STIMULATION OF GROWTH AND INDUCTION OF VARIABILITY IN MANGOSTEEN (*Garcinia mangostana* L.)

By

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THESIS

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DECLARATION

I hereby declare that the thesis entitled **"Stimulation of growth and induction of variability in mangosteen (*Garcinia mangostana* L.)"** is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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Dedicated to

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Introduction

1. INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is identified as one of the fruit crops deserving priority attention with a potential for increased acceptability (Ito and Hamilton, 1990; Osman and Milan, 2006). It is regarded as the "queen of tropical fruits" due to its instant visual and taste appeal (Cruz, 2001) and has recently been popularized for its medicinal benefits (Iinuma *et al.*, 1996; Sakagami *et al.*, 2005; Mahabusarakam *et al.*, 2006; Ngawhirunpat *et al.*, 2010). It is a very important crop of warm humid tropics. It belongs to the family Clusiaceae which contains about 35 genera and up to 800 species. Martin *et al.* (1987) considered four species of *Garcinia* to be widespread, well-known and often used in Asia. These are *Garcinia mangostana, G. cambogia, G. dulcis* and *G. tinctoria.* Of the species of *Garcinia,* about 40 produce edible fruits (Pynaert quoted by Bourdeaut and Moreuil, 1970; Yapwattanphum *et al*., 2002).

Mangosteen is indigenous to Malay Archipelago (Burkill, 1935) but the present distribution stretches from Southern India through the Malaysian region to as far as Philippines. The major producing countries are located in South- East Asia, namely, Thailand, Malaysia, Philippines and Indonesia. Thailand is the world's largest producer with an approximate production of 2.4 lakh metric tons annually accounting for 85 per cent of the total production, with exports recorded at 0.15 lakh MT in 2006 (Diczbalis, 2009).

In India, its cultivation has been attempted in many regions, but so far, it has successfully been established mainly in South India on the lower slopes of Nilgiris, between 1200 and 3500 feet above mean sea level and near Courtallum in Tamil Nadu. Another production centre in the country is concentrated in Kerala and the major cultivated areas are parts of Kottayam and Pathanamthitta

district and to a limited extent in Pariyaram village of Chalakkudi in Thrissur district.

It is a slow growing tree well-known for its fruits particularly in Southeast Asia. The fruits possess a sweet pulp which is eaten fresh, but also used in processed form. It is grown in other parts of the tropics and is one of the most praised tropical fruits. It yields profusely and fits very well as a component in the homesteads of Kerala. Though it is a crop with immense potential as a monocrop and as an intercrop in coconut gardens with very high domestic and foreign demand, its cultivation on a commercial basis is limited by its long gestation period of 10- 15 years (Lim, 1984; Richards, 1990; Wiebel *et al*., 1995). The seedlings used for commercial planting are extremely slow growing both at the nursery stage and in the orchards. The slow growth rate and consequent long pre-bearing phase has been a cause of concern wherever mangosteen is grown. Accelerating growth and reducing gestation period is a pre-requisite for extensive commercialization of the crop.

Mangosteen is unusual in that phenotypic differences are uncommon. Almost all trees are female (Morton, 1987). It is mainly propagated through seeds. Tree is an obligate apomict and hence seeds are not zygotic but are parthenogenetic, giving rise to true to type seedlings. It is assumed that presently cultivated trees may represent a single clone of female trees that produce fruits parthenocarpically. Hence variability is very limited in the cultivated types. Absence of genetic variability has become a major constraint in the conventional breeding of mangosteen which is apomictically propagated. Induction of mutation is an important tool for the production of new types in such vegetatively propagated species. As the seed is non-zygotic, there is better scope to induce variability through mutagenesis of seeds. Induction of variability in the crop, its

assessment and selection of promising types and popularization of the improved variety play major role in extending its commercial cultivation to more areas.

Cultivation of a perennial fruit tree crop like mangosteen certainly requires a scientific approach on accelerating growth and reducing juvenile phase to overcome constraints to production and induction of variability in the crop for evolving better genotypes. In this context, present investigation on "Stimulation of growth and induction of variability in mangosteen (*Garcinia mangostana* L.)" was taken up with the objective of developing techniques for accelerating seedling growth, reducing gestation period and inducing variability through mutation and polyploidy breeding in mangosteen.

Review of Literature

2. REVIEW OF LITERATURE

Mangosteen, the queen of tropical fruits is a very important crop of warm humid tropics (Almeyda and Martin, 1976; IBPGR, 1986; Kusumo and Verheij, 1994). It has been considered as the most delicious fruit of the tropics and hence called "the queen of the fruits" (Fairchild, 1915) or the "finest fruit of the world" (Dahlgren, 1947). Exceedingly slow growth of seedlings, long juvenile period of 10-15 years, lack of reliable method of propagation, few suppliers of planting materials and intensive labour requirements in harvesting are attributed as the reasons for the slow development and commercialization of the crop (Chong, 1992).

2.1. Description of species

The genus *Garcinia* was named by Linnaeus for Laurent Garcin (1683- 1757), a Swiss botanist with the Dutch Indies Company, who had published the first description of mangosteen (Corner, 1988). *Garcinia* is a large genus that consists of about 400 species (Campbell, 1967; Richards, 1990) distributed in the tropics of the world chiefly in Asia, Africa, Australia and Polynesia (Parthasarathy *et al.,* 2010). Lamoureux (1980), Corner (1988) and Verheij (1992) provided the following description on *Garcinia mangostana* L. It is a small or medium height evergreen tree, 6- 25 m with a strong trunk, symmetrically branched to form a conical crown. Leaves are opposite, entire and cuspidate at the apex, oblong elliptical, shortly petiolate (1-2 cm) and the apical pair of leaves on a branchlet are clasping to conceal the terminal bud. Leaves 15 - 25 cm long and 4.5 -13 cm wide, shining and coriaceous, dark green, rarely yellow green, glabrous above, dull pale green beneath. Central and lateral veins of leaves paler in colour than the lamina and obvious to the eye. Trees bearing male flowers are unknown, although described by Roxburgh in 1832, Richards (1990) states that this must have been the flower of a related species.

Mangosteen shows obligate agomospermy. Female flowers are solitary, paired or rarely 3 at apices of branchlets; pedicels 1.75 – 2.00 cm long and thick. Sepals 4 in 2 pairs, outer ones yellow-green 2 cm long, inner ones smaller with red margins. Petals 4, broadly ovate, 2-5 cm long and thick fleshy, yellow green with red margins or more or less entirely red. Staminodes many and shorter than the ovary, 1-2 seriate and 0.5 cm long. Ovary is broadly ellipsoid to globose, sessile and 4-8 celled. Stigma is sessile, 4-8 radiate and large in diameter. Fruit is a depressed globose shaped berry with thick pericarp, dark purple in colour with fleshy sweet aril. Fruits retain persistent sepals and stigma lobes. All parts of the plant contain yellow latex.

The species is not known in the wild. Records of occasional trees in forest area almost certainly represent relics of former cultivation or access points to the forest where fruits have been discarded or may be misidentification of a related species such as *Garcinia malaccensis* (Richards, 1990). Mangosteen was described as *Mangosteena garcinia* by Gaertner in 1790, but Linnaeus' description of the genus *Garcinia* meant that the valid taxonomic name is *Garcinia mangostana* L. (Osman and Milan, 2006).

2.2. Origin and distribution

Mangosteen as a cultigen is indigenous to the Malay Archipelago (Wester, 1921; Bailey, 1946; Ochse *et al.,* 1961; Gil *et al.,* 1972). Its cultivation extends throughout Southeast Asia, Myanmar and Indo-China, where it has diffused as a home garden and wayside species; although in recent times small orchards have been established in these regions, especially in Peninsular Malaysia, Borneo, Java and the Philippines.

Despite the many species with edible fruits, mangosteen is thought to be closely related to only two other species: *Garcinia hombroniana* and *Garcinia malaccensis*, themselves indigenous to Malaysia, although the distribution of *G.*

hombroniana extends to the Nicobar Islands. *Garcinia hombroniana* is mostly wild but also planted because its wood is valued and plant parts are used in medicine. *Garcinia malaccensis* is always wild never planted or cultivated and is a scarcer species with a scattered distribution.

Richards (1990) reported that mangosteen is an obligate agamosperm and both the close relatives of mangosteen are facultative agamospermous species and are diploid. It is suggested that the mangosteen is an allopolyploid derivative of these species which arose as a female form from a single hybridization event in cultivation, which has since reproduced asexually. *Garcinia hombroniana* is considered to be the female parent and *Garcinia malaccensis* as the male parent. Chromosome number has been found to be 2n= 56-76, 88-90-96, 120-130 (Verheij, 1992).

2.2.1. Spread of cultivation

Mangosteen is an important seasonal fruit throughout Southeast Asia. It is mainly consumed fresh and is regarded by many as one of the best known flavoured fruits in the world. Demand often exceeds supply and as with most tropical fruits, the main markets of mangosteen are close to the areas of production.

The major producing countries are in Southeast Asia, namely Thailand, Malaysia, Philippines and Indonesia. Thailand is the world's largest producer. About 85 per cent of the total production of the four countries is in Thailand (Diczbalis, 2009).

Apart from the cultivation of mangosteen in Southeast Asia and Myanmar, it was introduced into Sri Lanka around 1800 and thrives there in moist regions up to 600 m above sea level (Macmillan, 1935). It was first cultivated in India during the eighteenth century and between 1880 and 1890, plantings were made at the Kallar and Burliar stations in erstwhile Madras State (Krishnamurthy *et al.,* 1964). It is now

mostly seen on the lower slopes of the Nilgiri Hills between 360 and 1060 m and near Courtallam. The bulk of the production in India is concentrated in homesteads of Kerala and the major cultivated areas are parts of Kottayam and Pathanamthitta district. Another small production centre is in Pariyaram village of Chalakkudi in Thrissur district. The present centres of production are catchment areas of the rivers Pampa, Achancoil, Manimala and Meenachil and the low lying areas. Another point is the hill areas of Wyanad where also it is found in home gardens. The major harvesting season in the plains is from the end of May to mid June while in high altitudes it is during August – September. Thus in Kerala, fruits are available in two distinct periods (George *et al.,* 1996).

Mangosteen was introduced to the West Indies before 1955 and the seed was distributed through the Royal Botanic Gardens at Kew, UK (Popenoe, 1928). Outside Southeast Asia, cultivation was more in the West Indies. Karp (2010) reports that commercial cultivation and marketing in the United States started just recently. Native to Indonesia and Malaysia, mangosteen trees require a fully tropical climate and cannot be grown commercially in the contiguous United States. For more than a century, attempts have been made to cultivate mangosteen for the United States market; in the 1990s, small plantings were established in Hawaii and Puerto Rico, some of which are beginning to bear fruit. Interest in the purported health benefits of mangosteen has boosted public awareness and consumption of mangosteen products in recent years. Mangosteen was introduced in the Brazil in 1935 and currently is cultivated mainly in the states of Para and Bahia (Sacramento *et al.,* 2007). The principal harvesting season is usually from January to May and a small second crop in August and September.

2.2.2 Constraints to the spread of mangosteen

Mangosteen trees pass through a juvenile phase which can last anything up to 12 to 20 years. The tree has exacting ecology limiting it to 10 ° N and 10 ° S of the

equator, but up to 18° in frost free areas such as Malagasy and Queensland. A short dry season is needed to stimulate flowering but mangosteen can flower twice a year or sporadically or erratically. Annual rainfall of 1270 mm is necessary and the ideal temperature range is 25 to 35° C with RH over 80 per cent (Krishnamurthy and Rao, 1962; Bourdeaut and Moreuil, 1970).

The apomictic mangosteen seedlings used for commercial planting are extremely slow growing both at the nursery stage and in the orchards (Hume, 1947; Almeyda and Martin, 1976; Wiebel *et al*., 1991, 1992a, 1995). The slow growth rate and the consequent long pre-bearing phase has been a cause of concern wherever mangosteen is grown. Though the long gestation period ranging from 10-15 years (Lim, 1984; Richards, 1990; Wiebel *et al*., 1995) can be reduced by resorting to vegetative propagation, the problem of slow growth becomes all the more conspicuous (Wiebel *et al*., 1991, 1995). Mangosteen is referred to as a crop which lacks root hairs (Richards, 1990) one of the prime reasons for slow growth as this reduced the vital link responsible for absorption of nutrients and water. Low carbon acquisition capacity and prolonged dormancy of buds at the apex have also been listed as probable causes of the slow growth rate (Downton *et al*., 1990).

2.3 Ecology

Species of *Garcinia* are found mostly in the warm and humid tropics of South and Southeast Asia as second storey trees. All wild species are adapted to shade and mangosteen is regarded as a shade tolerant tree (Ochse *et al.,* 1961; Gil *et al.,* 1972; Verheij, 1992). Specific climatic requirement limit the distribution of mangosteen to the equatorial band between 10° N and 10° S latitudes (Duclos, 1950; Verheij, 1992). Mangosteen is associated with areas of low elevation i.e. less than 500 m above mean sea level (Galang, 1955; Gil *et al.,* 1972). It can be cultivated in higher elevations but has a slower growth rate (Nakasone and Paull, 1998). Cultivation in higher altitudes is characteristic of India.

Mangosteen appears to require an uninterrupted water supply with a short dry season of 15 – 30 days, the latter initiating flowering. Ideally rainfall should be well distributed throughout the year. Juanda and Cahyono (2000) reported a suitable rainfall of 1270 – 2500 mm per year, favourably with ten wet months. However mangosteen trees can grow successfully in other areas when a constant supply of water is provided through irrigation, especially during the dry season (Ochse *et al.*, 1961; Gil *et al*., 1972; Vietmeyer, 1975). Mangosteen thrives in the temperature range of 20 – 35 ° C when RH is over 80 per cent (Bourdeaut and Moreuil, 1970) but a 20 – 25 ° C range is also acceptable for cultivation (Vietmeyer, 1975). Temperatures below 5 ° C and above 38 – 40 ° C are lethal and since growth is slowed at temperatures 15 – 20 ° C, this is not recommended for cultivation.

Shade is essential during the first 2- 4 years of growth both in the nursery and during early field establishment (Nakasone and Paull, 1998). There are no reports of photoperiod response. The photosynthetic rate is steady over a 27 – 35 ° C range, under 20 – 50 per cent shade (Wiebel *et al.*, 1995). Juanda and Cahyono (2000) reported the suitable sunlight intensity to be 40 – 70 per cent. Foliage and fruits are susceptible to sunburn in direct sunlight (Verheij, 1992).

Mangosteen can grow successfully on a wide range of soils (Campbell, 1967; Almeyda and Martin, 1978). The best soils for mangosteen cultivation are porous, deep, wet but well drained, slightly acidic, clay loams rich in organic matter (Galang, 1955; Almeyda and Martin, 1978; Juanda and Cahyono, 2000). In spite of a relatively weak root system, the trees can tolerate heavy soils which impede water movement, provided that the transpiration is limited by high humidity and shade. Under dry conditions, irrigation is needed and thick mulches are very beneficial (Verheij, 1992).

2.4. Breeding and improvement

There are no reports of improvement of mangosteen by conventional breeding. There appear to be no distinct mangosteen cultivar. The different morphological characteristics of the fruit in different regions might be due to climatic and soil factors. Genetic erosion of mangosteen has probably been severe throughout Southeast Asia and there are no truly wild populations. There are several obvious breeding constraints such as low seed production, long gestation period, gamboge, sensitivity to drought, poor root system, slow growth and apomixis. The main breeding objectives in mangosteen are developing drought resistance, modification of tree architecture, improving fruit quality by overcoming gamboge and fruit cracking and identification of suitable rootstocks (Te-chato and Lim, 2005).

Mangosteen produces apomictic seeds and it has been suggested that all mangosteen trees belong to a widespread single clone. As a result, large germplasm collections have not been set up other than to maintain stock material. Nonetheless recent research shows that variation does indeed exist within mangosteen and there is possibility of increasing variation in mangosteen (Osman and Milan, 2006). Only limited germplasm collections are held by different institutions (Sastrpradja, 1975; Valmayor and Espino, 1975; Coronel, 1983; Saw *et al.,* 1991; IBPGR, 1992; IPGRI, 2003).

Research at MARDI, Malaysia indicated regional variations in mangosteen, especially in time of fruiting, growth rate and thickness of fruit rind (Osman and Milan, 2006). Out of the 830 accessions surveyed, 16 were identified as distinctively different. In 1987, Idris and Rukayah reported on the occurrence of a male mangosteen. Richards (1990) considered that this could be a hybrid between a female mangosteen and either one of its parents, *Garcinia malaccensis* or *G. hombroniana.* This has not been tested, but the possibility exists that such chance variation occurs within the areas of distribution of the parents. There have been unsuccessful attempts

to hybridise mangosteen with *G. hombroniana*, but Richards (1990) considers mangosteen to be substantially unhybridised. Both wild parents are facultative agamosperms.

Horn (1940a) reported a form cultivated in the Philippines, locally called "Jolo", which possessed larger fruits than normal, the seeds larger and the pulp more acid. Cadillat (1970) reported on a mangosteen from the Sulu Islands with thicker fruit shell and acidic pulp but the taste was inferior.

The National Seed Industry Council of the Department of Agriculture, Philippines has released two varieties of mangosteen namely 'UPLB Sweet' in 2006 and 'Roxas Purple' in 2007 (Namuco, 2007, 2008). Both the varieties are seedling selections and have semi-spreading growth habit. The variety 'Roxas Purple' recorded an average yield of 850 fruits per tree. The oblong-shaped fruits are small with an average weight of 65 g. The apex of the fruit is somewhat pointed with a narrow stigmatic lobe. Each fruit has 1 or 2 flat and small (1.3 g) seeds. The peel is smooth, 6.4 mm thick and turns purple when fully ripe. The flesh, which constitutes about 27 per cent of the fruit weight, is snowy white, acid sweet, has mild aroma and with total soluble solids (TSS) of 21.4° Brix. Titrable acidity (TA) of the flesh is 14 mg per 100 g sample. The variety 'UPLB Sweet' recorded an average yield of 1,148 fruits per tree. The ovate-shaped fruits of 'UPLB Sweet' mangosteen are small with an average weight of 87 g. About 30 per cent of the fruits are seedless; others have only 1 or 2 small seeds. Fruit peel is smooth, 7.5 mm thick and turns purple when ripe. The flesh, which constitutes about 29 per cent of the fruit weight, is snowy white, has mild aroma and is deliciously sweet, with soluble solids of 18.65° Brix. Titrable acidity of the flesh is only 9 mg per 100 g sample.

Since the diversity in mangosteen is limited, selection of trees with outstanding characteristics is also limited. However, based on morphological

characteristics followed by RAPD analysis, the Centre for Tropical Fruit Studies of Bogor Agricultural University, Indonesia has chosen two potential parent trees and released them as new varieties, namely, Wanayasa and Puspahiang (Sobir and Poerwanto, 2007). The Centre is also developing several approaches to improve existing trees and technology package to establish mangosteen orchard, which consist of introducing new mangosteen clone 'Wanayasa', improvement of rooting system using mycorrhiza and *Agrobacterium rhyzogenes*, enhancement of tree growth using double-rootstock system, cropping system, irrigation and fertilization system, trees husbandry and harvest and post-harvest technology. Other approaches include research on genetic variability, identification and development studies on cause of gamboge and methods to overcome the gamboge problem on fruits, developing non-destructive technology for detection of gamboge and improving technique for prolonging shelf-life of fruits (Poerwanto *et al.*, 2008).

2.4.1. Mutation breeding

Mutation breeding plays a vital role as this gives quicker results than by hybridization (Ray, 1999). Treatment of seeds and vegetative propagules commonly produce chimeras. Mutations usually occur in small sectors of the meristem and as a result only a part is affected. There have been few attempts to apply induced mutations in mangosteen. In order to generate genetic variation in mangosteen, methods such as treating seed with chemical mutagens or subjecting them to irradiation is employed (Sobir and Poerwanto, 2007).

Prommee *et al.* (1999) reported varietal improvement in mangosteen by mutagen application in *in vitro*. Nodular calli were treated with ethyl methane sulfonate (EMS) and gamma radiation at various concentrations. Gamma rays at 20 and 40 Gy gave survival percentages of 84.2 and 80.8, which were significantly different from that of the control (100% survival) (Te-chato and Prommee, 1999). M1R1 from EMS treated plants had fat stems, branching, three sets of leaves per

whorl and abnormal arrangement of leaves. Forked, serrate, abnormal arrangement of leaves and dark brown leaves were observed from irradiated plantlets. M1R1 plantlets obtained from calli treated with all concentrations of EMS and 10 and 20 Gy gamma radiation showed variation of peroxidase isoenzyme patterns (Prommee, 2000).

In Indonesia, Rostini *et al.* (2003) used gamma ray irradiation with 1- 3 kR dosages to broaden the genetic variability of mangosteen to improve desired mangosteen traits. The results revealed that over 80 per cent of seeds irradiated with 1 kR and 2 kR showed variations in growth rate, plant height, leaf size, leaf colour, chlorophyll content, number of lateral roots and root length.

Seeds were irradiated with gamma rays at four different doses i.e. 0 Gy, 10 Gy, 20 Gy and 30 Gy using Gamma Chamber 4000 A (Sobir and Poerwanto, 2007). The results of the study revealed that gamma ray irradiation affected seedling growth and their rooting system, several leaf anatomy parameters such as upper cuticle thickness, spongy mesophyll thickness and leaf thickness. Higher gamma ray doses seemed to inhibit seedling growth and their rooting systems, but the effect on leaf anatomy parameters was not affected by gamma ray dose. Further RAPD analysis on several irradiated seedlings revealed that gamma ray irradiation attempt had successfully increased genetic variability of mangosteen.

2.4.2 Polyploidy breeding

Te-chato and Sujaree (1999, 2000) reported the effect of colchicine on induction of polyploidy and mutation of adventitious shoot buds and callus in mangosteen. The results showed that leaves of regenerated shoots contained more chlorophyll ‘b’ than those of control. However, shoot size, leaf area and leaf number tended to decrease while number and length of roots increased. A high concentration of colchicine, 500 mg/l, produced shoots with brown-callused leaves. However, roots could be induced from these shoots to from complete plantlets. Some of the intact

leaves produced a large number of shoots. Colchicine at 750 mg/l gave some morphological abnormalities with 5 roots per shoot. The number of chromosomes following colchicine treatment could not be determined due to their small size, however, 0.075 to 0.1 per cent colchicine caused an increase in size and colour of guard cells. Peroxidase and esterase markers indicated that there were differences between treated and non-treated plantlets of mangosteen. Morphological abnormalities of plantlets induced from treated callus included the production of new shoots on intact leaves, a large number of roots and three leaves per whorl.

