.V., Janz, E.R. and Dharmadara, H.M. 1991. Studies on some chemical components of nutmeg (*Myristica fragrans* Houtt.) leaf directed at determination of sex of seedlings. *Journal of the natural science council of Sri Lanka* **19**:91-97

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

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

Panse, V.G. and Sukhatme, P.V. 1995. *Statistical Methods for Agricultural Workers* (4th ed.). Indian Council of Agricultural Research, New Delhi, p.347

Phadnis, N.A. and Choudhari, K.G. 1971. Sex determination in the seedling stage of nutmeg (*Myristica fragrans* Houtt.). *Trop. Sci.* **8**:265-274

Philip, J., Nair, G.S., Premalatha, T. and Sudha Devi, 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

Prasad, V.V. and Iyengar, E.R.R. 1982. Physiological differences in the male and female plants of jojoba. *Curr. Sci.* **51**(21):1039-1040

* Prestoe, 1948. Sex of nutmeg trees. *Gardeners Chron.* **148**(1):135

Purseglove, J.W. 1974. *Tropical Crops - Dicotyledons*, Vol.I and II combined. Longman group Ltd., London, p.409-414

Pursegolve, J.W., Brown, E.G., Green, C.L. and Robbins, S.R.J. 1981. *Spices* (1st ed). Longman, London pp.286-339

Rao, O.P., Singh, R.N. and Singh, B.P. 1985. Sex identification in papaya through colorimetric tests and morphological characters of leaf petiole. *Progressive Hort.* 17(4):340-346

Rema, J. and Krishnamoorthy, V. 1994. Allspice. *Spice India* 4(10):9-10

Rodriquez, D.W. 1969. *Spices, Pimento, A Short Economic History.* Minist. Agric., Jamaica: Commod. Bull. No.3.

Rojast, Y., Ramos, R.R. and Salazar, C.R. 1985. Possible relation between sex and morphological and agronomic characteristics of papaya. *Acta Agronomica* 35(2):20-33

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

Sadasivam, S. and Manickam, A. 1992. *Biochemical Methods.* New Age International Publishers and Tamil Nadu Agricultural University, Coimbatore, p.373

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

Sen, P.K., Sen, S. and Basu, R.N. 1965. Studies on rooting of cuttings. 11 effect of auxin. *Indian J. Pl. Physiol.* **8**:36-50

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

Sherly, R. 1994. Growth, flowering, fruit set and fruit development in Kodampuli (*Garcinia cambogia* Derr.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur.

Singh, J., Bindra, A.S. and Bhambota, I.R. 1977. Sex identification in papaya from seed and seedling characters. *Punjab hort. J.* 17(3):139-142

Singh, R.N. and Jindal, K.K. 1974. Studies on sex determination in papaya seedlings at nursery stage. *Proceedings of the XIX International Horticultural Congress. I Section VII. Fruits* (Ed. Antiszewski, R., Harrison, L. and Zych, C.C.) International Society for Horticultural Science, Warsaw, Poland p.457-542

Snell, F.D. and Snell, T.C. 1967. *Colorimetric Methods of Analysis.* D. Van. Nostran Co., New Delhi

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

* Talyshinskii, G.M. 1982. Banding patterns of proteins in the leaves of polyploid forms of mulberry. *Doklady Akademii Nauk SSR* **38**(5):62-64

Tepper, C.S. and Anderson, A.J. 1984. The genetic basis of plant pathogen interaction. *Phytopathology* **74**:1143-1145

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

Thomas, P. 1997. Characterisation of dioecy in nutmeg (*Myristica fragrans* Houtt.). M.Sc. (Hort.) thesis, Kerala Agricultural University, Vellanikkara, Thrissur

Tucker, A.O., Maciarello, M.J. and Landrum, L.R. 1992. Volatile oils of Caribbian Myrtaceae. *J. Essential Oils Res.* **2**(4):195-196

Vadivel, E., Ponnuswamy, V., Irulappan, I. and Dharmaraj, G. 1981. Vegetative propagation in cinnamon (*Cinnamomum zeylanicum*) *South Indian Hort.* **29**(4):231-232

* Vardar, Y. 1968. *Transport of Plant Hormones*. North-Holland Pub. Amsterdam, Netherlands, P.156-191

Variyar, P.S. and Bandyopadhay, C. 1995. Estimation of phenolic acids in cinnamon, clove, cardamom, nutmeg and mace by high performance liquid chromatography. *J. Spices Aromatic Crops* 4(2):129-134

Veek, M.E. and Russell, G.F. 1973. Chemical and sensory properties of pimento leaf oil. *J. Food Sci.* 38(6):1028-1031