2.4.3 Hybridisation

Mangosteen is self fertile but male sterile and since it has many related species which are fully fertile, hybridisation may be possible especially with closely related species such as *G. hombroniana.* In Vietnam, advances in tissue culture of mangosteen have been proved useful in embryo rescue from interspecific hybridisation (Sando *et al.*, 2001).

2.5 Variability studies

Due to its reproductive manner, mangosteen trees are essentially clonal. Variation of mangosteen in the field is predicted due to differences of environmental conditions (Sobir and Poerwanto, 2007). However several studies revealed that population from apomictic reproduction does not always carry the same genetic properties, even in obligate apomixis (Asker and Jerling, 1992). Variability in progeny of obligate apomixes plant has been reported in genus *Taraxacum* (Ford and Richards, 1985).

2.5.1 Morphological variability

Some distinct variations in morphological characters have been reported in mangosteen, such as, unusual growth habit, trees with distinct differences in yield, fruit size or length of juvenile phase (Almeyda and Martin, 1976). In Queensland,

Australia, there are several trees with an unusual growth habit. Branches point upwards and produce fruit with a distinct pointed shape (Almeyda and Martin, 1976; Verheij, 1992). Two types of mangosteen have been identified in terms of shape of fruit, one type producing a round shape with semi-flat bottom end and the other type with oblong shape fruit which cannot stand on its distal end (Steenis, 1981). A wild form containing only four carpels with fully developed seed was also found in north Borneo (Morton, 1987). In Yan Bukit Pinang, Malaysia, a tree bearing seedless fruits was reported (Thomas, 1997). Mansyah *et al.* (1999) found that mangosteen in West Sumatra show wide variability in leaf length, fruit weight and rind thickness. Mangosteen found in Tembilahan, Sumatra Island exhibit flattened fruit shape, very short peduncle and elliptic stigma lobes (Mansyah *et al.*, 2005).

In a study of four mangosteen populations in Java Island, variability was observed in several morphological characters such as tree shape, fruit shape and petal colour (Mansyah, 2002; Prabowo, 2002; Purwanti, 2002). Variation was also observed in fruit characters, such as, weight, length, diameter, length/diameter ratio, rind thickness, peduncle length, total soluble solids and presence of fruit latex. Number of locules and seed number per fruit did not vary significantly. Correlation analysis showed that TSS was correlated negatively with fruit diameter, fruit weight, fruit length, peduncle length and rind thickness whereas fruit diameter was positively correlated with fruit weight, fruit length, rind thickness and number of seed per fruit. A distinctive type which produces fruit with insignificant seed size (less than 1 cm in length), bigger fruit size, thicker rind, more acidic taste and larger leaf size was also found in Borneo (Sobir and Poerwanto, 2007).

2.5.2 Genetic variability

Mangosteen is a dioecious species and such plants are characterised by high levels of genetic diversity (Sando *et al.*, 2001). However mangosteen is unusual in that phenotypic differences are uncommon. It has been suggested by some

mangosteen growers in North Queensland that the occasional phenotypic variants observed in their plantations are as a result of cross pollination from wild related species. Whether these differences can be attributed to genetic variation or are purely a consequence of being cultivated at different geographical locations is unclear.

Of the several PCR based techniques developed during the last two decades, Random Amplified polymorphic DNA (RAPD) technique offers a best method for genotype characterisation (Williams *et al.,* 1990). Te-chato (2000) used RAPD markers to assess the genetic variation of 20 meristematic nodular callus lines and 8 somaclones from a single regenerated mangosteen leaf. Eight primers tested showed no polymorphism from each somaclone. A total of five to fifteen monomorphisms were common among those somaclones. Use of somatic embryogenesis to produce somaclones and genetic transformation may also aid in developing certain genetic base (Sanyal, 2001).

Te-chato *et al.* (2000) studied the diversity of *Garcinia* species and interspecies relationships using amplified chloroplast DNA. The results indicated that mangosteen is closely related to *Garcinia speciosa,* followed by *G. atroviridis, G. dulcis* and *G. schomburgkiana.*

A study to investigate the phenotypic and genotypic variability in mangosteen was conducted at several locations in Java and West Sumatra, Indonesia (Mansyah *et al.,* 2003). Data from 74 mangosteen tree samples were recorded for 13 phenotypic characters. Genetic studies were undertaken on 23 DNA samples from mangosteen tree samples by using RADP technique with 5 selected primers, i.e. SB 13 (AGTCAGCCAC), SB 19 (CAGCACCCAC), OPH 12 (ACGCGCATGT), OPH 13 (CACGCCACAC) and OPH 18 (GAATCGGCCA). Mangosteen exhibited significant phenotypic and genotypic variation. Three canopy shapes (pyramidal, elliptical and semicircular) and 3 fruit shapes (spheroid with acute fruit tip and prominent stigma,

spheroid with round tip and non-prominent stigma and ellipsoid with flat tip and non-prominent stigma) were observed. Genetic variation was observed by variation in DNA banding pattern. Dendrogram based on DNA banding patterns divided the accessions into 2 main groups, i.e. the first group consisted of accessions mostly originating from Wanayasa and Kaligesing, and the second group consisted of heterogenous accessions from other locations. There was no correlation between similarity based on morphological characters and similarity based on DNA banding patterns, which may be due to the fact that many quantitative characters and only a few primers were observed.

Ramage *et al.* (2004) studied the genetic diversity in 37 accessions of *Garcinia mangostana* (L.) and 11 accessions from eight other *Garcinia* species. It was observed that considerable genetic diversity exists within *Garcinia mangostana* accessions and between other *Garcinia* species. This provides a valuable framework for the genetic improvement of mangosteen.

The phylogenetic relationships among 17 *Garcinia* species including *G. mangostana*) were analysed by comparing sequences of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (Yapwattanaphun *et al.,* 2004). Both parsimonious and neighbour joining (NJ) analyses revealed that *G. mangostana* is closely related to *G. malaccensis* believed to be a progenitor of mangosteen. Another suspected progenitor of mangosteen, *G. hombroniana*, was more distant from *G. mangostana* than *G. malaccensis* phylogenetically. *G. hombroniana* formed a cluster with *G. rostrata*, *G. speciosa* and *G. sizygiifolia*, and this cluster was connected with a cluster of *G. mangostana* and *G. malaccensis*. The ITS sequence analysis showed that *G. atroviridis*, *G. cowa*, *G. dulcis*, *G. malaccensis*, *G. mangostana*, *G. rostrata* and *G. vilersiana* have nucleotide additivity (2 different nucleotides at the same nucleotide position) at several sites in the ITS region. The occurrence of these species

might be related to hybridization with ancestors, but the genomic compositions, even chromosome numbers, of these species are still unknown.

Sobir and Poerwanto (2007) reported variability in several morphological characters such as tree shape, fruit shape and petal colour though mangosteen trees have same genetic properties due to apomictic reproduction. Further studies using DNA markers confirmed genetic variability among mangosteen population. The variation may have arisen from accumulation of natural mutations. Another hypothesis is that mangosteen population may have developed from more than a single hybridization of its two wild progenitor species.

The genetic diversity of 22 accessions of the genus *Garcinia* was assessed using peroxidase, RAPD markers and gene sequence specific amplification polymorphism (GSSAP) (Wittayawannakull *et al.,* 2010). Among the 15 isozymes tested, only peroxidase produced reproducible, polymorphic bands with a polymorphism information content (PIC) of 0.79. A total of eight bands were generated forming three fingerprint patterns distinct for *G. mangostana, G. binucao, G. kydia* and *G. lateriflora*. No bands were observed for *G. livingstonei* and *G. xanthochymus*. The three RAPD primers showed high PIC of 0.92 (OPB-04), 0.78 (OPB-06) and 0.91 (OPB-07). For GSSAP markers, two sets of primers based on the conserved regions of acyl-ACP thioesterase (ACYL-ACP), and chalcone synthase (CHALCS) had relative PICs of 0.75 and 0.89 for ACYL-ACP and CHALCS, respectively. The high PICs indicate the capability of these techniques to quantify genetic diversity in *Garcinia* species. The dendrograms using UPGMA-SAHN cluster analysis based on peroxidase, RAPD and GSS amplification polymorphism showed that *Garcinia* species clustered into five groups at mean similarity coefficient 0.54. Group I consisted of all 17 *G. mangostana* accessions and was further classified into three subgroups. Group II composed of *G. kydia* and *G. lateriflora* showed a genetic similarity of 0.94. *G. livingstonei, G. xanthochymus* and *G. binucao* were unique in

their groups. This study showed that the *G. mangostana* accessions analyzed had low genetic variation and that the different species can be clearly distinguished by combined peroxidase, RAPD and gene sequence specific amplification polymorphism.

2.6. Culture

2.6.1 Seedling establishment

2.6.1.1 Seed and seed germination

The most common practice for the propagation of mangosteen is through seeds. They are not true seeds (sexual embryos) but are adventitious embryos (asexual embryos), since there is no fertilization involved. Since the seeds are formed from nucellar tissue (asexual origin), they produce seedlings that are identical to the mother plant, they are apomictic and breed true to type (Galang, 1955; Campbell, 1967). There may be little variation in the seedlings and later in the fruits produced.

Seed weight ranges from 0.5 g to 2.0 g per seed. Those which are mature are usually in the weight range of 1.0 – 2.0 g. Polyembryony is known to occur in mangosteen giving rise to up to three seedlings per seed. The phenomenon is reported to occur in as many as 11 per cent of mangosteen (Wester, 1920; 1926).

The germination of mangosteen seed is considered quite unique compared with seeds of other crop species (Vogel, 1980). During germination, swelling occurs at opposite ends of the seed and a radical and plumule emerge from opposite ends. The radical dies when adventitious roots develop from base of the plumule (Hume and Cobin, 1946; Chandler, 1958). Germination generally occurs in 14- 21 days but this ranges from 10 to 54 days depending on seed age and the growing medium. Seeds from the same plant often vary greatly in germination time in different years and seasons.

Seed handling techniques are important due to the short life of seeds. Any drying can drastically reduce germination and seeds generally lose viability in 3 – 5 days when removed from the fruit. When kept in the fruit, viability can be retained for 3 – 5 weeks (Chandler, 1958) but the resulting germination can be slower (Winters and Rodriguez-Colon, 1953).

Fresh seeds are covered by a very thin membrane which is the only protection against desiccation. Seeds can be temporarily stored in moist charcoal, peat moss or coconut fibre (Gonzales and Anoos, 1951; Winters and Rodriguez-Colon, 1953), in sealed tins with moistened charcoal, seeds survive for 3-5 weeks (Chandler, 1958). The type of temporary storage and the time of extraction from the fruit markedly affect germination (Gonzales and Anoos, 1951). Normally large (more than 1 g in weight) plump and fully developed seeds are chosen for planting. A number of studies have reported that large seeds are associated with higher viability and higher survival rates (Wester, 1916; Horn, 1940b; Hume and Cobin, 1946; Gonzales and Anoos, 1951).

2.6.1.2 Media and sowing

Seeds are usually sown directly in individual polybags or pots (Duclos, 1950). Various media may be used including soil high in organic matter (Hume, 1947) or a peat – sand (1:1 mixture) or a soil-sand (1:3) mixture. Other media with high moisture retention include shredded coconut fibres. When dealing with a large number of seeds, sowing is usually in a seed bed. A typical seedbed may be made of wood or cement and the sowing medium used can consists of a mixture of sand and soil in a 3:1 ratio by volume. The sowing medium should be deep up to 1m in depth.

Rukayah and Zabedah (1992) studied the growth of mangosteen in different potting mixtures. The best seedling growth was obtained with a 3:2:1 sand:soil:manure mixture. Increasing the organic content reduced growth. Wiebel *et*

al. (1991) observed that potting plants in a porous substrate increased growth and application of GA stimulated growth of dormant buds. Shade was found necessary for satisfactory growth.

Seeds are planted 5-10 mm in depth and spaced 2-3 cm apart if sown in a seed bed and are covered with sand. They are normally placed singly on the flat side in a horizontal position. Generally it has been reported that sprouting occurs in 2-3 weeks and is complete in about six weeks. However seeds may be sown in any position as germination still occurs when seeds are placed on their sides or even upside down. Under such conditions, seeds will germinate in about 20 – 30 days after sowing (Padolina, 1931; Gonzales and Anoos, 1951; Almeyda and Martin, 1978). By comparison, seeds which are sown without their testa removed require 4 – 5 weeks before they germinate and the seedlings derived from such seeds are normally not uniform.

2.6.1.3. Seedling growth

Under favourable conditions, seedlings can grow up to 25 cm in a year (Verheij, 1992) but normally slow growth is seen in the early stages. The slow growth is attributed to poor root development especially the development of lateral roots.

Mangosteen has a long juvenile phase. Good growth of seedling, especially leaf area, is promoted so that the juvenile phase is completed as quickly as possible; usually when the trunk has 16 pairs of laterals (Verheij, 1992) at about 5-7 years. Studies have shown that seedlings watered with nutrient solution supplemented with yeast extract grew better within 10 months than similarly treated plants receiving no yeast extract (Horn, 1940b). As the seedlings grew older and leaf area increased, the growth stimulation by yeast extract was less. Growth of all seedlings grown in dead sphagnum moss watered with nutrient solution with or without yeast extract was better than growth in soil. Trials conducted at the Bureau of Plant Industry in Davao,

Philippines showed that the application of gibberellic acid could also accelerate the early growth of mangosteen seedlings (Osman and Milan, 2006).

The growth of mangosteen seedlings was monitored over 24 months and the effects of mixtures of sand:soil:cow manure (3:2:1, 1:1:2 or 1:1:4) and container size (polyethylene bags 22 x 30 or 30 x 38 cm) on growth were studied (Rukayah and Zabedah, 1992). Seedling growth was slow for the first 12 months but then increased at a faster rate. There was a marked increase in the number of tertiary roots compared with secondary roots between months 6 and 14. After that, the weight of the tertiary roots increased markedly but the growth of secondary and tap roots was slower. The dry weight ratio of shoot and root was high during the first 18 months but fell to 4.94 after 24 months. Container size had no effect on growth. It was concluded that seedlings should be grown in the nursery for at least 2 years before transplanting.

Nutrient limitations in the production of mangosteen seedlings were studied based on foliar analysis (Moraes *et al.,* 2006). After 12 months in the nursery (initial development stage) under shaded conditions, a progressive yellowing of young leaves was observed, together with significant reductions in Fe and Mn concentrations in leaves. Foliar sprays of 0.8 mg Mn ($MnSO_4$) and 2.5 g urea $litre^{-1}$ was ineffective in initiating the recovery of the green colour of the leaf blades. However, the leaf blades turned green following the application of 0.8 mg Fe ($FeSO_4$) and 2.5 g urea $litre^{-1}$, suggesting the low Fe absorption efficiency of mangosteen despite the high concentration of Fe in the substrate of surface soil (223.7 mg dm^{-3}). This indicates the need for Fe incorporation in the substrate or Fe application on leaves during the nursery stage.

The influence of shading intensity on growth, morphology and leaf gas exchange of mangosteen seedlings in the nursery was investigated over a 2-year period (Wiebel *et al.,* 1994). Diurnal gas exchange studies revealed significantly

higher carbon gain for leaves grown in 20 or 50 per cent shade than for leaves grown in 80 per cent shade. Seedlings grown in 20 or 50 per cent shade accumulated significantly more DW than those in 80 per cent shade during the 2-year study period. Seedlings grown in decreased shade showed decreased leaf size, increased leaf thickness, lower specific leaf area (SLA) and higher stomatal frequency. Less shaded seedlings also allocated relatively more dry matter to roots than shaded seedlings and exhibited a significant reduction in leaf area relative to total plant DW (leaf area ratio). Increased leaf number, enhanced branching and shorter internodes resulted in less shaded seedlings having a more compact appearance. Irrespective of light conditions, mangosteen seedlings exhibited inherently slow growth because of low photosynthetic rates per unit leaf area, low SLA, low leaf area ratios and inefficient root systems.

The chlorophyll content of mangosteen leaves is high (Schaper and Chacko, 1991) and may confer a significant advantage by permitting increased absorption of light mainly in the green and far-red regions (Bjorkman, 1981; Chazdon and Fetcher, 1984). Mangosteen leaves in shaded condition may maintain a high chlorophyll content by compensating for the thinner palisade mesophyll through extensive grana formation as has been reported in several shade plants (Goodchild *et al.,* 1972).

Diurnal variations in soluble sugar and starch levels in mature leaves of 2-year-old seedlings with immature, semi-mature and mature terminal flushes were studied by Wiebel *et al.* (1995). Glucose, fructose, sucrose and starch concentrations in leaf extracts increased significantly after midday, reaching a maximum at 18.00 h, regardless of the ontogenic stage of the terminal flush. High translocation rates in flushing and non-flushing seedlings observed in this study suggest that assimilate production rather than translocation may be a major factor limiting the growth of mangosteen seedlings.

Young mangosteen seedlings were grown under 25, 40, 55 or 100 per cent of light intensity for two years (Issarakraisila and Settapakdee, 2008). An increase of light intensity increased the thickness of lamina resulting in an increase of palisade and spongy tissues and the stomata frequency also increased. Both chlorophyll a and b declined gradually as the light intensity increased and the average ratio was 0.808. The growth of seedlings described as leaf size, leaf number per plant, total leaf area, height, fresh weight and dry weight were dramatically reduced when exposed to 100 per cent light intensity condition. Maximum growth was found when exposed to 40 per cent light intensity condition. Dry weights of seedlings grown under 25, 40, 55 or 100 per cent of light intensity were 161.7, 201.5, 150.1 and 11.7 g per plant, respectively.

2.6.2 Propagation methods and growth of plants

Asexual propagation is currently limited and little used because plants propagated from seeds are usually more robust and reach fruiting earlier (Osman and Milan, 2006). Many attempts to root cuttings and layering have failed (Gonzales and Anoos, 1951; Campbell 1967).

2.6.2.1 Grafting

Grafting has been shown in other plant species to provide desirable characteristics such as precocity, dwarfness and plant architecture that promote economy for picking and pruning. The propagation of mangosteen could be considerably improved if a suitable and vigorous rootstock can be identified (Wester, 1926). Mangosteen seedlings themselves are not necessarily good rootstocks because of their slow growth (Winters, 1953). Several attempts have been made to identify other rootstocks that are fast growing and have vigorous root systems, but these attempts have generally failed because of incompatibility problems.

Studies have been conducted to develop better vegetative propagation techniques with the main objective of shortening the long juvenile period (Chong, 1992). The techniques developed for mangosteen are cleft grafting, saddle grafting, side-cleft grafting and approach grafting. Among these techniques, cleft grafting is the most promising. Early field evaluation has shown that the growth of cleft grafted plants is much more vigorous and possesses better tree form than saddle, side-cleft and approach materials. Moreover, some of the cleft and approach materials have flowered and fruited within four years after field planting when seedling materials planted in the same area still showed no signs of flowering. This clearly indicates that grafting can shorten the juvenility of mangosteen. In mangosteen propagation, cleft grafting is preferred over budding because the crop has no "visible" bud to be used for budding, bark does not "slip" well and success for grafting is much better than for budding. In another study, softwood grafting using sweet or sour mangosteen as rootstocks and scion from one year old shoots with current season's sprout gave promising results with an initial success of 60 – 80 per cent and final success of 50 per cent (George *et al.*, 1998).

There is no documented information on effects of rootstocks on production and stock – scion relationships. Generally all *Garcinia* species, except mangosteen, can adapt well to drought and semi-arid zones since they have good root systems, which develop horizontally (soil surface) and vertically (deep beneath the surface). At their early growth stage, they require no shade and frequent watering is unnecessary. Because of these characteristics, these species have been used as rootstocks of mangosteen for dry areas (Te-chato and Lim, 2005).

Mangosteen is compatible when grafted to itself but the resulting cleft grafted plants usually exhibit rather unique characteristics which are different from plants propagated from seeds. Grafted plants exhibit extremely stunted growth, usually together with non-upright shoot development. Cleft grafted plants show a tendency to

bend in a sideways direction when they grow, even observable at the nursery stage and plants may need to be staked to keep them upright, especially when they grow taller. The bending becomes more pronounced if the scions used in cleft grafting are taken from side shoots instead of terminal shoots of the mother plant. However, at times, cleft grafted plants can show precocity and come into first bearing very early. The first bearing sometimes occurs in saplings still at the nursery stage. When mangosteen grafted onto mangosteen seedlings, the best technique is cleft grafting as practised in Malaysia (Osman and Milan, 2006).

Webster (1915) obtained successful union by shield budding mangosteen on *Calophyllum inophyllum* and *Garcinia venulosa* using well matured but green and smooth, non-petioled bud wood, but the bud was not able to sprout and was gradually callused over. Inarching trials have shown that mangosteen is not graft compatible with plants belonging to the genera *Calophyllum, Cratoxylon* and *Rheedia* (Gonzales and Anoos, 1951). However, there are several reports which indicate success with rootstocks of the genera *Garcinia, Platonia, Pentadesma* and *Clusia* (Osman and Milan, 2006).

Most *Garcinia* species are not compatible with mangosteen. Of the Asian species of *Garcinia* used as rootstocks, only a few appear to be compatible with mangosteen. The percentage of union is very low (10 per cent for *Garcinia kydia* and 12 per cent for *Garcinia venulosa*). *Garcinia lateriflora* and *G. tinctoria* have shown, as have *G. hombroniana, G. livingstonei* and *G. morella*, fairly successful results as rootstocks (Fairchild, 1915; CSIR, 1948; Galang, 1955; Ochse *et al.*, 1961). *Garcinia speciosa* may be potential rootstock according to research at the Prince of Songkla University, Thailand. Attempts to graft mangosteen onto *Garcinia malaccensis*, one of its other parents have not been tried. (Osman and Milan, 2006). John *et al.* (2008) reported that *G. hombroniana* is an ideal rootstock for mangosteen by virtue of its well developed root system and fast growth. Graft compatibility is good and success

rate is also high in polythene mist houses. By grafting to a highly adapted rootstock like *G. hombroniana*, the cultivation of mangosteen can be extended to diverse soil types especially laterite uplands of Kerala.

Mathew *et al.* (2004) conducted grafting trials on *Garcinia spp.* to induce adaptability to marshy or wet soils. For kokum (*Garcinia indica*), the rootstocks used were seedlings of its own, of *kodampuli* (*G. gummi-gutta*), and of *G. cowa*. Scions were non-precured shoots with 2-4 leaves. In *G. gummi-gutta*, scions were leafy shoots and precured green shoots. In mangosteen (*G. mangostana*), grafting was done on its own seedlings, *G. tinctoria, G. hombroniana, G. cowa* and *G. gummi-gutta. G. gummi-gutta* and *G. cowa* were considered good alternate rootstocks for *G. indica*. Meanwhile, for *G. gummi-gutta*, aside from its own seedlings, *G. hombroniana* was considered a good rootstock. For *G. mangostana*, however, *G. tinctoria, G. hombroniana, G. gummi-gutta* and *G. cowa* were not suitable rootstocks; hence, new combinations need to be developed to induce faster growth and early bearing since grafts on its own seedlings grew very slowly. Three methods of grafting were evaluated in yellow mangosteen (*Garcinia xanthochymus*) (cleft graft, splice graft and whip and tongue graft) and 2 types of rootstocks - those with and those without active photosynthetic leaves (Almeida *et al.,* 2008). The analysis revealed no statistical difference between any two propagation methods. However, the procedure of leaving two pair of leaves below the grafting point showed a negative effect on the grafting success.

Poerwanto (2002) reported the use of nurse stock plants as a new technique to enhance growth of the crop. To improve the root system of mangosteen and enhance growth, a nurse stock plant technique has been developed. Giving an additional nurse stock plant to the mangosteen seedling provides the tree with a double root system. Nurse stock plants of *G. dulcis* and *G. fructicosa* on non-grafted seedlings enhanced seedling growth as much as twice compared to seedlings without a nurse stock plant.

Other experiments using mangosteen as the nurse stock plant of three types of mangosteen seedlings (non-grafted, grafted with juvenile scion, grafted with mature scion) showed that seedlings with a nurse stock plant, especially non-grafted plants and those grafted with juvenile scion, grew better than seedlings without a nurse stock plant. The growth of non-grafted seedlings was better than grafted seedlings. Seedlings grafted with mature scion showed the poorest growth. He observed that nurse stock plants of *Garcinia dulcis* and *G. fructicosa* on non-grafted seedlings enhanced seedling growth as much as twice compared to seedlings without a nurse stock plant.

The mangosteen, varieties 'UPLB Sweet' and 'Roxas Purple', responds very well to cleft grafting with rootstocks 1 to 2 years old (Namuco, 2007; 2008). The scions should be taken from the orthotropic (vertical) shoots that are at least 4 months old from flushing. Seed is also advisable since mangosteen is an obligate apomict; however, start of fruiting is expected to be delayed by about 5 years compared to grafted plants.

2.7. Growth studies

2.7.1 Vegetative growth

The crop like most other polyaxial tropical and subtropical trees such as rubber, mango, cashew and citrus exhibit a rhythmic growth habit each under relatively constant environmental conditions of the tropics (Alvim, 1964; Wareing, 1970; Borchert, 1973). The relationship between vegetative growth and fruiting was studied in deciduous fruit trees by various workers like Gustafon (1926), Reed (1929), Barnard (1932) and McMunn (1939). Later these studies were continued in tropical and subtropical tree crops like mango, jack, guava, sapota, citrus etc.

A detailed study on Malabar tamarind (*Garcinia cambogia* Desr.), a related species of mangosteen showed that shoot growth is seasonal with one main flushing

period commencing from January and extending up to May. However, scattered flushes occurred throughout the year with mean growth varying significantly from month to month and having a peak during the summer months (George *et al*., 1992; Sherly, 1994).

Bourdeaut and Moreuil (1970) reported production of three vegetative flushes in mangosteen from Malagasy Republic. Alex (1996) in a study on vegetative, floral and fruit characters in mangosteen observed that shoot growth in mangosteen coincided with the main flushing season from June to August and with a second one from January to February. Maximum shoot growth was observed during July. The growth of the tree was slow with an extension of 6.91 cm in a year.

The effects of canopy manipulation on water use and yield of mangosteen were studied (Sakdiseata *et al.,* 2000). Results showed that significant differences in water use among treatments occurred during the period of fruit-setting and fruit development. Significant differences in some characters of fruit quality among the treatments were also found. It is suggested that canopy manipulation affects water use and yield of mangosteen.

To optimize crop load of mangosteen, fruit density and leaf number : fruit were assessed using a framework of quadrant cube (0.5 x 0.5 x 0.5 m). Relationship between fruits per quadrant and fruit number per plant was found, and leaf number : fruit was also related to fruit yield per plant. These results indicate that the assessment of fruit density and leaf number : fruit is of benefit for crop load management. Thus, nine fruits per quadrant and 18 leaves : fruit are recommended to optimize crop load of mangosteen (Sdoodee and Phonrong, 2006).

In another study to assess crop load effect on yield and quality of mangosteen fruits, it was found that the mangosteen trees in the treatment of 1001-1500 fruit per

plant provided a significantly high yield (84.23 kg per plant) with a high percentage (66%) of standard fruit size (>70 g), while the mangosteen trees in the treatment of <500 fruit per plant gave the lowest yield (Phonrong and Sdoodee, 2005; Sdoodee *et al.,* 2008). Although the significantly highest yield (119.89 kg per plant) was found in the treatment of >1500 fruit per plant, most of the fruits were of small size. It was remarkable that the mangosteen trees in the treatment of >1500 fruit per plant exhibited high physiological response with high stomatal conductance and water uptake. After harvesting, leaf flushing and root growth of the plants in the treatment of >1500 fruit per plant were poor. This would lead to an occurrence of alternate bearing in the consecutive year.

A pruning trial was established to investigate the effect of canopy manipulation on growth and yield of seven year old mangosteen trees (Hadloh and Sdoodee, 2007). The treatments included control or no-pruning (T1), cutting upper one along one side of each tier of branches along the main stem (T2), cutting one tier of branches with the upper tier along the main stem remaining (T3) and top-cutting at 3-meter plant height T(4). It was found that one year after pruning, the trees in T2 exhibited highest relative plant height and longest branch length after pruning. The plant growth in T4 was greater and the mangosteen trees in T4 also exhibited high root proliferation. From the result, it is suggested that canopy manipulation of T4 is an appropriate method.

Sdoodee and Phunkied (2008) introduced a concept of high-density planting in the cultivation of mangosteen. Under closer planting system, tree form has to be modified and the application of root restriction by artificial barrier to control canopy size was investigated. Root restriction provided an effective control of canopy size and canopy size decreased with an increase of root restriction. It was also found that root restriction caused detrimental effects on fruit yield and fruit size.

2.7.2 Flowering, fruit maturation, fruit characters and yield

There are reports of existence of male and hermaphrodite flowers in mangosteen (CSIR, 1948; Veeraragavathatham and Balashanmugham, 1989). Krishnamurthy *et al.* (1964) had reported female trees with staminodes. Mangosteen was reported to be unisexual and dioecious, but only female trees with infertile staminodes had been found in Malaya and Java (Purseglove, 1969). Richards (1990) observed mangosteen to be invariable and almost all being females.

Very little work has been done on flower characters and floral biology of mangosteen. Krishnamurthy *et al.* (1964) described two main seasons of flowering in mangosteen, the seasons being April to May and October to November. Flowers of mangosteen were borne terminally on branchlets, mostly single to three (Steenis, 1981), 5-6 cm in diameter, sepals 4 in 2 pairs, inner pair reddish, petals 4, yellowish, edge red, falling early, ovary 4-8 celled, stigma sessile with as many lobes as cells of ovary (Purseglove, 1969). There are reports of trees producing flowers in clusters of up to 12 (Rai, 2004). Veeraragavathatham and Balashanmugham (1989) described male flowers in mangosteen borne in 3 to 9 flowered terminal fascicles. Parthenocarpy and apomixes in mangosteen had been suggested by Lim (1984). Singh (1985) also reported parthenocarpic fruit development in mangosteen.

The understanding of flower initiation, development, and maturation in mangosteen is of paramount importance to shorten its long juvenile phase and to synchronize its flowering or fruiting time. In this study, Chan *et al.* (2009) identified 97 tentative unique genes with higher expression levels in young flower buds compared to young shoots by using suppressive subtraction hybridization and reverse northern analysis. Sequence analysis showed that 63.9% of these transcripts had non-significant matches to sequences in the non-redundant protein database in GenBank, 19.6% had significant matches to unknown proteins while the remaining 16.5% had putative functions in transcription, stress, signal transduction, cell wall biogenesis,

photosynthesis and miscellaneous. Real-time PCR analysis revealed that three genes have different transcript profiles in flowers of different developmental stages and young shoots.

Mangosteen trees are slow to come to bearing. They usually produce their first fruits 10 – 15 or more years after planting (Hume, 1947; Gonzales and Anoos, 1951; Ochse *et al.*, 1961). Te-chato and Lim (2004) reported early fruit setting from tissue culture-derived mangosteen plants. Tissue culture-derived plants were field planted and morphological characters of these plants were observed in comparison with seed-derived plants. The results showed that tissue culture-derived plants were more bushy and started blooming 5 years after planting while the seed-derived plants still had tall canopy (not bushy) and were not bearing fruit in the same period of time. However, the blooming of cultured plants did not give the fruit setting in the first blooming year. All flowers dropped off completely. Heavy fruit setting was observed in the following year. Tissue culture trees had smaller but healthier leaves whereas seed-derived trees had pale yellowish green leaves. Fruit qualities in terms of total soluble solids (TSS) and total acids (TA) were not much different between the two types of these mangosteen trees.

Mangosteen is a seasonal fruit. Gil *et al.* (1972) reported a duration of 5 – 6 months from flowering to fruit ripening. The fruiting season in the Philippines is from June to December (Galang, 1955). The fruit is available in Malaysia during June to August and December to February (Osman and Milan, 2006). The main harvesting season in Indonesia is from September to April (Poerwanto *et al.*, 2008). In low land areas of Sri Lanka, the harvest is from May to July and in high land areas from September to October. In Sri Lanka, plants have been reported to produce two crops a year, a light crop in January from flowers produced in August and a heavy crop in July and August from flowers produced in January (Fairchild, 1915; Popenoe, 1928). In Puerto Rico, the harvest period is July and August for unshaded trees or November

to December if shaded (Almeyda and Martin, 1976). However, depending on zone, weather conditions and farm management practices, fruiting season can begin four to six weeks earlier.

Reports from Indonesia showed that, in mangosteen, the maximum physical growth of fruit reached at 103 days from full bloom, when pulp acidity attained its highest value and there were red patches on the skin. Soluble solid content in pulp increased with increasing days from full bloom until the skin became purple and fruit ripened on the tree within 114 days (Daryno and Sosrodiharjo, 1986). Poonnachit *et al.* (1992) reported that fruit development takes 100-120 days from anthesis and up to 180 days in cooler areas or at higher elevations. The pattern of fruit growth follows a sigmoid curve. Initially, fruit growth is dominated by the pericarp with aril dry matter not increasing until 20 days from anthesis and then continuing throughout the fruit development. At 13 weeks, the fruit shows the highest percentages of pulp, rind, sugar and acid: 29.37 per cent, 69.14 per cent, 18 per cent and 0.49 per cent respectively (Kanchanapom and Kanchanapom, 1998).

Mangosteen fruits are produced singly at the end of the branchlets and usually do not mature and ripen uniformly. The fruits ripen over a period of 6-12 weeks and each tree will bear coexisting generations of fruits resulting from successive generations of flowers. Therefore not all the fruits will reach maturity or ripen at the same time. The interval between harvests should not be too long to avoid the fruits from becoming overripe. Harvesting should be done every second or third day to obtain top quality fruit with the degree of ripeness demanded by the market. Thus harvesting of a single tree may take from 40 to 60 days. The high cost of picking appears to be a major constraint for the commercialization of the crop (Cox, 1976).

Anabesa (1992) conducted a study to establish the optimal harvest period for mangosteen based on days after flower opening as determined by the physico-

chemical and sensory characteristics of mangosteen fruit. Results showed that mangosteen fruits harvested as early as 113 to 116 days after flower opening had qualities comparable to those harvested at full ripeness (119 days from flower opening). Fruits which were harvested on the 113th day from flower opening had total soluble solids of 20.05 degree Brix; an edible portion of 23.95 per cent and a peel colour of greenish purple. On the other hand, those which were harvested on the 116th day from flower opening had total soluble solids of 20.73 degree Brix; an edible portion of 24.85 percent and a peel colour of grey purple with green streaks. The sensory evaluation showed that fruits harvested at 113 and 116 days from flower opening had flesh flavour and texture comparable to the fully ripe ones.

Fruits are at the edible, ripe stage when the skin has darkened to a reddish purple, no latex remains in the skin and the flesh segments separate easily from the skin (Tongdee and Suwanagul, 1989) and soluble solid content ranges from 17 to 20 per cent and titrable acidity from 0.7 to 0.8 per cent (Kader, 2002). Kanchanapom and Kanchanapom (1998) reported that the earliest mangosteen fruits can be harvested after fruit set is 11- 12 weeks (77 – 84 days).

Each mangosteen fruit weighs approximately 55 – 75 g and contains 2 – 3 well developed seeds (Osman and Milan, 2006). The fruit is a globular, indehiscent berry and either spherical or slightly flattened (Yaacob and Tindall, 1995). The fruits are mostly eaten fresh as a dessert fruit. The quality of mangosteen fruit is affected by differences in climatic conditions (Popenoe, 1928). The fruit has high moisture content. The soft fruit aril, the edible portion makes up about 25 – 30 per cent of the fruit. Intengan (1968) reported an average of 26 per cent of the fruit as edible portion. Another report indicates that mangosteen presents an average of 32.5% of pulp, 18.17° Brix and 1% of acidity (Sacramento, 2007). The fruits on a dry weight basis are made of aril 20 %, rind 37 %, seed 26 % and calyx with peduncle 17 % (Nakasone and Paull, 1998). Aril contains a high percentage of carbohydrates,

mostly in the form of sugars (Verheij, 1992). The sugars present are sucrose, glucose and fructose (CSIR, 1948). It is generally low in minerals (Tongdee and Suwanagul, 1989) and vitamins, but calcium, phosphorous and ascorbic acid levels are comparatively high (Leung *et al.,* 1952; Intengan, 1968; Coronel, 1983; Morton, 1987; Poomipamorn and Kumkong, 1997; Juanda and Cahyono, 2000). The per cent of total soluble solids range from approximately 13 – 20 per cent depending on the maturity stage of the fruit. When immature, the range is 13 – 15.2 per cent, but when ripe, it is about 18.3 – 19.0 per cent (Tongdee, 1985; Pankasemsuk *et al.*, 1996; Nakasone and Paull, 1998). Kondo *et al.* (2002) reported that mangosteen is a non-climacteric fruit.

The mangosteen aril possesses a delicate flavour. MacLeod and Pieris (1982) analyzed the volatile compounds that contribute to the aroma and detected about 52 compounds. The seeds possess a nutty flavour (Coronel, 1983). The seed is reported to contain about 30 per cent oil (Pratt and Rosario, 1913). Compounds isolated from the fruit peel of mangosteen contain abundant xanthones, especially alpha-mangostin (Yodhnu *et al.,* 2009). It has a long history of use as a medical plant, mostly in Southeast Asia (Obolskiy *et al.,* 2009). It has been used as traditional medicine such as anti-inflammatory and antibacterial and is popularly applied to cosmetic and pharmaceutical products.

Under optimum conditions in Malaysia, mangosteen trees begin to fruit 8- 10 years after planting. The yield varies from tree to tree and from season to season. The first crop may yield 100 – 300 fruits per tree and about 500 in a fully grown tree. The yield steadily increases up to 1000 – 2000 fruits per tree in the 10 – 20th years of cropping. In Thailand, the average yield of 400 fruits per tree is reported (Osman and Milan, 2006). Kay-ming (1990) observed that in Hainan, China, average yield from 18-20 year old bearing tree was 23-25 kg indicating that the yield of mangosteen tree is rather low and not stable. Singh (1985) reported an average yield of 200 to 400

fruits per tree in India as compared to 500 to 1500 fruits in other countries. In the Nilgiri hills in Southern India, trees in two small orchards produced an average of 360 fruits per year over a period of 18 years, the best trees yielding consistently up to 500 fruits per year (Verheij, 1992). In Australia, yields are variable and about 400 – 900 fruits can be harvested from each mature tree (Chay-Prove, 2004). Older trees (45 years) can yield 3000 fruits per tree and then decline in yield (Kanchanapom and Kanchanapom, 1998). In Indonesia, the yield increases from average of 10 – 20 fruits per tree after the 5th year to more than 1000 fruits per tree after the 15th year (Juanda and Cahyono, 2000).

It was found that in mangosteen, the total yield per tree was higher in trees grown in the river belts. The trees grown in the rocky terrain were stunted and yield per tree was significantly poor (250 to 500 fruits per tree). On the other hand, an adult healthy tree grown in the river belts yielded up to 1500 fruits (George *et al.,* 1996). Trees tend to bear in alternate years (Vietmeyer, 1975). An estimated light crop is 100 fruits per plant while a heavy crop is 500 – 600 or more fruits per plant (Fairchild, 1915; Popenoe, 1928; Galang, 1955). Yields of 200 – 800 fruits per full grown plant have been reported in places with good soils and up to 2000 fruits per tree have been noted. Average crops to aim for are 400 – 700 fruits per tree (Osman and Milan, 2006).

2.7.3 Plant nutrients and growth

In mangosteen, leaf nutrients transferred to accumulate in fruits leading to increase in fruit size and peel thickness (Patarapiyapun, 1995). Poowarodom *et al.* (2002) also reported that N, P, K and Mg concentration in the mangosteen leaf decreased with increasing leaf age. Fruits required K during fruit development, therefore, K in leaves decrease with the progress of fruit development. From results of nutrients analysis in mangosteen leaves, Poowarodom *et al*. (2002) reported that N, P, K, Ca and Mg concentrations were 1.33, 0.09, 1.27, 1.01 and 1.05 g/100g (dried

weight), respectively, and Fe, Mn, Cu and Zn concentrations were 32.05, 90.60, 22.30 and 22.20 mg kg^{-1}, respectively. A study conducted in Malaysia revealed that P, K, Ca, Mg, Fe and Na concentrations in mangosteen fruits were 0.013, 0.045, 0.007, 0.013, 0.001 and 0.007 g/100g, respectively (MAO, 2002).

The imbalance or deficiency of essential nutrients in soils and plants may cause poor fruit quality. To classify this issue, the pattern of plant nutrient accumulation and nutrient requirement in soils and mangosteen trees during fruit development period were investigated (Pechkeol *et al.,* 2007). Mangosteen fruit qualities were not significantly different between the outer and inner canopy fruits. Likewise, most of the plant nutrients accumulation in mangosteen leaf, peel and flesh were not significantly different between two fruit positions. It was remarkable that nutrient accumulation in the fruit decreased from blooming to harvesting period. Mangosteen (leaf, peel and flesh) required higher amounts of N, P, K, Ca and Mg for growth in the early stage of fruit development period (from bloom to 6th week after bloom) and S and B in the late stage of fruit development period (from 6th week after bloom) compared with other growth periods.

Leaf analysis was used as a guide to diagnose nutritional status and as a fertilizer tool for mangosteen plant (Liferdi *et al.,* 2008). Leaf age is the main important factor to estimate plant nutritional status. It was found that leaves of four and five month ages were the best to be used as leaf samples to diagnose P status since they have the highest correlation (above 0.7) between P concentration in the leaf and fruit yield. P concentration decreased as the age of the leaves increased.

2.7.4. Plant growth regulators and growth

Plant growth regulators or plant bioregulators control the mechanisms of growth and development in plants. The alteration in the plant growth and

development is possible by manipulating the endogenous levels of hormones through exogenous applications.

Goh *et al.* (1994) reported effect of auxins on root induction of *in vivo* produced mangosteen shoots of 10-15 mm, which were established in vermiculite/sand mixture in pots. Yusuf (2002) in a study on selection index and activation of seedling growth in mangosteen tried growth regulator sprays of IAA, GA and BA, each, at 50, 150 and 250 mg/l for activating the growth of nursery plants. The study revealed that the best treatment was GA 150 mg/l followed by IAA 250 mg/l. He also observed that a combination of *Glomus fasciculatus* 5 g + *Azospirillum* 10 g + single super phosphate 10 g had a positive effect on seedling growth.

Wiebel *et al.* (1992b) studied the influence of applied growth regulators on bud dormancy and growth of mangosteen. GA, BA and NAA were tried in different combinations and concentrations in 1 to 3 year old mangosteen seedlings (at the nursery stage) and 4 year old orchard trees. Among the different growth regulators applied, all treatments with gibberellins were effective in overcoming bud dormancy, but only when application was made directly to the bud. GA_{4+7} + BA in lanolin paste applied on to the buds of four year old field grown trees significantly increased the number of new flushes as well as leaf area. GA_{4+7} and BA when applied individually were less effective in inducing bud break than when applied together. Branched seedlings responded poorly to BA but the combinations of GA_{4+7} with BA was the most effective, causing 100 per cent bud break within a week. BA was effective only on seedlings, which had not yet begun branching. GA_{4+7} + BA in four year old mangosteen trees produced significantly more shoots. They are of the view that it may be possible to enhance the growth of field- grown trees during their pre-bearing age by inducing one or two extra flushes per year with PGRs, such as GA_{4+7} + BA provided sufficient reserves are available to enable normal growth.

Nakorn *et al.* (1997) conducted studies on the effects of gibberellic acid at different concentrations on the growth of mangosteen seedlings. Two experiments were conducted to evaluate the effects of gibberellic acid at 50, 100, 150, and 200 ppm with spraying to leaf and stem drawing. For the first method of GA3 application (spraying), the treatment of 100 ppm was found to be most effective compared with control. For the stem drawing method of GA3 application also, the treatment of 100 ppm showed the highest growth rate compared with the control. There was significant difference in the stem diameter and leaf area of mangosteen seedlings after application of GA3 at the different concentrations with both methods compared to the control.