* Original not seen

Appendices

APPENDIX I

Monthly averages of weather data for 1998-1999 at Ambalavayal

Month	Temperature °C		Soil temperature °C				RH		Rainfall (mm)	No. of rainy days	Evaporation (mm)	Wind speed Km/hr
	Max	Min	FN 7.25		AN 2.25		Morning 7.25	AN 2.25				
			5 cm	10 cm	5 cm	10 cm						
April 1998	31.9	20.8	26.4	26.4	38.6	34.1	91	56	61.2	8	4.2	3.1
May	30.5	20.8	25.0	25.6	37.8	33.6	92	67	96.8	7	3.6	2.6
June	26.1	19.7	22.9	23.3	30.3	28.1	95	81	353.2	18	1.9	5.2
July	24.8	19.2	21.5	21.7	27.5	25.5	96	84	524.8	27	1.0	2.9
August	25.6	19.5	22.0	22.5	28.8	26.8	97	84	200.0	17	1.8	1.7
September	25.6	18.8	21.8	22.3	29.0	26.9	96	82	182.8	15	1.9	2.4
October	25.6	18.5	21.4	22.0	29.1	26.8	95	78	167.6	14	1.9	2.7
November	26.1	18.1	21.1	21.9	30.1	27.5	94	76	120.6	8	2.4	2.3
December	25.9	16.7	19.9	21.0	30.8	27.3	92	62	34.4	3	2.8	4.8
January 1999	27.5	15.0	19.6	21.5	35.8	30.5	89	42	-	-	3.4	3.0
February	30.1	16.8	22.0	23.5	37.7	32.4	86	45	16.0	2	4.3	4.2
March	31.5	19.00	24.3	26.0	40.9	35.5	94	44	18.4	2	4.7	3.0
April	29.7	19.3	23.7	24.8	36.8	33.1	92	59	100.2	4	3.9	3.0
May	26.3	19.2	22.5	23.2	30.8	28.5	95	79	162.6	13	2.8	3.8
June	26.1	18.6	21.5	22.0	30.8	28.2	92	76	187.2	12	2.2	3.1
July	24.3	18.4	21.0	21.4	26.8	25.2	95	85	429.8	24	1.0	3.4
August	27.0	18.6	21.0	21.6	29.5	27.3	94	80	192.8	13	2.1	2.4

APPENDIX II

Analysis of variance for plant characters of mature male and female plants

	df	Mean sq.	F value
Height of plant	1	3.113	22.808
Spread	1	0.020	0.016
Height of 1st branching	1	270.480	3.531
No. of primary branches	1	194.80	5.217
No. of secondary branches	1	120.050	4.475
No. of leaves/flush	1	0.000	999.990
Petiole length	1	0.002	0.200
Leaf area	1	230.249	9.449

CHARACTERIZATION OF DIOECY AND STANDARDIZATION OF PROPAGATION THROUGH CUTTINGS IN ALLSPICE (*Pimenta dioeca* (L.) Merr.)

By

V. S. SREEJA

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Horticulture

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ABSTRACT

Investigation on "Characterisation of dioecy and standardisation of propagation through cuttings in allspice [*Pimenta dioeca*(L.) Merr.]" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara and there Regional Agricultural Research Station, Ambalavayal, during 1997-1999 with the objectives to characterize the dioecious nature of allspice and to standardize vegetative propagation through cuttings so as to overcome the problems due to dioecy.

Among the different morphological characters studied, height of the plant and number of primary and secondary branches were the only two characters which showed significant difference between male and female plants. The female plants were taller with lesser primary and secondary branches. With respect to the leaf characters, female plants recorded a higher leaf area than the males.

The biochemical characters analysed showed that the females had a higher content of total free amino acids, essential oil and oleoresin. On the other hand phenols, nitrogen and carbohydrate were higher in males.

The TLC profile of carbohydrate was same for both male and female plants. There were seven different amino acids for females and only six in the case of males. The TLC profile of one year old seedlings showed no similarity to any of the sex but the profile of the four year old seedlings could be compared to that of sex differentiated plants.

The results on biochemical characterization of dioecy can be considered as a basic information for identifying a specific biochemical factor such as enzyme for sex determination in allspice.

The results of the investigations on propagation through stem cuttings suggested leafy softwood cuttings as the comparatively better plant part for rooting (30%) as compared to other three types of cuttings. Treatment with growth regulator in general gave higher rooting and among the various growth regulators, IBA (1000 to 1500 ppm) was found to be superior. The study also showed that the presence of leaves was essential for enhancing rooting efficiency in allspice cuttings.

Biochemical studies revealed that a higher carbohydrate and amino acid contents and a higher C/N ratio had a positive correlation with rooting. On the other hand, total phenol and nitrogen contents in low amounts enhanced rooting and higher amounts disrupted rooting.