Rai *et al.* (2006) studied the changes of gibberellic acid and total sugar content in flower developmental stages of mangosteen. The result showed that flower development of mangosteen consisted of four stages: induction, differentiation, maturation of flower organs, and anthesis. Floral induction was microscopically characterized by the swelling of the basal structure of the new shoot. It was found that induction stage of mangosteen flowering was characterized by sharp decrease of gibberellic acid (GA3, GA5, GA7) and increase of total sugar content of leaf. On the other hand, it was found that leaf of the non-flowering shoot apices had high gibberellic acid and low total sugar.

With the objective to induce apical bud sprouting in young mangosteen seedlings, an experiment was carried out with the application of the plant growth regulators kinetin (0, 100 and 400 mg per litre) and gibberellin GA_{4+7} (50 mg per litre) (Moraes *et al.*, 2009). To obtain rooted cuttings, two other experiments were performed: (a) application of indole butyric acid at the concentrations of 0, 100, 500 and 1000 mg per litre and (b) application of alpha naphthalene acetic acid, at 6000 mg per litre. In these trials, cutting from orthotropic branches were utilized. The

results indicated the possibility of using plant growth regulators to obtain more uniform root stocks in a shorter nursery stage and, despite the low percentage of rooting, the planting material obtained from cuttings has a great practical potential, since the size of the rooted cuttings is equivalent to that of one year old seedlings.

Growth retardants, particularly paclobutrazol, are being used to stimulate enhanced or early flowering in mango. Unlike the other classes of growth retardants which are normally applied as foliar sprays, paclobutrazol is usually applied to the soil due to its low solubility and long residual activity (Davenport and Elisea, 1997). This growth retardant is most efficacious as it reduces shoot elongation and promotes flowering. As a result, paclobutrazol is being promoted to control flowering and vegetative growth in commercial mango orchards of Indo-China, Australia and South Africa (Voon *et al.*, 1991). The main effects of paclobutrazol are induction of regularity, reduction in tree height, more number of flowering shoots, improved number of perfect flowers, fruit set, retention and growth and ultimately increased yield. Paclobutrazol is a systemic growth regulator which acts by reducing gibberellin production. Tree vigour is controlled by reducing the internode lengths of new shoots and earlier formation of terminal buds. Fruit bud production, fruit quality and yield can also be favourably influenced (Davenport and Elisea, 1997).

An experiment was conducted to induce early bearing in five year old mangosteen at Thailand (Sdoodee and Saelim, 1991). The five treatments included no application or control and paclobutrazol application at four rates: 2.0, 3.0, 4.0, 5.0 g/plant). Prior to the application, all plants were imposed to a water-withholding period of 21 days. After the application, they were adequately irrigated by mini sprinkler system. Total leaf nitrogen of all plants imposed to water deficit decreased markedly. After re-watering, the control plants recovered with an increase in total leaf nitrogen to normal level. Total leaf nitrogen of the other treatments with paclobutrazol slowly increased and they were significantly lower than the control.

The application rate of 4.0 and 5.0 g/plant caused significantly shorter branch than that of the control. There was no significant effect on leaf area of leaf flushing and diameter of internodes. The application rate of 5.0 g/plant apparently caused twisted leaves. Early bearing was found only in the treatments of 2.0 and 3.0 g/plant, however, the numbers of fruit-setting were only 1 and 2 fruits per plant, respectively. Therefore 5-year mangosteen was not suitable to induce early bearing by paclobutrazol application.

Effect of four rates of nitrogen 0, 1, 2, and 3 kg per tree and four timing of paclobutrazol application (control, 3 months before flowering, 2 months before flowering and 1.5 months before flowering) were evaluated in 22-year-old mangosteen trees (Chalumpuk *et al*., 1998). Paclobutrazol application 3 months before flowering reduced shoot length and promoted earlier flowering, gave the highest yield, the highest number and weight of fruits per tree, leaf fresh and dry weight, while fruit development and fruit quality were not affected. Higher rates of nitrogen promoted growth in terms of shoot length, leaf area, aril weight and sweetness. Three kg N per tree gave the highest yield in terms of number and fruit weight per tree. When nitrogen and paclobutrazol were applied together increased the total number and fruit weight per tree and fruit quality in terms of sweetness. The interaction effect was more pronounced at higher rate of N and when paclobutrazol was applied at an earlier period before flowering. Trees treated with paclobutrazol also had higher C/N ratio in their shoot relative to control. The highest values were obtained from trees treated three months before flowering. The increase in C/N ratio was associated with flower bud initiation.

A study was conducted to determine the effect of paclobutrazol application combined with potassium nitrate or bicomine on flowering and fruiting of mangosteen (Omran and Semiah, 2006). Paclobutrazol was applied either by soil application at 2 g a.i. per tree or foliar spray at 1000 mg L^{-1} followed by foliar spray

of potassium nitrate or bicomine on preset timings prior to paclobutrazol application. The results showed that application of paclobutrazol combined with potassium nitrate or bicomine enhanced flowering and fruiting of mangosteen. However, only foliar spray of paclobutrazol followed by applications of KNO_3 or bicomine at one month after PBZ spraying and then repeated applications of this chemical during flowering and fruit development showed significant results. The proportions of fruits of different sizes at harvest (i.e. percentage of small, medium or large fruits) demonstrated certain differences among the treatments. However, fruit quality except fruit size during harvesting was not affected regardless of the treatments imposed to the trees.

Anbu *et al.* (2001) studied the effects of paclobutrazol in induction of flowering in mango variety Neelum. Soil drenching of 5 ml of PP^{333}, 90 days before bud break recorded the maximum number of fruits and fruit yield per tree in both the years of application. The next best treatment was 10 ml of PP^{333} applied 90 days before bud break. Murti *et al.* (2001) studied the influence of paclobutrazol on tree vigour and flowering in mango cv. Alphonso. The treated trees showed inhibition of gibberellin biosynthesis, enhancement in the level of ABA and cytokinin in xylem sap and leaves, increase in phenol content and decline in IAA content of leaves, higher phloem to xylem ratio and decline in xylem sap volume. These results show that paclobutrazol induced inhibition in tree vigour and promotion of flowering in mango is not only associated with gibberellin biosynthesis inhibiting character of paclobutrazol, but also with its influence on other hormones such as ABA, IAA and also on phenol.

2.7.5 Microbial inoculations and growth

According to Butler's review (1939), Arbuscular Mycorrhizal (AM) fungi root infections are more abundant in orchard and plantation crops. Its presence and beneficial effects in cultivated crops of Kerala have been reported by various workers

(Potty, 1978; Sivaprasad *et al.,* 1995a; Sivaprasad *et al.,* 1995b; Girija and Nair, 1985; Nair and Girija, 1986).

Masri *et al.* (1998) reported that AM fungi inoculation in mangosteen resulted in length related characteristics. An increase in root length density by 59 – 87 per cent, root branching density by 20 – 30 per cent, number of root tips by 22 – 25 per cent and number of laterals by 15 – 26 per cent was reported in mangosteen. These positive alterations to root systems were accompanied by tremendous increase in nutrient uptake. Uptake of P increased by 67 – 88 per cent in inoculated seedlings (Masri and Azizah, 1998). They also observed that the root to shoot ratio was not influenced by AM inoculation. But their study revealed that the improved growth and increased nutrient uptake of AM inoculated seedlings were due to positive alterations of root system characteristics by symbiosis. They concluded that arbuscular mycorrhiza could enhance growth and reduce the nursery period of mangosteen seedlings.

Only scanty information is available on the influence of *Azospirillum* on perennial crops and most of the studies are on field crops (Rao, 1982; Rao and Dass, 1989). Nearly 10 per cent of the soils and roots from temperate region and more than 50 per cent of the tropical soils were positive for *Azospirillum* (Neyra and Dobereiner, 1977). *Azospirillum* have been reported in the rhizosphere and rhizoplane of cocoa (Govindan and Nair, 1984) and in pepper cuttings (Govindan and Chandy, 1985).

Enhanced root elongation, root hair development and branching in a number of crops have been reported by *Azospirillum* inoculation (Kapulnik *et al*., 1983). Rao and Dass (1989) found that soil inoculation with pure cell suspension of *Azospirillum brasilense* or *Azotobacter chrocaccum* resulted in growth enhancement of ber and pomegranate. They argued that the growth enhancement could be due to production

of growth regulators and nitrogen fixation. For non-leguminous crops, *Azospirillum* has been demonstrated to be beneficial for nitrogen fixation and plant nutrition (Bashan and Holguin, 1997).

Materials and Methods

3. MATERIALS AND METHODS

The present investigation on "Stimulation of growth and induction of variability in mangosteen (*Garcinia mangostana* L.)" was taken up with the objective of developing techniques for accelerating seedling growth, reducing gestation period and inducing variability through mutation and polyploidy in mangosteen.

3.1 Experiment site

The present investigation was carried out at the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara, Thrissur, Kerala during 2006-2009.

3.1.1 Location

The College of Horticulture, Vellanikkara is situated at a latitude of 10^{o} 31' N and longitude of 76^{o} 3' E. The area lies 22.25 m above MSL and enjoys the typical warm humid tropical climate of Kerala.

3.1.2 Climate

The climatalogical data during the period of investigation are given in Appendix I.

3.2 Plants

Mangosteen seedlings were raised in polythene bags containing the growing media and were used for nursery studies. For growth studies in the main field, plants of two age groups were selected. One group consisted of 27 two year old grafted plants and the other 33 five year old orchard trees. Two year old seedlings of *Garcinia mangostana*, *G. indica*, *G. hombroniana*, *G. gummi-gutta*, *G. xanthochymus* and *G. cowa* were used for rootstock studies. Seeds and scions collected from the mangosteen trees available in the college orchard were used for studies on induction of variability.

3.2.1 General management in the nursery and main field

The nursery plants were maintained under uniform shade with proper maintenance and plant protection measures. Stray incidence of leaf eating caterpillars, thrips and disease like rust were noticed in the nursery which were controlled by spraying recommended doses of plant protection chemicals. In the main field, plants were maintained with proper shading, irrigation, manuring and other intercultural operations. No serious pests or diseases were noticed in the main field.

3.3 Experimental design

The investigations were conducted in four experiments.

3.3.1 Experiment I

Experiments were conducted to find out the effect of growing media and application of nutrients, bio-products, bio-agents and bio-regulators to enhance growth and to reduce juvenile phase.

3.3.1.1 Effect of media and growth regulator

Seedlings were raised in polythene bags containing 2: 1: 1 [soil (laterite): sand: organic manure] mixture. Four organic manures were used.

M1 – Well rotten cow dung

M2 – Poultry manure

M3 – Vermicompost

M4 – Coir pith compost (enriched containing 40 % coir pith compost, 10 % Rajphos, 10 % groundnut cake, 20 % neem cake and 20 % bone meal)

Four growth regulators at three concentrations were sprayed at fortnightly interval @ 50 ml per plant for fifteen months.

IAA – 150 ppm (GR1), 300 ppm (GR2), 450 ppm (GR3)

IBA – 150 ppm (GR4), 300 ppm (GR5), 450 ppm (GR6)

GA – 100 ppm (GR7), 200 ppm (GR8), 300 ppm (GR9)

BA – 100 ppm (GR10), 200 ppm (GR11), 300 ppm (GR12)

Design: CRD

No. of treatments: 52 [48 + 4 control (without growth regulator)]

No. of replications: 10

3.3.1.2 Effect of growth promoting substances

Seedlings were raised in 2:1:1 [soil (laterite): sand: cow dung] growing media. Nutrient solutions, bio-agents, bio-products and bio-regulators were used as growth promoters.

B1 – *Pseudomonas* sp. (2 %)

B2 – *Azospirillum* sp. (10 g per plant)

B3 – AMF (10 g per plant)

B4 – Fresh cow dung solution (3g/ l)

B5 – Cow's urine (25 times dilution with water)

B6 – Vermiwash (5 times dilution)

B7 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 %

B8 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %

B9 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm

B10 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + BA 100 ppm

B11 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm

B12 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm

B13 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm + BA 100 ppm

B14 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm

B15 - Control

Standard preparations of B1, B2 and B3 collected from TNAU, Coimbatore were used. B1 was used as foliar spray and soil drench and B2 and B3 were

applied in the polybags and gently mixed with the potting mixture without disturbing the roots. All other treatments were given as foliar spray @ 50 ml per plant. The treatments were given at fortnightly interval for fifteen months.

Design: CRD

No. of treatments: 15

No. of replications: 10

3.3.2 Experiment II

3.3.2.1 Accelerating plant growth in the main field

Plants of two age groups were used.

3.3.2.1.1 Two year old grafted plants

GA, BA and combinations of GA + BA were applied directly to the bud in lanolin paste at monthly interval for one year.

C1 – GA 1 µg/10 mg (GA 100 ppm)

C2 – GA 2 µg/10 mg (GA 200 ppm)

C3 – BA 1 µg/10 mg (BA 100 ppm)

C4 – BA 2 µg/10 mg (BA 200 ppm)

C5 – GA + BA (1 µg + 1 µg)/10 mg (GA 100 ppm + BA 100 ppm)

C6 – GA + BA (1 µg + 2 µg)/10 mg (GA 100 ppm + BA 200 ppm)

C7 – GA + BA (2 µg + 1 µg)/10 mg (GA 200 ppm + BA 100 ppm)

C8 – GA + BA (2 µg + 2 µg)/10 mg (GA 200 ppm + BA 200 ppm)

C9 – control (no growth regulator)

Design: RBD

Replications: 3

3.3.2.1.2 Five year old orchard trees

GA, BA, combinations of GA + BA and paclobutrazol were applied.

D1 – GA 1 µg/10 mg (GA 100 ppm)

D2 – GA 2 µg/10 mg (GA 200 ppm)

D3 – BA 1 µg/10 mg (BA 100 ppm)

D4 – BA 2 µg/10 mg (BA 200 ppm)

D5 – GA + BA (1 µg + 1 µg)/10 mg (GA 100 ppm + BA 100 ppm)

D6 – GA + BA (1 µg + 2 µg)/10 mg (GA 100 ppm + BA 200 ppm)

D7 – GA + BA (2 µg + 1 µg)/10 mg (GA 200 ppm + BA 100 ppm)

D8 – GA + BA (2 µg + 2 µg)/10 mg (GA 200 ppm + BA 200 ppm)

D9 – Paclobutrazol 1.5 g a.i./tree

D10 – Paclobutrazol 2.0 g a.i./tree

D11 – Control (no growth regulator)

Treatments D1 to D8 were given directly to the buds in lanolin paste at monthly interval for one year (September - October 2007 to September – October 2008). Treatment D9 and D10 were prepared in 25 litres of water and was given as soil drench as well as spray as single dose during September – October 2007 and 2008.

Design: RBD

Replications: 3

3.3.3 Experiment III

3.3.3.1 Use of nurse stocks and rootstocks to enhance growth

3.3.3.1.1 Nurse stock

Additional nurse stocks namely seedlings of *Garcinia mangostana, G. indica, G. hombroniana, G. gummi-gutta, G. xanthochymus and G. cowa* were approach grafted/ inarched to two year old seedlings to provide a double root system. Seedling growth was measured bimonthly for two years.

3.3.3.1.1.1 Method of grafting

3.3.3.1.1.1.1 Approach grafting technique

The technique as described by Hartmann *et al.* (2002) was followed. Healthy seedlings of mangosteen and other nurse stocks of about two years old, raised in polythene bags, with a height of about 25 – 30 cm were selected for grafting. The selected mangosteen seedlings as well as nurse stock seedlings were of approximately

same size. At the point where union is to occur, a slice of bark and wood 2.5 to 5 cm long was cut from both the stems. The two cut surfaces were then bound tightly together with a polythene tape first and with a plastic twine above it so as to keep it well united. After the successful union, the nurse stock portion above the union was cut in a step wise manner to avoid competition with the mangosteen seedlings.

3.3.3.1.1.1.2 Inarching

Inarching was also followed and it differs from approach grafting in that top of the nurse stock plants is not extending above the point of graft union (Hartmann *et al.*, 2002). Here upper portion of the nurse stock was cut about one third through one side adjacent to the mangosteen seedlings to be inarched. On the opposite side, a short cut is made at the top of the seedling bringing it to a wedge. A similar cut was made on the mangosteen seedlings also. The two cut surfaces were then bound tightly together with a polythene tape first and with a plastic twine above it so as to keep it well united for successful graft union.

3.3.3.1.2 Use of related species as rootstock

In addition to seedlings of *Garcinia mangostana*, related species *viz. G. indica, G. hombroniana, G. gummi-gutta, G. xanthochymus and G. cowa* were used as rootstocks. Softwood grafting was followed and growth of grafts was recorded bimonthly.

3.3.3.1.2.1 Method of grafting

The softwood grafting technique in mangosteen as described by Chong (1992) was followed. Healthy seedlings of mangosteen and other related species of about two years old with a height of about 25 – 30 cm were selected as rootstocks. Seedling rootstock was decapitated at the top with two terminal leaves. A vertical cut of about 4-5 cm deep was made from top downwards on the stock. Scion sticks of 6 – 8 months old, 10 – 15 cm length were collected from healthy mother plants on the day of grafting. The lower end of the scion was shaped into a wedge by removing the bark with a little wood from both the sides with a sharp knife. The leaves were also

trimmed to reduce transpiration loss. The scion was inserted into the vertical cut of the stock and secured in place firmly with a polythene strip. The grafts were placed in the mist chamber to maintain humidity and to prevent the scions from drying. Retention of green colour in the grafted portion was taken as the indication of perfect union.

3.3.4 Experiment IV

3.3.4.1 Induction of variability through induced mutation and polyploidy

3.3.4.1.1 Material for treatment

3.3.4.1.1.1 Seeds

Seeds were collected from freshly harvested fruits and used for irradiation as well as colchicine treatment. Treated seeds were sown on the same day in polythene bags containing the potting mixture for further studies.

3.3.4.1.1.2 Scion

Scion sticks of 6 – 8 months old, 10 – 15 cm length were collected from healthy mother plants on the day of grafting. The material was subjected to gamma irradiation and was grafted on to mangosteen seedlings immediately employing softwood grafting technique Chong (1992).

3.3.4.1.2 Source of treatment

3.3.4.1.2.1 Gamma irradiation

The seeds were exposed to gamma radiation in a Gamma Chamber (GC-900). First LD_{50} dose of exposure was found and dose to be exposed was fixed accordingly. Five to 50 Gy were tried on seeds as well as scion materials.

3.3.4.1.2 Colchicine

3.3.4.1.2.1 Preparation of colchicine solution

Colchicine powder was first mixed with small quantity (10 per cent of final volume) of glycerol and was dissolved in distilled water and volume was made up to achieve required concentration.

3.3.4.1.2.2 **Dose of colchicine**

Dose of colchicine was fixed based on sensitivity studies. A dosage of 0.1 to 3.5 percent was used for treating the seeds as well as application to the buds.

3.3.4.1.2.3 Method of application

It was applied as seed soaking (overnight) and application at the growing apex (for 4 hours) of one year old seedlings.

3.3.4.2 Assessment of induced variability through RAPD Analysis

This includes DNA isolation from leaves of mangosteen samples, purification, optimization of PCR protocols for RAPD, screening of random primers and assessment of genetic variability if any due to mutagenesis.

3.3.4.2.1 DNA extraction

Quality of DNA is an important factor which influences the PCR reactions. DNA isolation method reported by Doyle and Doyle (1990) and Dellaporta *et al*. (1983) was slightly modified and tried for the extraction of genomic DNA. Tender leaves were taken from the selected plants using sterile blades. The leaf samples were stored in ice boxes until reaching laboratory.

3.3.4.2.2 Reagents used

1. Extraction buffer
    a. 100 mM Tris pH 8.0
    b. 50 mM EDTA pH 8.0
    c. 500mM NaCl
    d. 10 mM β-Mercaptoethanol (added immediately before use)
2. 20% SDS
3. 5 M Potassium acetate pH 5.5
4. TE buffer
    a. 10 mM Tris pH 8.0
    b. 1 mM EDTA pH 8.0
5. 3 M Sodium acetate pH 5.2

6. Isopropanol
7. Ethanol (70% and 100%)
8. Tris-saturated phenol
9. Chloroform: Isoamyl alcohol (24:1 v/v)

3.3.4.2.3 Procedure

Tender leaf sample (1g) was collected on ice, cut into pieces with a sterile blade and transferred to a mortar and ground into fine powder using liquid nitrogen. The powder was then transferred to a 50 ml centrifuge tube kept on ice containing 7 ml extraction buffer and 20µl β-Mercaptoethanol. 0.5 ml of 20 per cent SDS solution was added to the tube, mixed well and incubated at 65^{o} C for 20 minutes. To the above suspension, 2.5 ml of 5 M potassium acetate was added, shaken vigorously and left on ice for 20 minutes with periodic shaking. The mixture was then centrifuged at 12,000 rpm, 4^{o} C for 10 minutes and the supernatant was filtered using sterile muslin cloth into a clean 50 ml centrifuge tube. The filtrate was mixed with 5 ml ice-cold isopropanol and was incubated at -20^{o} C for 30 minutes. After the incubation the mixture was centrifuged at 12,000 rpm for 10 minutes at 4^{o} C to pellet the DNA. The supernatant was discarded and the DNA in the pellet form was washed with 70 per cent alcohol and air-dried. The pellet was then resuspended in 500 µl TE buffer and transferred to a sterile eppendorff tube. DNA was again precipitated with 50 µl of 3 M sodium acetate and 300 µl of isopropanol. The DNA pellet was washed with 70 per cent alcohol and air-dried and resuspended in 300 µl of TE. The DNA was further purified by adding equal volume of chloroform : isoamyl alcohol mixture. The top aqueous layer was taken into a sterile eppendorff tube, 200 µl of isopropanol was added and mixed gently, and kept at -20^{o} C for 30 minutes for precipitation. The DNA was pelleted by centrifuging at 12,000 rpm for 10 minutes at 4^{o} C. The DNA in the pellet form was then washed with 70 per cent alcohol and then with absolute alcohol. The pellet was air-dried and dissolved in 300 µl sterile double distilled water.

3.3.4.2.4 Purification of DNA

The DNA isolated will also contain RNA and Protein in it. To exclude the RNA, the sample was treated with RNase (1% solution in 10 mM sodium acetate, pH 5.2) and for protein, proteinase K (2% solution in distilled water).

The extracted DNA suspended in double distilled water was treated with 5 µl of RNase solution (1%) and incubated at 37^{o} C for one hour. Then it was further treated with 1 µl of proteinase K solution (2%) and incubated at 45 ^{o} C for one hour. After the incubation, sample was made upto 500 µl using distilled water. Equal volume of phenol : chloroform : isoamyl alcohol (25:24:1, v/v/v) mixture was added to the sample and mixed gently. The aqueous layer was taken and added equal volume of chloroform : isoamyl alcohol (24:1) mixture. Mixed gently and after a brief centrifugation, saved the top aqueous layer, 2/3 volume of chilled isopropanol was added, mixed gently until the DNA was precipitated and centrifuged at 10,000 rpm for 3 minutes at room temperature. The isopropanol was poured off and the DNA pellet was washed first with 70 per cent alcohol and then with absolute alcohol. The DNA was then allowed to air dry, redissolved in 60 µl sterile double distilled water and stored at -20^{o} C for further use.

3.3.4.2.5 Agarose gel electrophoresis of DNA samples

3.3.4.2.5.1 Materials and equipment used

1. Agarose
2. TAE buffer 50X
    a. Tris base - 242 g
    b. Glacial acetic acid - 57.1 ml
    c. EDTA (0.5 M) - 100 ml (pH 8.0)

Made up with distilled water to 1 litre.

3. Gel loading dye 2X (100 ml)
    a. Glycerol - 40 ml
    b. 4X TAE buffer - 50 ml

c. Bromophenol - 0.5 %

4. Ethidium bromide solution (0.1 %)
5. Electrophoresis unit, power supply unit, casting tray and comb

3.3.4.2.5.2 Procedure

Gel buffer (TAE 1X) was taken in a conical flask (100 ml for large gel and 30 ml for small). Agarose (1 % for DNA and 1.2 % for RAPD samples) was weighed, added to the flask, stirred and boiled with frequent stirring till the agarose dissolved completely. Ethidium bromide was added into the flask and it is allowed to cool to 65^{o}C. The open end of the gel casting tray was sealed with cello tape and placed on a horizontal surface and the comb was placed properly on the tray. The dissolved agarose was poured gently into the tray. The gel was allowed to solidify for 30 minutes and then the comb was removed carefully. The gel was then placed in the electrophoresis unit with the well side directed towards cathode. 1X TAE buffer was added to cover the gel with a few mm of buffer. 10 µl of DNA sample (15 µl in case of RAPD products) was pipetted out onto a parafilm and mixed well with 4 µl of loading dye. The samples were then loaded carefully into the well by using micropipette. Standard DNA molecular weight markers were also added in one well. The cathode and anode of the electrophoresis unit were then connected to the power supply and the gel was run at constant voltage (60 mA). The power supply was turned off when the loading dye moved to required distance (1 ½ to 2 hours).

3.3.4.2.5.3 Gel documentation

The gel was taken from electrophoresis unit and viewed under UV light in a UV transilluminator. The ethidium bromide stain intercalates between the nitrogen bases of DNA and fluoresces in orange colour under UV light. The image of the gel was monitored and stored in a gel documentation system (Alpha Imager-2000, Alpha Infotech, USA).

3.3.4.2.5.4 Random Amplified Polymorphic DNA (RAPD) analysis

RAPD is a technique in which a single short oligonucleotide primer, which binds to many different loci, is used to amplify random sequences from a template

DNA. The number of amplified products in RAPD depends on the length of primer and the size of the target genome, and is based on the probability that a given DNA sequence (complementary to that of the primer) will occur in the genome on opposite strands of the DNA, in opposite orientation within a distance readily amplifiable by PCR. The products can be easily separated by standard electrophoresis techniques and visualized by ultraviolet illumination of ethidium bromide stained gels. PCR amplification process involves repeated thermal cycles. The procedure reported by Raghunathachari *et al*. (2000) was slightly modified and attempted for amplification of DNA.

The cycles included,

Step no.	Temperature (^{o}C)	Duration (min)	Steps involved	No. of cycles
1	94	3	Initial denaturation	1
2	92	1	Denaturation	40
3	37	1	Annealing	
4	72	2	Initial extension	
5	72	5	Final extension	1

The reaction mixture (25µl) consisted the following:

1. 10X assay buffer with 15 mM $MgCl_2$ - 2.5 µl
2. dNTPs Mix - 1.0 µl
3. Taq DNA polymerase - 2.0 µl (0.6 units)
4. Primer - 2.0 µl (10 p moles)
5. Template DNA - 5.0 µl (50 ng)
6. Sterile Milli-Q water - 12.5 µl

A master mix without the template DNA was prepared using the reaction mixture for the required number of reactions. From this master mix, 20 µl was pipetted into each PCR tube and 5 µl of the template DNA were added. The reaction

mix was overlaid with 25 µl of sterile mineral oil. The PCR tubes were loaded in the Thermal Cycler (PTC 200, MJ Research, USA) and the programme was run. The programme was completed in 3 hours 45 minutes. The amplified products were electophoresised on 1.2 per cent agarose gel. The gel was viewed under UV light and documented.

3.3.4.2.5.5 Screening of random primers for RAPD

Eleven decamer primers were screened (Table 1) for amplification of genomic DNA extracted from the mangosteen leaf samples, using the Thermal Cycler mentioned under RAPD. Out of the eleven primers used, only OPA-18 and OPC-O3 yielded scorable bands. These two primers were selected and utilized for further characterization. The total number of bands along with the number of polymorphic bands obtained in all five isolates with OPA-18 and OPC-O3 primers was recorded.

Table 1. List of primers used for screening

Sl. No.	Primer code	Primer sequence
1	OPE-03	CCAGATGCAC
2	OPE-04	GTGACATGCC
3	OPA- 18	AGGTGACCGT
4	OPA-19	CAAACGTCGG
5	OPA - 20	GTTGCGATCC
6	OPC-02	GTGAGGCGTC
7	OPC-03	GGGGGTCTTT
8	OPB-01	GTTTCGCTCC
9	OPB-02	TGATCCCTGG
10	OPBB-01	ACACTGGCTG
11	OPBB-02	CCCCCGTTAG

3.3.4.2.5.6 Data analysis

The pattern of DNA amplification for the two primers was scored as 1 or 0 by the presence or absence of bands respectively and the data was fed to the NTSYS PC 2.0 software package. Similarity indices (pair wise similarity coefficient) were computed as JACCARDS's similarity coefficient

$$GS_{ij} = a/a+b+c$$

where,

GS_{ij} = the measure of genetic similarity between individuals i and j

a = the number of polymorphic bands shared by i and j

b = the number of bands present in i and absent in j

c = the number of bands present in j and absent in i

The DNA fingerprint data were used to construct dendrogram by employing unweighted pair group method of arithmetic averages (UPGMA) using NYSTS pc version 2.01 programme (Rohlf, 1998) using SAHN coefficient.

3.4 Observations

The main items of observations recorded are detailed below.

3.4.1 Experiment I

3.4.1.1 Plant, leaf and root characters

3.4.1.1.1 Plant height

Seedling height was measured from the collar region to the tip of the main stem using a metre scale and expressed in centimetre.

3.4.1.1.2 Plant spread

The spread of the plant in East-West and North-South directions were measured and recorded in centimetre.

3.4.1.1.3 Number of leaves

The total number of leaves present at the time of each observation was counted and recorded.

3.4.1.1.4 Length and breadth of leaf

The leaf length from the base of the petiole to the leaf tip and width at the centre of the leaf was measured in centimetres.

3.4.1.1.5 Total leaf area

Leaf area was calculated by multiplying the length, breadth and the factor (0. 62) and average was expressed as cm^2. The factor was pre-standardised for this purpose by taking hundred leaves and length and breadth of the leaves were measured. The leaf area of the corresponding leaf was measured by leaf area meter to work out the factor value. Thus the factor value was derived using the formula

Factor = (Leaf area/ Length x Breadth)

Using this factor value, leaf area of a leaf was calculated. Total leaf area was estimated by summing up the individual leaf area of all the leaves on the plant over a given period of experiment.

3.4.1.1.6 Petiole length

The length of the petiole from the point of its emergence to the base of the leaf lamina was measured and recorded in cm.

3.4.1.1.7 Leaf thickness

Thickness of the leaves was measured using vernier calipers and expressed in mm.

3.4.1.1.8 Colour development of leaves

Colour of the newly emerged leaves and colour development of young leaves were noted.

3.4.1.1.9 Duration from leaf emergence to senescence

Duration of leaf from emergence to senescence was noted and recorded.

3.4.1.1.10 Leaf senescence

The number of leaves drying due to senescence per plant per year was recorded.

3.4.1.1.11 Internodal length

The distance between two adjacent nodes was measured using a metre scale and the average expressed in centimetre.

3.4.1.1.12 Number of branches per plant

The number of branches emerging from the main stem was recorded. Secondary and tertiary branches produced were also recorded.

3.4.1.1.13 Number of primary, secondary and tertiary roots and total root number

After carefully removing the potting mixture using water spray, the number of primary, secondary, tertiary roots and total number of roots were counted and recorded.

3.4.1.1.14 Length of longest root

Length of the longest root was measured from the collar region to growing tip of the root using a scale and expressed in centimetre.

3.4.1.1.15 Root spread

The maximum root spread was measured and expressed in centimetres.

3.4.1.1.16 Fresh and dry weight of plant parts

The seedlings were uprooted at six months interval. Immediately fresh weight of leaves, shoot, roots and whole plant was recorded separately using an electronic balance and the average expressed in gram.

The samples collected for recording the fresh weight were dried in an oven maintained at 60° C till the weight of the samples remained constant. The dry weights were recorded separately and average expressed in gram.

Plant and leaf characters were recorded at monthly interval and root characters were recorded by sampling at six months interval.

3.4.1.2 Physiological parameters

3.4.1.2.1 Leaf Area Index (LAI)

Leaf Area Index was calculated using the equation

$$LAI = \frac{\text{Total leaf area of the plant}}{\text{Land area occupied by the plant}}$$

LAI was recorded at monthly interval.

3.4.1.2.2 Specific Leaf Weight (SLW)

Specific Leaf Weight was worked out using the equation

$$SLW = \frac{\text{Total dry weight of leaves}}{\text{Total leaf area}}$$

SLW was recorded at six month interval.

3.4.1.2.3 Relative Growth Rate

Relative growth rate (RGR) is the rate of increase in dry weight per unit dry weight per unit time expressed in g g^{-1} day. It is calculated by the formula suggested by Blackman (1919).

$$RGR = \frac{\text{Log e } W_2 - \text{Log e } W_1}{(t_2 - t_1)}$$

Where, W_1 and W_2 are the dry weight of the whole plant at time t_1 and t_2 respectively.

RGR was recorded after a period of one year of study.

3.4.1.3 Chemical analysis

Nutrient as well as other chemical analysis was conducted at six months interval.

Samples of plant components collected for recording dry weight were used for nutrient analysis. The dried samples were ground, mixed and then chemically analysed for major nutrients, *viz*., nitrogen, phosphorus, potassium, calcium and magnesium and micro nutrients, *viz*., Fe, Cu, Mn and Zn.

3.4.1.3.1 Nitrogen

One gram dried leaf sample was digested using concentrated sulphuric acid; oxidized using 30 % H_2O_2 and the N content was estimated by Microkjeldahl method (Jackson, 1958).

3.4.1.3.2 Phosphorus

The leaf sample (0.5 g) was digested using diacid mixture of nitric acid and perchloric acid taken in the ratio of 9:4 (Johnson and Ulrich, 1959). Finally phosphorus was estimated using vanadomolybdophosphoric yellow colour method (Jackson, 1958). The intensity of yellow colour was read in Spectronic-20 at 470 nm.

3.4.1.3.3 Potassium

From the digested sample as mentioned above, an aliquot was prepared and K content was estimated using a flame photometer (Cheng and Bray, 1951).

3.4.1.3.4 Micro nutrients

Micro nutrients (Cu, Fe, Zn, Mn) and Secondary nutrients (Ca, Mg) were estimated by Atomic Absorption Spectrophotometer (Jackson, 1958; Sims and Johnson, 1991).

3.5.5.3.5 Chlorophyll content

The chlorophyll content of the leaves was determined using Dimethyl sulphoxide (DMSO) (Shoaf and Livm, 1976). The most recent, fully developed leaf was taken and cut into small pieces. Incubated the sample in 7.0 ml of DMSO at 65^{o} C for 30 minutes. At the end of the incubation period the supernatant solution was decanted and the leaf tissue was discarded. The volume was made up to 10 ml with DMSO. The absorbance was read at 645 and 663 nm using DMSO as blank. Chlorophyll a, b and total ratio was calculated using the formula, and expressed in mg g $^{-1}$ leaf weight.

$$\text{Chlorophyll 'a'} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W \times a}$$

$$\text{Chlorophyll 'b'} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W \times a}$$

$$\text{Total chlorophyll} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W \times a}$$

Where,

A = Absorbance at specific wave lengths 645 and 663 nm.

V = Final volume of the chlorophyll extract (ml),

W = Fresh weight of the sample (g),

a = Path length of light (1 cm).

3.5.5.3.6 Nutrient uptake

The total nutrient uptake by the plant was calculated based on nutrient content and dry weight of plant component and expressed as mg per plant.

3.4.2 Experiment II

3.4.2.1 Height of the plant

Height of the plant was measured from the collar region to the tip of the main stem using a metre scale and expressed in centimetre in the case of two year old plants. For orchard trees, a graduated long pole was used to measure tree height.

3.4.2.2. Girth of the tree

Girth of the plant was measured at 10 cm and 30 cm from the base for two year old grafted plants and five year old orchard trees respectively and expressed in centimetres.

3.4.2.3. Number of branches

Number of primary and secondary branches was counted and noted.

3.4.2.4. Spread of plants

The spread of the plants in East-West and North-South directions was measured and recorded in centimetre for two year old plants and in metres for orchard trees.

3.4.2.5. Time of bud break and season of flushing

Periodic observations were made to determine the time of flushing and number of flushes produced in a year.

3.4.2.6. Leaf area

Leaf area was calculated by multiplying the length, breadth and the factor (0. 62) and average was expressed as cm^2 as described under experiment I.

3.4.2.7. Growth of shoots and leaf production in unit time

Twenty lateral shoots, five each from north, south, east and west directions were tagged on individual trees and extension growth was measured in centimetre

scale. Number of secondary branches produced on these tagged shoots was also recorded. In the case of two year old plants, as the number of lateral shoots was limited, all the available lateral shoots were tagged and extension growth measured. Number of leaves produced in these shoots was also noted to observe the growth pattern of plants.

3.4.2.8 Flowering

3.4.2.8.1 Pattern of flowering

Observations on time of flowering, duration of flowering, number of flowered shoots per unit area of the tree canopy and days from flowering to harvest were noted.

3.4.2.8.2. Flower drop

Number of flower and fruit drop was recorded periodically.

3.4.2.9 Fruit characters

3.4.2.9.1 Fruit weight

Individual fruit was labelled immediately after harvest. Then fruit weight was recorded using an electronic balance and average expressed as gram.

3.4.2.9.2 Fruit length, breadth and circumference

Length and breadth of the fruit were measured using a centimetre scale. Circumference was measured using a thread and its length measured using a centimetre scale and the average expressed as centimetre.

3.4.2.9.3 Pulp weight

The fruit hull was carefully removed and white segmented pulp weight of each fruit was taken using an electronic balance.

3.4.2.9.4 Number of segments

Number of white juicy segments was counted immediately after the fruit was opened and was categorized into big, medium and small segments.

3.4.2.9.5 Percentage of edible portion

Percentage of edible portion was calculated using the formula

$$\text{Percentage of edible portion} = \frac{\text{Pulp weight}}{\text{Weight of the fruit}} \times 100$$

3.4.2.9.6 Thickness of rind

The pulp was carefully removed from the fruit hull and the rind thickness was measured from the four opposite sides and the average worked out and expressed in centimetres.

3.4.2.9.7 Cavity size of fruits

The pulp was carefully removed from the fruit hull and the maximum and minimum cavity size was measured using a centimetre scale and expressed in centimetres.

3.4.2.9.8 Colour of ripe fruits

The ripe fruits were classified into purplish red, purple, dark purple or black as per the colour indices developed by MAO (2002).

3.4.2.9.9 Number of seeds per fruit

The number of seeds per fruit was counted and the average expressed as number.

3.4.2.9.10 Seed weight

The seeds were extracted from the pulp and the weight of individual seeds in a fruit was taken using an electronic balance. The seeds were then categorized into big (> 1.5 g) and small seeds (< 1.5 g).

3.4.2.9.11 Total Soluble solids (TSS)

Total soluble solids was determined in freshly extracted juice of ripe fruits using Erma hand refractometer with a range of 0 to 30° brix and expressed in degree brix (AOAC, 1980).

3.4.2.9.12 Incidence of gamboge

Individual fruits were examined to note the incidence of gamboge and percentage of incidence worked out.

3.4.2.10 Yield

Number and weight of fruits obtained from each tree during each harvest was recorded to arrive at the total yield from individual trees. Time and duration of harvesting and number of harvests were also noted.

3.4.3 Experiment III

3.4.3.1 Percentage success of grafts

Grafts that retained green colour in the grafted portion after one month of grafting was counted as successful graft and its percentage was worked out as per the formula

$$\text{Percentage success of grafts} = \frac{\text{Number of successful graft}}{\text{Total number of grafting carried out}} \times 100$$

3.4.3.2. Time taken for graft take

The period between time of grafting and emergence of a new leaf from the scion portion is counted and expressed as days as the time taken for graft take.

3.4.3.3. Number of sprouts/ leaves produced by the graft

Number of new sprouts/ leaves produced by the grafts was recorded bimonthly for a period of two years.

3.4.3.4. Rate of growth

Extension growth of the grafts was measured in centimetre scale and recorded. The number of branches produced by the grafts was also noted.

3.4.4 Experiment IV

3.4.4.1 Vegetative characters of individual seedlings and grafts

3.4.4.1.1 Percentage germination of irradiated seeds

Seeds after irradiation were sown immediately in polybags containing potting mixture. Number of seeds germinated was recorded periodically and the percentage of germination was worked out using the formula

$$\text{Percentage of germination of seeds} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

3.4.4.1.2 Number of days taken for seed germination

The number of days taken from date of sowing to date of germination of seeds was counted and the average worked out.

3.4.4.1.3 Percentage success of grafts

The irradiated scions were grafted immediately on the rootstocks. Time taken for sprouting of scions and percentage of sprouting were noted. The final success of grafting was worked out as per the formula

$$\text{Percentage success of grafts} = \frac{\text{Number of successful graft}}{\text{Total number of grafting carried out}} \times 100$$

3.4.4.1.4 Plant height

Seedling height was measured from the collar region to the tip of the main stem using a metre scale and expressed in centimetre.

3.4.4.1.5 Size of leaf

The leaf length from the base of the petiole to the leaf tip and width at the centre of the leaf was measured in centimetres. Abnormality of leaves if any due to irradiation is also noted.

3.4.4.1.6 Colour development of leaves

Colour of the newly emerged leaves and colour development of young leaves were noted to observe for any variation in leaf colour compared with the normal seedlings.

3.4.4.1.7 Internodal length

The distance between two adjacent nodes was measured using a metre scale and the average expressed in centimetre.

3.4.4.1.8 Rate of growth

Extension growth of seedling and grafts was measured in centimetre scale and recorded. The number of branches produced by the seedlings and grafts was also noted.

3.4.4.1.9 Root length in two months

After carefully removing the potting mixture using water spray, length of the root was measured from the collar region to growing tip of the root using a scale and expressed in centimetre.

3.4.4.2 RAPD Analysis

Different primers were screened for amplification of genomic DNA extracted from the mangosteen leaf samples, using the Thermal Cycler mentioned under RAPD. From these, primers that gave good amplification were selected and utilized for further characterization. The total number of bands along with the number of polymorphic bands obtained in all five isolates with OPA-18 and OPC-O3 primers was recorded. The DNA fingerprint data were used to construct dendrogram by employing unweighted pair group method of arithmetic averages (UPGMA) using NYSTS pc version 2.01 programme (Rohlf, 1998) using SAHN coefficient to understand the genetic variability or relatedness of samples studied.

3.5 Statistical analysis

The data recorded on the various experiments were subjected to statistical analysis following the methods of Panse and Sukhatme (1985). Treatment means were compared using DMRT.

Results

4. RESULTS

The results of the experiments entitled “Stimulation of growth and induction of variability in mangosteen (*Garcinia mangostana* L.)” are presented in this chapter.

4.1 Experiment I: Enhancing seedling growth in nursery

4.1.1 *Effect of media and growth regulators*

4.1.1.1 *Plant, leaf and root characters*

4.1.1.1.1 Plant height

Table 2 shows the effect of media, growth regulator and their interactions on plant height. The interaction between media and growth regulator was found to be significant in all the stages.

At three month stage (Table 2), among the media, M4 (coir pith compost as organic manure) recorded the maximum plant height (13.66 cm) which was significantly superior to M2 (poultry manure as organic manure) but was on par with M1 (well rotten cow dung as organic manure) and M3 (vermicompost as organic manure). During this initial period, GR9 (GA 300 ppm) showed the highest plant height (15.98 cm) in the M4 medium but was on par with rest of the treatments including control. None of the growth regulator treatments showed any significant difference from the control in the respective media with reference to plant height at three month stage.

During six, nine, twelve and fifteen month stages, among the media, M3 (vermicompost) was the most superior recording the maximum plant height followed by M4 (coir pith compost), M1 (well rotten cow dung) and M2 (poultry manure).

Table 2. Influence of media and growth regulators on plant height (cm)

Growth Regulators	3 months[@]				6 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	13.66^{aAB}	10.56^{abB}	11.60^{abAB}	14.96^{aA}	13.78^{aAB}	12.56^{aA}	14.40^{cdAB}	17.90^{abA}
GR2	10.52 ^{aB}	11.80^{abAB}	11.60^{abAB}	14.52^{aA}	11.04^{aB}	13.44^{aAB}	14.90^{bcdAB}	17.30^{abA}
GR3	10.90 ^{aB}	10.68^{abB}	12.16^{abAB}	14.40^{aA}	11.52^{aB}	12.24^{aAB}	16.28^{bcd}	16.06^{abAB}
GR4	10.90 ^{aA}	12.64^{aA}	10.68^{bA}	12.64^{aA}	11.30^{aA}	14.30^{aA}	13.78^{dA}	14.86^{bA}
GR5	11.96 ^{aA}	10.56^{abA}	13.14 ^{abA}	13.66^{aA}	12.86^{aA}	11.90^{aA}	16.14^{bcdA}	15.94^{aA}
GR6	12.10 ^{aB}	9.64^{abB}	11.10 ^{abB}	15.78^{aA}	12.70^{aB}	10.50^{aB}	15.04^{bcdAB}	17.96^{abA}
GR7	12.26^{aAB}	9.98^{abB}	10.96^{abAB}	13.74^{aA}	12.90^{aAB}	10.80^{aB}	16.22^{bcdA}	16.00^{abA}
GR8	11.40 ^{aB}	9.30^{abB}	14.30 ^{aA}	15.34^{aA}	11.90^{aB}	9.90^{aB}	19.18^{abA}	16.62^{abA}
GR9	13.74 ^{aA}	8.80^{bB}	13.98 ^{abA}	15.98^{aA}	15.40^{aBC}	10.82^{aC}	21.66^{aA}	16.66^{abB}
GR10	12.00 ^{aB}	10.40^{abB}	11.70 ^{abB}	15.72^{aA}	12.86^{aB}	11.60^{aB}	15.72^{bcdAB}	17.40^{abA}
GR11	12.72^{aAB}	10.04^{abB}	12.52^{abAB}	14.84^{aA}	13.30^{aB}	10.60^{aB}	18.76^{abcA}	19.76^{aA}
GR12	10.52 ^{aB}	11.16^{abB}	12.54^{abAB}	15.00^{aA}	11.58^{aB}	12.92^{aB}	18.86^{abcA}	18.06^{abA}
Control	11.94^{aAB}	9.46^{abB}	12.16^{abAB}	13.66^{aA}	13.52^{aAB}	10.00^{aB}	17.32^{abcdA}	14.32^{bAB}
Significance	S				S			
CV (%)	16.9				19.8			

@ Experiment was initiated with six month old polybag plants
Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period
M1- medium with well rotten cow dung as organic manure, M2- medium with poultry manure as organic manure, M3- medium with vermicompost as organic manure, M4- medium with coir pith compost as organic manure
GR1- IAA 150 ppm, GR2- IAA 300 ppm, GR3- IAA 450 ppm, GR4- IBA 150 ppm, GR5- IBA 300 ppm, GR6- IBA 450 ppm, GR7- GA- 100 ppm, GR8- GA 200 ppm, GR9- GA 300 ppm, GR10- BA 100 ppm, GR11- BA 200 ppm, GR12- BA 300 ppm

Table 2. continued

Growth Regulators	9 months				12 months				15 months			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
GR1	16.00^{aA}	17.32^{abA}	20.22^{eA}	22.24^{abA}	21.20^{aA}	23.66^{aA}	24.46^{cA}	25.84^{abcA}	25.04^{aA}	31.18^{aA}	30.72^{bA}	30.16^{abcA}
GR2	12.96^{aB}	16.14^{abAB}	21.92^{deA}	22.34^{abA}	18.60^{aB}	19.50^{abcB}	29.44^{abcA}	29.60^{aA}	23.26^{aB}	24.14^{abcB}	38.72^{abA}	37.74^{aA}
GR3	14.34^{aC}	16.60^{abAB}	23.42^{cdeA}	19.94^{abAB}	16.32^{aB}	20.52^{abcB}	32.46^{abcA}	23.68^{abcB}	18.78^{aC}	24.34^{abcBC}	42.92^{aA}	28.16^{bcdB}
GR4	13.06^{aA}	19.58^{aA}	19.54^{eA}	18.38^{abA}	14.72^{aB}	21.86^{abAB}	28.48^{bcA}	21.16^{bcAB}	17.94^{aC}	30.68^{abAB}	39.00^{abA}	25.20^{cdBC}
GR5	17.48^{aAB}	13.46^{abB}	22.14^{deA}	20.94^{abAB}	21.20^{aBC}	17.00^{abcC}	29.98^{abcA}	25.58^{abcAB}	25.24^{aBC}	22.18^{bcdC}	39.70^{aA}	32.24^{abcAB}
GR6	14.96^{aB}	14.40^{abB}	20.94^{eAB}	23.28^{abA}	20.34^{aB}	18.00^{abcC}	30.86^{abcA}	27.04^{abAB}	25.50^{aB}	23.74^{acB}	42.84^{aA}	32.40^{abcB}
GR7	14.22^{aB}	14.70^{abB}	25.36^{bcdeA}	21.08^{abAB}	16.52^{aB}	15.66^{bcB}	31.82^{abcA}	25.12^{abcA}	19.90^{aB}	17.48^{cdB}	39.44^{abA}	30.94^{abcA}
GR8	16.64^{aB}	19.44^{aB}	28.70^{abcdA}	22.12^{abAB}	17.80^{aB}	20.34^{abcB}	34.42^{abA}	23.26^{abcB}	20.06^{aB}	22.00^{bcdB}	41.18^{aA}	24.94^{cdB}
GR9	16.64^{aBC}	11.88^{bC}	34.82^{aA}	23.74^{abB}	16.84^{aC}	12.82^{cC}	37.28^{aA}	26.52^{abB}	17.24^{aC}	14.92^{dC}	40.36^{aA}	29.56^{abcB}
GR10	14.94^{aB}	14.26^{abB}	25.32^{bcdeA}	22.38^{abA}	16.96^{aB}	14.96^{bcB}	30.98^{abcA}	26.84^{abA}	19.44^{aB}	16.14^{cB}	38.64^{abA}	32.36^{abcA}
GR11	14.88^{aB}	12.38^{abB}	31.24^{abA}	25.12^{aA}	18.84^{aB}	13.18^{cB}	36.18^{abA}	29.00^{abA}	22.66^{aB}	14.18^{dB}	42.64^{aA}	34.36^{ab}
GR12	14.14^{aC}	17.14^{abBC}	30.16^{abcA}	22.38^{abB}	16.64^{aC}	18.30^{abcBC}	36.28^{abA}	26.26^{abB}	20.16^{aC}	20.30^{cdC}	43.70^{aA}	31.00^{abcB}
Control	15.00^{aB}	12.84^{abB}	26.60^{abcdeA}	16.44^{bB}	15.78^{aB}	13.42^{cB}	35.58^{abA}	18.02^{cB}	16.74^{aB}	14.20^{dB}	46.70^{aA}	19.68^{dB}
Significance	S				S				S			
CV (%)	23.9				21.1				19.1			

Means with same lower case letter as superscript within a column are homogeneous, means with same upper case letter as superscript within a row are homogeneous for a period

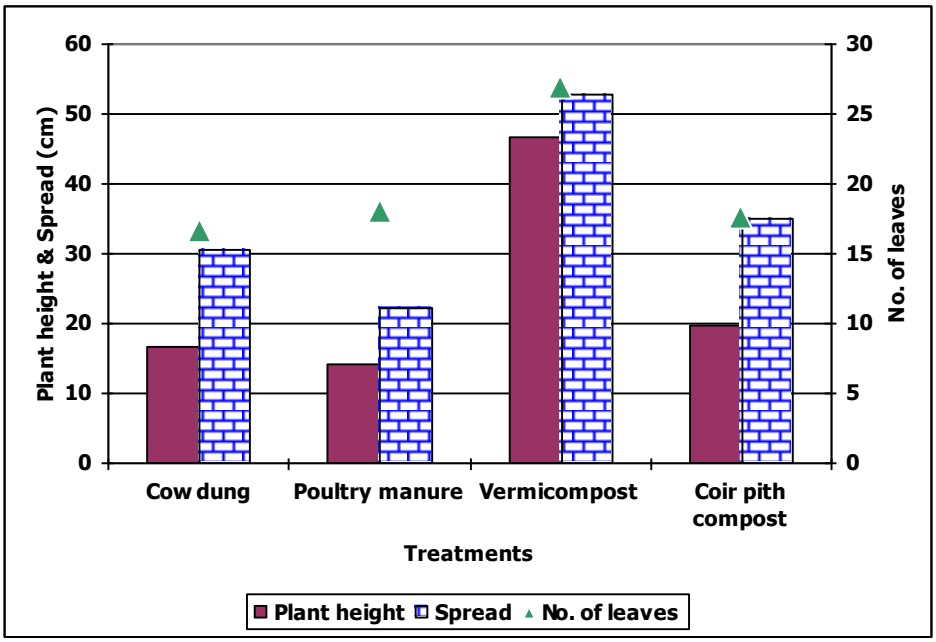


Fig. 1. Effect of different media on growth parameters of mangosteen seedlings at 15th month

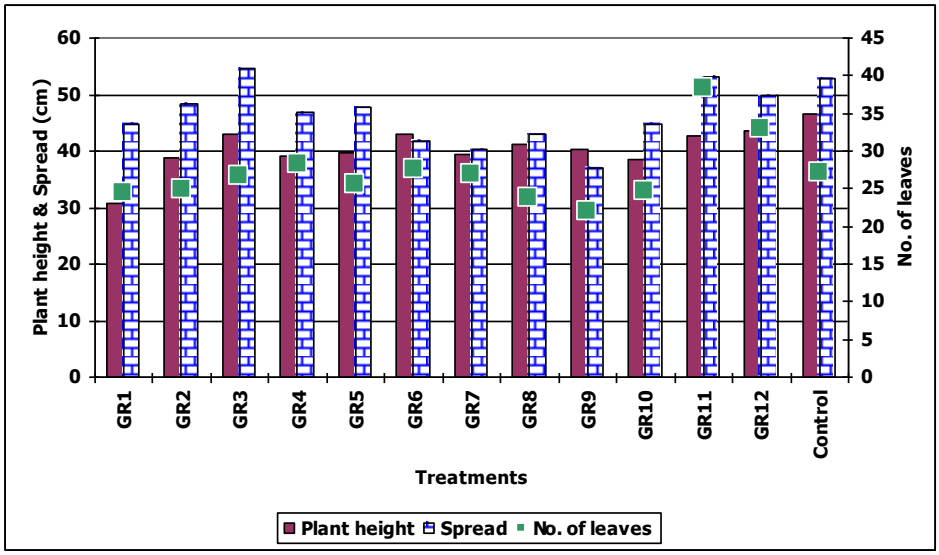


Fig. 2. Effect of growth regulators on growth parameters of mangosteen seedlings in vermicompost medium at 15th month

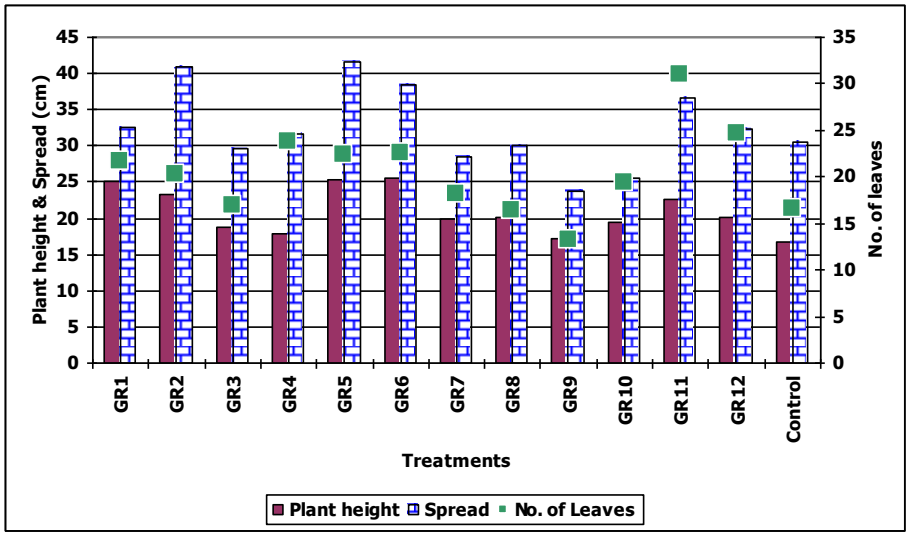


Fig. 3. Effect of growth regulators on growth parameters of mangosteen seedlings in cow dung medium at 15th month

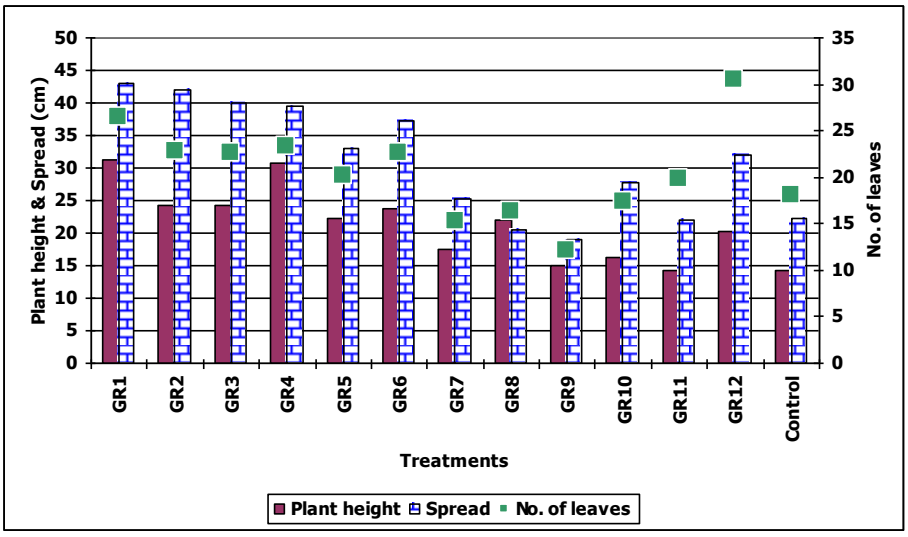


Fig. 4. Effect of growth regulators on growth parameters of mangosteen seedlings in poultry manure medium at 15th month

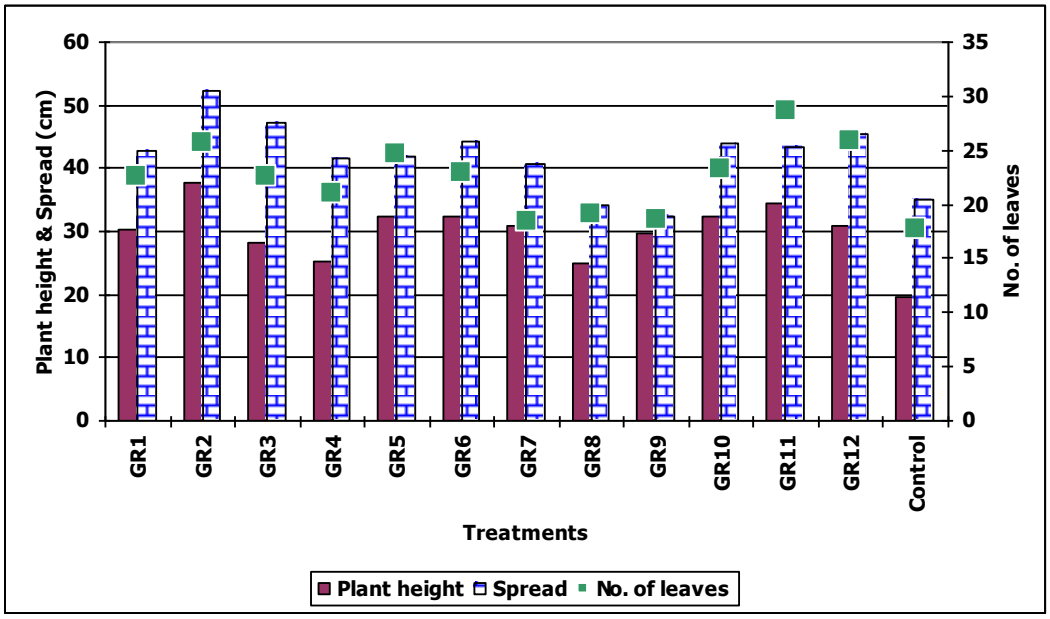


Fig. 5. Effect of growth regulators on growth parameters of mangosteen seedlings in coir pith compost medium at 15th month

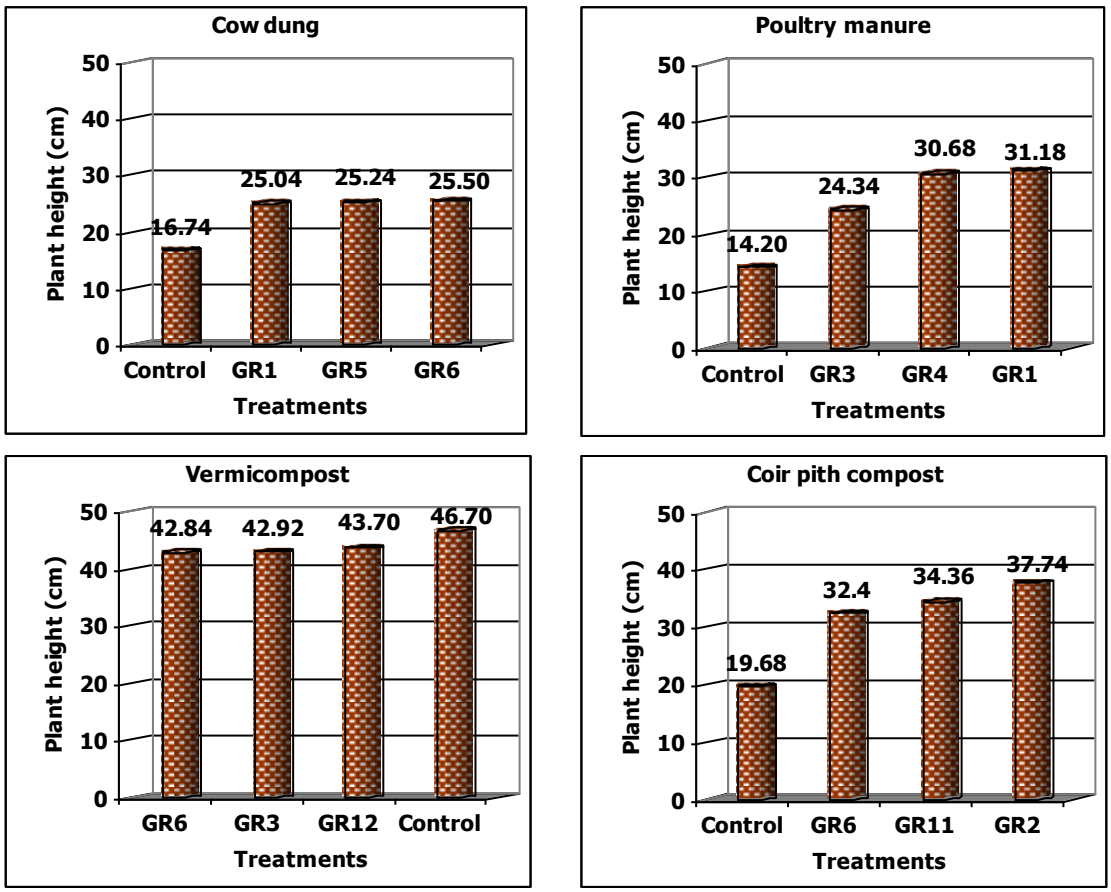


Fig 6. Comparison of superior treatments influencing plant height at 15th month in different media

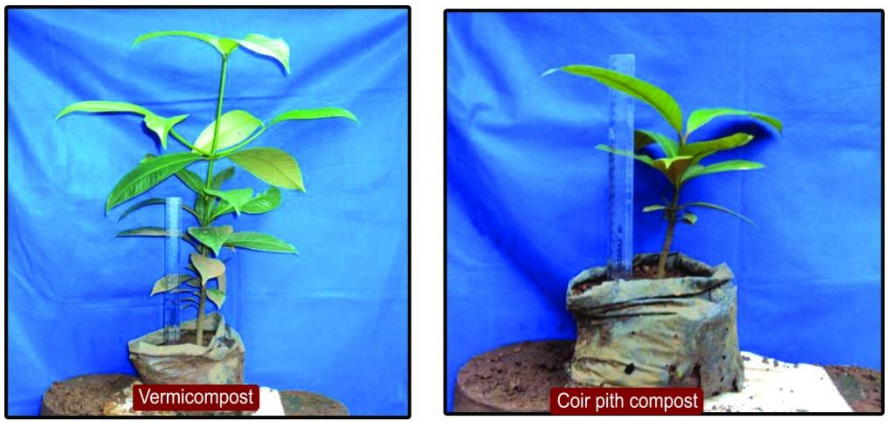
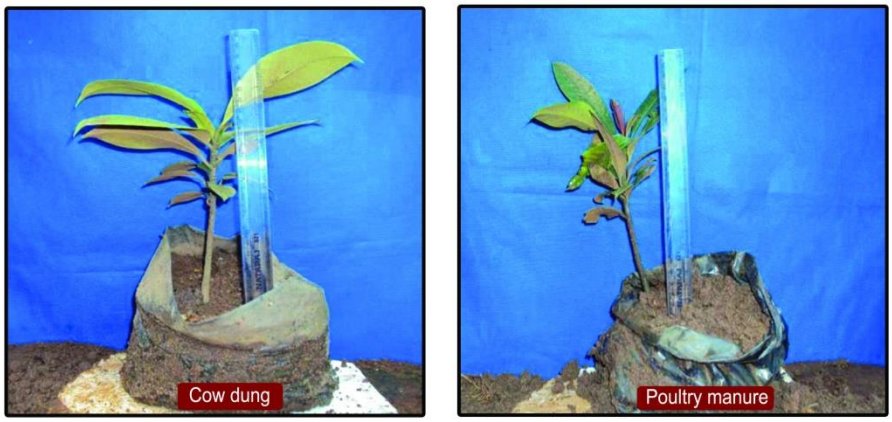


Plate 1. Comparison of plant height in different media at fifteen month stage



In the M3 medium, GR9 (GA 300 ppm) recorded the highest plant height at six, nine and twelve month stages while control was the most superior at fifteen month period. In the case of other media, effect of growth regulators varied widely during different periods without a definite pattern of influence.

At the end of fifteen months, among the media, M3 (vermicompost as organic manure) recorded the maximum plant height (46.70 cm) which was significantly superior to all other media *viz.* M1 (16.74 cm), M2 (14.20 cm) and M4 (19.68 cm). The media M1, M2 and M4 were homogeneous at this period with respect to plant height (Fig.1 and Plate 1).

At this stage, in the M3 medium, control recorded the highest plant height (46.70 cm) which was on par with all other treatments except GR1. In the medium M1, GR6 (IBA 450 ppm) recorded the highest plant height (25.50 cm), but was homogeneous with rest of the treatments including control. GR1 (IAA 150 ppm) recorded the maximum plant height (31.18 cm) in M2 which was on par with GR2, GR3, GR4 and GR6 and differed significantly with all other treatments. In the medium M4, GR2 (IAA 300 ppm) had the maximum plant height (37.74 cm) which was significantly superior to GR3, GR4, GR8 and control (Fig. 2 to 6).

4.1.1.1.2. Plant spread

The effect of media, growth regulator and their interactions on plant spread is presented in Tables 3 to 5. The interaction between media and growth regulator was found to be significant only at six and fifteen month stages. During other periods, interaction was non significant indicating that the effect of growth regulator was not altering with change in media. However, the main effects due to media were found to be significant. This indicates that there was significant influence of the media on plant spread.

Table 3. Plant spread for each medium averaged over all growth regulators (cm)

Media	3 months[@]	9 months	12 months
M1	20.81[b]	26.19[c]	29.22[c]
M2	17.69[c]	26.24[c]	28.77[c]
M3	22.06[ab]	37.69[a]	41.25[a]
M4	22.68[a]	33.59[b]	37.32[b]
Significance	S	S	S

Table 4. Plant spread for each growth regulator averaged over all media (cm)

Growth Regulators	3 months[@]	9 months	12 months
GR1	21.73	33.97[abc]	36.67[ab]
GR2	21.08	34.53[ab]	39.41[a]
GR3	20.61	35.60[a]	38.85[a]
GR4	20.50	32.63[abc]	36.13[ab]
GR5	21.68	33.86[abc]	36.93[ab]
GR6	20.48	33.23[abc]	36.77[ab]
GR7	19.56	26.71[de]	29.91[cd]
GR8	21.13	27.26[de]	29.17[cd]
GR9	19.46	22.63[e]	24.82[d]
GR10	20.71	30.04[bcd]	32.30[bc]
GR11	21.53	28.82[cd]	33.30[bc]
GR12	21.25	31.89[abcd]	35.43[ab]
Significance	NS	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 5. Influence of media and growth regulators on plant spread (cm)

Growth Regulators	6 months				15 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	23.70^{aA}	25.36^{abcA}	24.92^{abcA}	26.92^{abA}	32.48^{abcdeB}	43.12^{aAB}	44.66^{abcdeA}	42.82^{abcdAB}
GR2	21.40^{aA}	23.20^{bdA}	26.84^{abcA}	27.04^{abA}	41.00^{abA}	42.04^{aA}	48.44^{abcdeA}	52.42^{aA}
GR3	23.04^{aB}	31.28^{aA}	30.08^{abAB}	26.44^{abAB}	29.54^{cdeC}	39.98^{aBC}	54.62^{aA}	47.12^{abcAB}
GR4	21.96^{aA}	26.40^{abA}	25.52^{abcA}	26.64^{abA}	31.76^{abcdeB}	39.58^{aAB}	46.72^{abcdeA}	41.48^{abcAB}
GR5	23.56^{aAB}	20.20^{bdeB}	29.12^{abA}	26.68^{abAB}	41.54^{aAB}	33.02^{abcB}	47.84^{abcdeA}	41.80^{abcdAB}
GR6	22.38^{aAB}	18.18^{cdeB}	28.88^{abA}	27.88^{abA}	38.50^{abcA}	37.14^{abA}	41.80^{cdeA}	44.36^{abcA}
GR7	20.76^{aAB}	18.00^{cdeB}	26.12^{abcA}	21.74^{bAB}	28.42^{cdeB}	25.28^{cdB}	40.32^{deA}	40.70^{bcdA}
GR8	20.60^{aA}	18.82^{cdeA}	23.80^{bcA}	22.50^{bA}	30.00^{bcdeBC}	20.52^{dC}	43.12^{bcdeA}	34.02^{cdAB}
GR9	22.40^{aA}	14.98^{eA}	20.62^{cA}	21.84^{bA}	23.80^{eBC}	19.08^{dC}	37.06^{eA}	32.28^{dAB}
GR10	21.40^{aB}	21.60^{bdeB}	32.34^{aA}	27.74^{abAB}	25.56^{deB}	27.72^{bcdB}	44.88^{abcdeA}	43.90^{abcA}
GR11	23.80^{aBC}	17.90^{cdeC}	31.50^{aA}	29.10^{abAB}	36.62^{abcdBC}	21.96^{cdC}	53.28^{abA}	43.38^{abcdAB}
GR12	21.54^{aB}	22.26^{bdeB}	31.38^{aA}	30.44^{aA}	32.34^{abcdeB}	31.90^{abcB}	49.92^{abcdA}	45.54^{abA}
Control	21.90^{aB}	17.00^{deB}	32.20^{aA}	23.34^{abB}	30.56^{abcdeBC}	22.14^{cdC}	52.90^{abcA}	35.12^{bcdB}
Significance	S				S			
CV (%)	18.9				18.5			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

During the initial period, up to three months, (Table 3) M4 (coir pith compost) was superior in terms of plant spread (22.68 cm) which was on par with (22.06 cm) M3 (vermicompost). M2 (poultry manure) recorded the lowest plant spread (17.69 cm) which was significantly different from all other media. During all other stages, vermicompost was the most superior recording the highest plant spread followed by coir pith compost. Effect of M1 and M2 did not vary significantly at these stages.

Comparison of the effect of each growth regulator on plant spread averaged over all media (Table 4) showed that in third month, effect of all the growth regulators was homogeneous with respect to plant spread. During ninth and twelfth months where interaction was not significant, GR3 (IAA 450 ppm) and GR2 (IAA 300 ppm) respectively showed superiority over other growth regulators in plant spread.

At the end of fifteen months (Table 5), among the media, M3 (vermicompost) recorded the maximum plant spread (52.90 cm) which was significantly superior to all other media *viz.* M1 (30.56 cm), M2 (22.14 cm) and M4 (35.12 cm). M4 was homogeneous with M1 but was superior to M2. The media M1 and M2 were homogeneous at this period.

At this stage, in the M3 medium, GR3 (IAA 450 ppm) recorded the highest plant spread (54.62 cm) which differed significantly with GR6, GR7, GR8 and GR9. GR5 (IBA 300 ppm) recorded the maximum plant spread (41.54 cm) in M1 which was on par with all the treatments except GR3, GR7, GR8, GR9 and GR10. In the medium M2, GR1 (IAA 150 ppm) recorded the highest plant spread (43.12 cm), which was significantly superior to GR7, GR8, GR9, GR10, GR11 and control. In the medium M4, GR2 (IAA 150 ppm) had the maximum plant spread (52.42 cm) which was homogeneous with all other treatments except GR7, GR8, GR9 and control.

4.1.1.1.3 Number of leaves

The interaction between media and growth regulator was found to be significant only at fifteen month stage. But during other stages, significant difference was observed among the growth regulators and also among the media.

Among the different media (Table 6), influence of M4 (coir pith compost) and M3 (vermicompost) on number of leaves was uniform and significantly superior to other two media up to six month stage. Thereafter, M3 recorded the highest value of 21.75, 24.05 and 27.00 respectively for nine, twelve and fifteen month periods and was significantly superior to all other media. M2 (poultry manure) recorded the lowest number of leaves during the entire period.

Effect of each growth regulator on number of leaves averaged over all media (Table 7) where interaction was not significant revealed that GR11 (BA 200 ppm) recorded the highest number of leaves at six (17.70), nine (23.10) and twelve (26.65) month stages. At three month stage, effect of all the growth regulators was homogeneous with respect to number of leaves.

At the end of fifteen months (Table 8), among the media, M3 recorded the highest number of leaves (27.00) which was significantly superior to all other media *viz.* M1 (16.60), M2 (18.00) and M4 (17.60). The media M1, M2, and M4 were homogeneous at this period.

GR11 (BA 200 ppm) recorded the highest number of leaves in M1 (31.00), M3 (38.20) and M4 (28.60) media while in M2, GR12 (BA 300 ppm) recorded the maximum number of leaves (30.40) at the end of fifteen months. In M3, GR11 was significantly superior to all other treatments while in other media, its influence did not vary much with other treatments. Effect of GR12 in M2 was also homogeneous with GR1, GR2, GR3, GR4 and GR6.

Table 6. Number of leaves for each medium averaged over all growth regulators

Media	3 months[@]	6 months	9 months	12 months
M1	13.45 (3.79)[b]	12.85 (3.70)[b]	15.12 (3.94)[d]	18.37 (4.33)[c]
M2	12.88 (3.71)[b]	14.18 (3.85)[b]	17.28 (4.22)[c]	19.17 (4.43)[c]
M3	14.77 (3.96)[a]	17.88 (4.33)[a]	21.75 (4.75)[a]	24.05 (4.99)[a]
M4	14.90 (3.98)[a]	16.63 (4.19)[a]	19.35 (4.50)[b]	20.92 (4.67)[b]
Significance	S	S	S	S

Table 7. Number of leaves for each growth regulator averaged over all media

Growth Regulators	3 months[@]	6 months	9 months	12 months
GR1	14.60 (3.95)	16.35 (4.15)[ab]	18.90 (4.44)[b]	21.50 (4.72)[b]
GR2	14.35 (3.91)	16.00 (4.10)[abc]	18.70 (4.42)[b]	20.85 (4.66)[bc]
GR3	13.55 (3.80)	15.95 (4.09)[abc]	18.55 (4.39)[b]	19.90 (4.54)[bcd]
GR4	14.30 (3.91)	15.40 (4.02)[abc]	18.50 (4.40)[b]	21.15 (4.69)[b]
GR5	14.45 (3.92)	16.50 (4.15)[ab]	19.30 (4.47)[ab]	20.95 (4.65)[bc]
GR6	14.45 (3.92)	16.45 (4.16)[ab]	19.40 (4.50)[ab]	21.80 (4.76)[b]
GR7	13.45 (3.78)	13.60 (3.79)[cde]	15.15 (3.97)[cd]	17.40 (4.25)[cde]
GR8	13.45 (3.78)	12.50 (3.65)[de]	15.05 (3.97)[cd]	16.90 (4.20)[de]
GR9	13.10 (3.74)	11.90 (3.56)[e]	13.40 (3.73)[d]	14.50 (3.88)[e]
GR10	13.30 (3.77)	14.90 (3.94)[bcd]	17.60 (4.24)[bc]	19.80 (4.50)[bcd]
GR11	14.65 (3.94)	17.70 (4.30)[a]	23.10 (4.86)[a]	26.65 (5.20)[a]
GR12	14.35 (3.91)	17.40 (4.27)[ab]	22.85 (4.86)[a]	26.10 (5.18)[a]
Significance	NS	S	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous
Values in the parenthesis are mean of square root transformed values

Table 8. Influence of media and growth regulators on number of leaves

Growth Regulators	15 months			
	M1	M2	M3	M4
GR1	21.60 (4.74)^{bcA}	26.40 (5.17)^{abA}	24.40 (5.03)^{bcA}	22.60 (4.85)^{abcA}
GR2	20.20 (4.58)^{bcA}	22.80 (4.88)^{abcA}	24.80 (5.08)^{bcA}	25.60 (5.16)^{abA}
GR3	16.80 (4.17)^{cB}	22.60 (4.84)^{abcAB}	26.60 (5.25)^{bcA}	22.60 (4.86)^{abcAB}
GR4	23.80 (4.98)^{abA}	23.20 (4.90)^{abcA}	28.20 (5.39)^{bcA}	21.00 (4.69)^{abcA}
GR5	22.40 (4.79)^{bcA}	20.20 (4.57)^{bcdA}	25.60 (5.16)^{bcA}	24.60 (5.06)^{abcA}
GR6	22.60 (4.83)^{bcA}	22.60 (4.85)^{abcA}	27.60 (5.35)^{bcA}	22.80 (4.87)^{abcA}
GR7	18.20 (4.34)^{bcdB}	15.20 (3.98)^{deC}	26.80 (5.27)^{bcA}	18.40 (4.39)^{bcB}
GR8	16.40 (4.12)^{cB}	16.20 (4.14)^{cdeB}	23.80 (4.96)^{cA}	19.00 (4.47)^{bcA}
GR9	13.20 (3.75)^{dB}	12.00 (3.58)^{eB}	22.00 (4.79)^{cA}	18.60 (4.42)^{bcA}
GR10	19.40 (4.40)^{bcdAB}	17.40 (4.24)^{cdeB}	24.60 (5.06)^{bA}	23.20 (4.92)^{abcAB}
GR11	31.00 (5.65)^{aAB}	19.80 (4.49)^{bcdC}	38.20 (6.23)^{aA}	28.60 (5.44)^{aBC}
GR12	24.60 (5.01)^{abB}	30.40 (5.59)^{aAB}	33.00 (5.83)^{abA}	25.80 (5.17)^{abAB}
Control	16.60 (4.14)^{cB}	18.00 (4.33)^{cdeB}	27.00 (5.29)^{bcA}	17.60 (4.30)^{cB}
Significance	S			
CV (%)	10.2			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period
Values in the parenthesis are mean of square root transformed values

4.1.1.1.4 Total leaf production and senescence for fifteen months

The interaction between media and growth regulator was found to be significant with respect to total leaf production and senescence for fifteen months (Table 9). Among the media, M3 (vermicompost) recorded the highest total leaf production (20.00), which was significantly superior to M1 (9.20) and M4 (10.40) and was on par with M2 (14.40). The media M1, M2 and M4 were homogeneous at this period.

GR11 (BA 200 ppm) recorded the highest total leaf production in M1 (23.60), M3 (29.20) and M4 (18.00) while GR12 (BA 300 ppm) had the maximum total leaf production (24.40) in M2 during the same period. In M3, effect of media alone was on par with that of growth regulators, while in other media, effect of some of the growth regulators was superior to effect of media alone.

With respect to leaf senescence for the same period, (Table 9), among the media, M3 recorded the lowest leaf senescence (4.00) but was on par with all other media *viz.* M1 (4.40), M2 (6.80) and M4 (4.40).

Among the growth regulators, both GR2 (IAA 300 ppm) and GR6 (IBA 450 ppm) recorded the lowest leaf senescence (1.60) in M1, while GR2 alone showed the lowest leaf senescence (1.40) in M3. In M2, GR3 (IAA 450 ppm) recorded the minimum (0.80) and control showed the maximum leaf senescence (6.80) for the same period. In the medium M4, GR5 (IBA 300 ppm) had the lowest leaf senescence (1.40).

4.1.1.1.5 Leaf length

The interaction between media and growth regulator was found to be significant only at fifteen month stage. But during other stages, significant difference was observed among the growth regulators and also among the media.

Table 9. Influence of media and growth regulators on total leaf production and leaf senescence for fifteen months

Growth Regulators	Total leaf production for 15 months[@]				Total leaf senescence for 15 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	12.40 (3.70)^{bcA}	17.20 (4.18)^{abA}	15.60 (4.07)^{bA}	13.60 (3.79)^{abA}	3.60 (2.13)^{abcA}	3.40 (2.05)^{bcA}	2.20 (1.77)^{cdA}	2.80 (1.93)^{abcA}
GR2	10.00 (3.28)^{bcdA}	14.80 (3.95)^{bcA}	14.40 (3.91)^{bA}	15.60 (4.07)^{abA}	1.60 (1.57)^{cB}	4.40 (2.3)^{abcA}	1.40 (1.52)^{dB}	1.80 (1.66)^{abcAB}
GR3	8.00 (2.90)^{cdB}	11.20 (3.48)^{bcAB}	18.00 (4.35)^{bA}	12.00 (3.6)^{abAB}	2.60 (1.81)^{bcA}	0.80 (1.31)^{dA}	2.40 (1.81)^{cdA}	2.20 (1.77)^{abcA}
GR4	12.80 (3.7)^{bcA}	13.20 (3.74)^{bcA}	19.60 (4.52)^{abA}	12.20 (3.62)^{abA}	2.40 (1.82)^{bcA}	2.20 (1.76)^{cdA}	2.40 (1.81)^{cdA}	3.00 (1.96)^{abcA}
GR5	11.60 (3.48)^{bcdA}	11.60 (3.5)^{bcA}	15.60 (4.07)^{bA}	13.40 (3.79)^{abA}	2.40 (1.78)^{bcA}	3.00 (1.97)^{bcdA}	2.20 (1.68)^{cdA}	1.40 (1.54)^{cA}
GR6	10.40 (3.29)^{bcdB}	15.00 (3.97)^{bcAB}	18.00 (4.35)^{bA}	12.40 (3.64)^{abAB}	1.60 (1.57)^{cB}	4.80 (2.33)^{abcA}	2.20 (1.75)^{cdAB}	1.80 (1.62)^{abcB}
GR7	10.60 (3.37)^{bcdB}	9.60 (3.18)^{cdB}	19.60 (4.53)^{abA}	9.00 (3.16)^{bB}	4.40 (2.3)^{abA}	5.20 (2.48)^{abA}	4.40 (2.32)^{abcA}	4.40 (2.25)^{abA}
GR8	8.80 (3.09)^{cdB}	10.00 (3.31)^{bcdAB}	16.60 (4.17)^{bA}	9.20 (3.19)^{bB}	3.40 (2.06)^{abcA}	4.20 (2.24)^{abcA}	5.60 (2.56)^{abA}	3.80 (2.18)^{abcA}
GR9	6.00 (2.59)^{dB}	5.60 (2.46)^{dB}	16.80 (4.21)^{bA}	10.60 (3.39)^{abAB}	4.80 (2.31)^{abA}	3.40 (2.05)^{bcA}	6.40 (2.71)^{aA}	3.80 (2.17)^{abcA}
GR10	13.20 (3.56)^{bcA}	10.40 (3.3)^{bcdA}	15.60 (4.07)^{bA}	12.80 (3.71)^{abA}	6.20 (2.67)^{aA}	3.60 (2.12)^{abcAB}	3.00 (1.92)^{bcdB}	1.60 (1.59)^{bcB}
GR11	23.60 (4.95)^{aAB}	13.20 (3.54)^{bcC}	29.20 (5.44)^{aA}	18.00 (4.35)^{aBC}	3.80 (2.17)^{abcA}	4.60 (2.32)^{abcA}	4.00 (2.15)^{abcdA}	2.00 (1.69)^{abcA}
GR12	17.60 (4.23)^{abA}	24.40 (5.01)^{aA}	23.80 (4.96)^{abA}	16.00 (4.12)^{abA}	4.20 (2.26)^{abcA}	5.60 (2.5)^{abA}	2.80 (1.92)^{bcdA}	3.60 (2.12)^{abcA}
Control	9.20 (3.06)^{cdB}	14.40 (3.87)^{bcAB}	20.00 (4.57)^{abA}	10.40 (3.37)^{bB}	4.40 (2.29)^{abA}	6.80 (2.77)^{aA}	4.00 (2.18)^{abcdA}	4.40 (2.31)^{aA}
Significance	S				S			
CV (%)	15.7				21.1			

@ Experiment was initiated with six month old polybag plants, means with same lower case letter as superscript within a column are homogeneous, means with same upper case letter as superscript within a row are homogeneous for a period, values in the parenthesis are mean of square root transformed values

Among the different media (Table 10), M4 (coir pith compost) recorded the highest leaf length both at three (11.15 cm) and six month (13.17 cm) stages. Thereafter, M3 (vermicompost) recorded the highest value with a leaf length of 23.73 cm at the end of the study. But both the media were uniform in their influence except for the initial and final periods. M2 (poultry manure) recorded the lowest value during the entire period.

Effect of each growth regulator on leaf length averaged over all media (Table 11) where interaction was not significant revealed that GR3 (IAA 450 ppm) recorded the highest leaf length both at six (12.95 cm) and nine (17.08 cm) month stages. At twelve months, GR6 (IBA 450 ppm) recorded the highest leaf length (18.93 cm). Effect of all the growth regulators was uniform at third month.

At fifteen month stage (Table 12), among the media, M3 recorded the maximum leaf length (23.73 cm) which was significantly superior to all other media *viz.* M1 (15.71 cm), M2 (13.43 cm) and M4 (17.69 cm). The media M1, M2 and M4 were homogeneous at this period.

Growth regulators showed significant variation among the media at this period. GR6 (IBA 450 ppm) recorded the maximum leaf length in M1 (19.21 cm) and M4 (25.56 cm) at this stage while in M3 medium, GR10 (BA 100 ppm) recorded the highest leaf length (25.53 cm). In M2, GR3 (IAA 300 ppm) recorded the maximum leaf length of 19.07 cm at this period.

4.1.1.1.6 Leaf breadth

The interaction between media and growth regulator was found to be significant at ninth, twelfth and fifteen month stages. But during other stages also, significant difference was observed among the growth regulators and also among the media.

Table 10. Leaf length for each medium averaged over all growth regulators (cm)

Media	3 months[@]	6 months	9 months	12 months
M1	9.98[b]	10.39[b]	12.12[b]	13.64[b]
M2	8.22[c]	9.78[b]	11.93[b]	13.17[b]
M3	10.20[b]	13.03[a]	17.76[a]	19.71[a]
M4	11.15[a]	13.17[a]	16.38[a]	19.12[a]
Significance	S	S	S	S

Table 11. Leaf length for each growth regulator averaged over all media (cm)

Growth Regulators	3 months[@]	6 months	9 months	12 months
GR1	10.21	11.95[ab]	16.32[ab]	18.42[a]
GR2	9.91	11.61[ab]	15.93[ab]	17.83[ab]
GR3	9.87	12.95[a]	17.08[a]	18.57[a]
GR4	9.59	12.03[a]	15.93[ab]	17.84[ab]
GR5	10.17	11.80[ab]	16.35[ab]	18.18[ab]
GR6	10.05	12.19[a]	16.63[ab]	18.93[a]
GR7	9.60	10.31[bc]	11.63[c]	13.88[cd]
GR8	9.98	10.25[bc]	11.47[c]	12.84[d]
GR9	9.13	8.90[c]	9.08c	10.58[d]
GR10	10.16	12.01[ab]	14.92[a]	17.05[ab]
GR11	10.03	12.40[a]	14.18[b]	15.76[bc]
GR12	9.97	12.73[a]	15.03[a]	17.07[ab]
Significance	NS	S	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 12. Influence of media and growth regulators on leaf length (cm)

Growth Regulators	15 months			
	M1	M2	M3	M4
GR1	17.06^{abcB}	18.98^{aAB}	22.47^{abAB}	24.10^{aA}
GR2	17.50^{abcA}	18.20^{aA}	21.21^{abA}	21.86^{abcA}
GR3	14.56^{abcB}	19.07^{aAB}	23.65^{abA}	22.90^{abA}
GR4	16.06^{abcB}	18.69^{aAB}	21.47^{abAB}	22.84^{abA}
GR5	19.04^{abAB}	14.66^{abcd}	22.83^{abA}	24.36^{aA}
GR6	19.21^{aB}	16.83^{abB}	24.99^{abAB}	25.56^{aA}
GR7	13.29^{bcB}	11.85^{bcdeB}	19.20^{bA}	19.88^{abcdA}
GR8	13.97^{abcB}	8.38^{deB}	19.82^{abA}	14.92^{dAB}
GR9	11.85^{cAB}	7.90^{eB}	11.47^{cAB}	16.23^{cdA}
GR10	13.41^{abcB}	13.96^{abcdB}	25.53^{aA}	23.12^{abA}
GR11	16.22^{abcB}	10.36^{cdeC}	21.19^{abA}	20.10^{abcA}
GR12	14.79^{abcB}	14.00^{abcdB}	22.50^{abA}	24.61^{aA}
Control	15.71^{abcB}	13.43^{abcdeB}	23.73^{abA}	17.69^{bcdB}
Significance	S			
CV (%)	19.7			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Among the different media (Table 13), M4 (coir pith compost) recorded the highest leaf breadth at three (3.91 cm) and six month (4.39 cm) stages but was on par with M3 at both these stages. Thereafter, M3 (vermicompost) recorded the highest values at all the stages with a leaf breadth of 8.21 cm at the end of the study. At all these stages, M3 was significantly superior to all other media.

Effect of each growth regulator on leaf breadth averaged over all media (Table 14) where interaction was not significant revealed that GR1 (IAA 150 ppm) and GR3 (IAA 450 ppm) recorded the highest leaf breadth at three (3.67 cm) and six (4.24 cm) month stages respectively.

At fifteen month stage (Table 15), among the media, M3 recorded the maximum leaf breadth (8.21 cm) which was significantly superior to all other media. Effect of other three media on leaf breadth was identical this period.

Growth regulators showed significant variation among the media in their effect on leaf breadth at this period. GR6 (IBA 450 ppm) recorded the maximum leaf breadth in M1 (7.80 cm) and M3 (9.40 cm) at this stage while in M2 medium, GR1 (IAA 150 ppm) recorded the highest value (7.65 cm). In the medium M4, GR5 (IBA 300 ppm) had the maximum leaf breadth (8.64 cm) at this period.

4.1.1.1.7 Leaf area

The interaction between media and growth regulator was found to be significant at twelve and fifteen month stages. But during other stages also, significant difference was observed among the growth regulators and also among the media.

Table 13. Leaf breadth for each medium averaged over all growth regulators (cm)

Media	3 months[@]	6 months
M1	3.39[b]	3.52[b]
M2	2.79[c]	3.17[c]
M3	3.76[a]	4.34[a]
M4	3.91[a]	4.39[a]
Significance	S	S

Table 14. Leaf breadth for each growth regulator averaged over all media (cm)

Growth Regulators	3 months[@]	6 months
GR1	3.67[a]	4.09[ab]
GR2	3.52[a]	4.03[ab]
GR3	3.41[ab]	4.24[a]
GR4	3.46[a]	4.03[ab]
GR5	3.58[a]	4.01[ab]
GR6	3.49[a]	4.00[ab]
GR7	3.32[ab]	3.49[bc]
GR8	3.31[ab]	3.37[c]
GR9	3.00[b]	2.94[c]
GR10	3.51[a]	3.94[ab]
GR11	3.60[a]	4.01[ab]
GR12	3.65[a]	4.10[a]
Significance	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 15. Influence of media and growth regulators on leaf breadth (cm)

Growth Regulators	9 months				12 months				15 months			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
GR1	4.80^{abA}	5.14^{abA}	5.92^{abcA}	6.18^{abA}	5.71^{abcdA}	7.03^{aA}	6.78^{abcA}	6.97^{abA}	6.79^{abcA}	7.65^{aA}	7.72^{bcA}	7.87^{abcA}
GR2	4.36^{abB}	4.52^{abcB}	6.67^{aA}	6.63^{aA}	5.25^{bcdefB}	6.37^{abAB}	7.54^{abA}	6.74^{abAB}	6.37^{abcA}	7.48^{abA}	7.68^{bcA}	6.91^{bcdA}
GR3	4.27^{abB}	5.53^{aB}	7.19^{aA}	5.95^{abAB}	5.54^{abcdB}	6.67^{abAB}	7.85^{aA}	7.11^{aAB}	5.64^{bcdeB}	7.64^{aA}	8.46^{abcA}	8.35^{abA}
GR4	4.53^{abB}	5.12^{abAB}	6.38^{abA}	5.65^{abAB}	5.32^{bcdeB}	6.20^{abcAB}	7.48^{abA}	6.57^{abAB}	5.76^{bcdeB}	7.36^{abAB}	7.94^{abcA}	8.07^{abA}
GR5	5.35^{aAB}	4.06^{abcB}	6.70^{aA}	6.02^{abA}	6.36^{abcAB}	5.26^{bcB}	7.81^{aA}	7.56^{aA}	6.97^{abcB}	6.17^{abcB}	8.68^{abA}	8.64^{aA}
GR6	4.56^{abB}	4.30^{abcB}	6.69^{aA}	6.66^{aA}	7.10^{aAB}	5.81^{abcB}	7.98^{aA}	7.45^{aA}	7.80^{aBC}	6.66^{abcC}	9.40^{aA}	8.59^{abAB}
GR7	3.43^{bA}	3.19^{cdA}	4.47^{cA}	4.01^{cdA}	3.82^{efB}	3.68^{deB}	5.71^{cA}	5.10^{cdAB}	4.63^{deB}	4.16^{deB}	6.93^{cA}	6.36^{cdeA}
GR8	3.26^{bBC}	2.16^{dC}	5.00^{bcA}	3.85^{cdAB}	4.64^{defAB}	2.66^{eC}	6.01^{bcA}	4.28^{dBC}	5.78^{bcdeB}	3.04^{eC}	7.78^{bcA}	4.59^{fBC}
GR9	3.25^{bA}	2.20^{dA}	2.36^{dA}	3.51^{dA}	3.63^{fA}	2.84^{eA}	2.79^{dA}	4.25^{dA}	4.25^{eAB}	3.31^{eB}	3.34^{dAB}	4.95^{efA}
GR10	3.75^{abB}	3.86^{bcB}	6.33^{abA}	5.72^{abA}	4.78^{cdefB}	5.06^{bcdB}	7.31^{abcA}	7.65^{aA}	5.70^{cdeB}	6.01^{bcB}	8.44^{abcA}	8.34^{abA}
GR11	3.68^{bB}	3.09^{cdB}	6.14^{abA}	5.32^{abcA}	6.76^{abA}	3.90^{deB}	6.68^{abcA}	6.17^{abcA}	7.35^{abA}	4.10^{deB}	7.10^{bcA}	7.34^{abcdA}
GR12	3.54^{bC}	4.02^{abcBC}	6.23^{abA}	5.43^{abcAB}	5.14^{bcdefB}	4.72^{cdB}	7.66^{abA}	7.02^{abA}	6.07^{bdB}	5.10^{cdB}	8.49^{abcA}	8.31^{abA}
Control	3.96^{abB}	3.98^{abB}	7.41^{aA}	4.78^{bcdB}	5.54^{abcdB}	4.17^{deB}	7.92^{aA}	5.42^{bcdB}	6.15^{bdB}	4.34^{deB}	8.21^{abcA}	5.93^{defB}
Significance	S				S				S			
CV (%)	20.6				17.0				14.9			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Among the different media (Table 16), M4 (coir pith compost) recorded the highest mean leaf area at three (27.33 cm^2) and six month (36.98 cm^2) stages but was on par with M3 at six month stage. Thereafter, M3 (vermicompost) recorded the highest values at all the stages with a mean leaf area of 121.37 cm^2 at the end of the study. At all these stages, M3 was significantly superior to all other media.

Effect of each growth regulator on mean leaf area averaged over all media (Table 17) where interaction was not significant revealed that GR3 (IAA 450 ppm) recorded the highest mean leaf area at six (34.64 cm^2) and nine (63.45 cm^2) month stages. At three month stage, effect of all the media was identical.

At fifteen month stage (Table 18), among the media, M3 recorded the maximum mean leaf area (121.37 cm^2) which was significantly superior to all other media *viz.* M1 (61.14 cm^2), M2 (37.71 cm^2) and M4 (65.09 cm^2). Effect of M1, M2 and M4 was homogeneous at this period.

At the same period, in M1, M3 and M4 medium, GR6 (IBA 450 ppm) recorded the highest mean leaf area of 95.74 cm^2, 147.03 cm^2 and 137.33 cm^2 respectively. GR1 (IAA 150 ppm) recorded the maximum mean leaf area (91.01 cm^2) in M2 at this period. In the case of M1 and M3, GR6 was on par with control in the respective media with reference to mean leaf area at fifteen month stage. In the M2 medium, effect of control was significantly lower compared to GR1, GR2, GR3 and GR4. In M4, all the treatments except GR2, GR7, GR8, GR9 and GR11 were superior to control. In all the GA concentrations, mean leaf area was relatively less in all the media compared to other growth regulator applications.

Table 16. Leaf area for each medium averaged over all growth regulators (cm^2)

Media	3 months[@]	6 months	9 months
M1	21.22^{c}	23.06^{b}	33.14^{c}
M2	14.64^{d}	20.37^{c}	31.79^{c}
M3	24.17^{b}	36.64^{a}	67.99^{a}
M4	27.33^{a}	36.98^{a}	57.78^{b}
Significance	S	S	S

Table 17. Leaf area for each growth regulator averaged over all media (cm^2)

Growth Regulators	3 months[@]	6 months	9 months
GR1	23.79	31.10^{ab}	58.24^{abc}
GR2	22.55	30.88^{ab}	58.77^{ab}
GR3	21.37	34.64^{a}	63.45^{a}
GR4	20.82	31.42^{ab}	55.58^{abc}
GR5	23.26	31.23^{ab}	59.78^{ab}
GR6	22.32	32.24^{a}	60.98^{ab}
GR7	20.24	23.29^{bc}	30.19^{de}
GR8	21.05	22.11^{bc}	27.62^{e}
GR9	17.97	17.12^{c}	17.26^{e}
GR10	22.61	30.37^{ab}	48.75^{abc}
GR11	23.18	32.98^{a}	43.36^{cd}
GR12	22.94	33.77^{a}	48.10^{bc}
Significance	NS	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 18. Influence of media and growth regulators on leaf area (cm^2)

Growth Regulators	12 months				15 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	54.97^{abcB}	78.62^{aAB}	85.27^{abAB}	91.60^{aA}	72.99^{abcB}	91.01^{aAB}	108.01^{abcAB}	118.38^{abcA}
GR2	48.00^{abcC}	62.43^{abcB}	98.51^{abA}	87.69^{abAB}	71.17^{abcdA}	84.21^{abA}	103.20^{bcA}	94.39^{bcdA}
GR3	50.05^{abcC}	73.93^{abBC}	110.53^{aA}	87.93^{abAB}	52.55^{bcdB}	90.80^{aAB}	124.90^{abcA}	118.78^{abcA}
GR4	51.43^{abcB}	69.17^{abcAB}	96.89^{abA}	80.33^{abAB}	59.31^{abcdB}	88.81^{aAB}	108.07^{abcA}	116.37^{abcA}
GR5	71.69^{abAB}	45.63^{abcdB}	102.23^{aA}	100.72^{aA}	86.44^{abBC}	59.50^{abcdC}	123.68^{abcAB}	130.80^{abA}
GR6	73.65^{aBC}	52.71^{abcdC}	111.41^{aA}	105.44^{aAB}	95.74^{aB}	70.80^{abcB}	147.03^{aA}	137.33^{aA}
GR7	28.43^{cB}	25.31^{deB}	66.84^{bA}	53.33^{bcAB}	41.51^{cdBC}	31.46^{cdeC}	85.97^{cA}	80.49^{cdeAB}
GR8	33.48^{cAB}	13.46^{eB}	66.58^{bA}	38.08^{cAB}	52.52^{bcdB}	15.85^{eB}	95.63^{bcA}	44.14^{eB}
GR9	24.51^{cA}	13.65^{eA}	18.41^{cA}	38.10^{cA}	32.35^{dA}	16.57^{eA}	24.45^{dA}	51.94^{eA}
GR10	37.74^{bcB}	42.20^{bcdeB}	103.03^{aA}	96.28^{aA}	50.28^{bcdB}	53.78^{abcdeB}	133.62^{abA}	120.06^{abcA}
GR11	58.32^{abcBC}	23.97^{deC}	84.67^{abA}	72.05^{abcAB}	73.04^{abcA}	26.64^{deB}	93.96^{bcA}	92.62^{bcdA}
GR12	38.57^{abcB}	39.23^{bcdeB}	101.10^{abA}	96.35^{aA}	55.80^{abcdB}	45.90^{bcdeB}	119.29^{abcA}	127.17^{abA}
Control	51.30^{abcB}	34.55^{cdeB}	110.62^{aA}	53.95^{bcB}	61.14^{abcdB}	37.71^{cdeB}	121.37^{abcA}	65.09^{deB}
Significance	S				S			
CV (%)	33.5				30.6			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.8 Total leaf area

The interaction between media and growth regulator was found to be significant at twelve and fifteen month stages. But during other stages, significant difference was observed among the growth regulators and also among the media.

Among the different media (Table 19), M4 (coir pith compost) recorded the highest total leaf area (409.97 cm^2) at three month (36.98 cm^2) stage. M3 (vermicompost) recorded the highest values at all other stages with a total leaf area of 3275.98 cm^2 at the end of the study. At all these stages except during sixth month, M3 was significantly superior to all other media.

Effect of each growth regulator on total leaf area averaged over all media (Table 20) where interaction was not significant revealed that GR11 (BA 450 ppm) recorded the highest total leaf area at three (349.84 cm^2) and six (628.52 cm^2) month stages. At nine month stage, GR3 (IAA 150 ppm) recorded the highest total leaf area (1252.84 cm^2).

At fifteen month stage (Table 21), among the media, M3 recorded the maximum total leaf area (3275.98 cm^2) which was significantly superior to all other media. M1 (1104.83 cm^2), M2 (739.21 cm^2) and M4 (1143.33 cm^2) were homogeneous at this period.

At the same period, in M1 and M3 medium, GR6 (IBA 450 ppm) recorded the highest total leaf area of 2351.11 cm^2 and 4061.00 cm^2 respectively. GR1 (IAA 150 ppm) recorded the maximum total leaf area (2514.00 cm^2) in M2 at this period. In the medium M4, GR5 (IBA 300 ppm) had the maximum total leaf area (3224.01 cm^2). In all the four media, GR7, GR8 and GR9 recorded relatively lower values compared to rest of the treatments.

Table 19. Total leaf area for each medium averaged over all growth regulators (cm^2)

Media	3 months[@]	6 months	9 months
M1	289.53[c]	306.86[b]	564.50[c]
M2	191.98[d]	313.47[b]	603.84[c]
M3	356.98[b]	680.83[a]	1518.67[a]
M4	409.97[a]	638.53[a]	1158.82[b]
Significance	S	S	S

Table 20. Total leaf area for each growth regulator averaged over all media (cm^2)

Growth Regulators	3 months[@]	6 months	9 months
GR1	345.42[a]	528.11[ab]	1164.87[a]
GR2	329.21[a]	528.90[ab]	1168.82[a]
GR3	292.06[ab]	575.39[a]	1252.84[a]
GR4	300.06[ab]	519.21[ab]	1075.33[a]
GR5	343.00[a]	538.54[a]	1233.30[a]
GR6	328.39[a]	555.39[a]	1225.86[a]
GR7	283.99[ab]	343.91[bc]	528.59[b]
GR8	287.67[ab]	283.21[c]	449.81[b]
GR9	237.85[b]	206.24[c]	234.95[b]
GR10	310.89[ab]	491.12[ab]	962.04[a]
GR11	349.84[a]	628.52[a]	1094.27[a]
GR12	337.02[a]	620.52[a]	1146.84[a]
Significance	S	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 21. Influence of media and growth regulators on total leaf area (cm^2)

Growth Regulators	12 months				15 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	1076.24^{abcA}	1952.42^{aA}	1946.02^{abcA}	1986.57^{aA}	1608.51^{abcA}	2514.00^{aA}	2676.40^{bcdA}	2738.20^{abA}
GR2	895.26^{abcB}	1312.03^{abcdAB}	2232.04^{abcA}	1996.76^{aA}	1507.62^{abcA}	1929.08^{abcA}	2605.70^{cdA}	2445.21^{abcA}
GR3	837.98^{abcC}	1544.26^{abBC}	2594.14^{abA}	1840.64^{abAB}	985.86^{bcA}	2084.50^{abAB}	3324.69^{abcdA}	2680.56^{abA}
GR4	1092.99^{abcB}	1450.99^{abcAB}	2376.95^{abA}	1610.44^{abcAB}	1417.32^{abcB}	2156.33^{abAB}	3104.48^{abcdA}	2456.25^{abAB}
GR5	1485.07^{abAB}	920.46^{bcdefB}	2368.95^{abA}	2300.92^{aA}	2085.75^{abAB}	1282.39^{abcdeB}	3128.10^{abcdA}	3224.01^{aA}
GR6	1597.30^{aBC}	1123.85^{abcdefC}	2737.45^{aA}	2256.16^{aAB}	2351.11^{aBC}	1585.09^{abcdC}	4061.00^{aA}	3179.58^{aAB}
GR7	478.27^{bcB}	389.92^{defAB}	1607.63^{bcA}	969.60^{bcAB}	844.84^{bcB}	520.53^{deB}	2383.72^{cdA}	1501.53^{bcdAB}
GR8	494.30^{bcAB}	203.62^{efB}	1341.04^{cdA}	675.22^{cAB}	918.65^{bcB}	255.32^{eB}	2266.32^{dA}	851.66^{dB}
GR9	281.51^{cA}	130.38^{fA}	372.22^{dA}	626.36^{cA}	459.52^{cA}	190.56^{eA}	538.92^{eA}	975.09^{dA}
GR10	792.17^{abcB}	730.87^{bcdefB}	2408.13^{abA}	2130.78^{aA}	1225.95^{abcB}	992.33^{bcdeB}	3294.73^{abcdA}	2798.84^{abA}
GR11	1656.91^{aB}	491.42^{cdefC}	2908.40^{aA}	1774.25^{abB}	2297.27^{aB}	558.54^{deC}	3625.71^{abcA}	2616.29^{abAB}
GR12	889.77^{abcB}	1150.44^{abcdeB}	2898.26^{aA}	2344.62^{aA}	1425.56^{abB}	1391.79^{abcdeB}	3927.98^{abA}	3311.12^{aA}
Control	855.06^{aB}	687.94^{bcdefB}	2566.19^{abA}	889.08^{bcB}	1104.83^{abcB}	739.21^{cdeB}	3275.98^{abcdA}	1143.33^{cdB}
Significance	S				S			
CV (%)	43.5				40.5			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

In the case of M1 and M3, effect of media alone was identical with that of superior growth regulator treatments. In M2 medium, GR1, GR3 and GR4 were significantly superior to control. All the treatments except GR7, GR8 and GR9 were significantly superior to control in M4.

4.1.1.1.9 Petiole length

The interaction between media and growth regulator was found to be non significant at all the stages. But significant difference was observed among the growth regulators and also among the media.

Among the different media (Table 22), M4 (coir pith compost) recorded the highest petiole length (0.48 cm) up to third month. M3 (vermicompost) recorded the highest values at all other stages with a petiole length of 1.08 cm at the end of the study. At all these stages except third month, M3 and M4 were significantly superior to other two media.

Effect of each growth regulator on petiole length averaged over all media (Table 23) revealed that GR5 (IBA 300 ppm) recorded the highest petiole length at three (0.48 cm) and nine (0.86 cm) months. At six (0.60 cm) and fifteen (1.08 cm) month periods GR6 (IBA 450 ppm) recorded the highest value. At twelve months, effect of both the treatments was identical (0.96 cm).

4.1.1.1.10 Internodal length

The interaction between media and growth regulator was found to be significant at all the stages except in the third month. But during third month also, significant difference was observed among the growth regulators and also among the media.

Table 22. Petiole length for each medium averaged over all growth regulators (cm)

Media	3 months[@]	6 months	9 months	12 months	15 months
M1	0.45[a]	0.50[b]	0.58[d]	0.67[c]	0.76[b]
M2	0.39[b]	0.49[b]	0.63[c]	0.69[c]	0.74[b]
M3	0.46[a]	0.61[a]	0.92[a]	1.02[a]	1.08[a]
M4	0.48[a]	0.60[a]	0.79[b]	0.89[b]	0.97[a]
Significance	S	S	S	S	S

Table 23. Petiole length for each growth regulator averaged over all media (cm)

Growth Regulators	3 months[@]	6 months	9 months	12 months	15 months
GR1	0.44[ab]	0.58[a]	0.83[a]	0.93[a]	1.00[ab]
GR2	0.45[ab]	0.54[abc]	0.79[ab]	0.88[ab]	0.97[ab]
GR3	0.47[ab]	0.57[a]	0.85[a]	0.94[a]	1.02[ab]
GR4	0.45[ab]	0.54[abc]	0.75[abc]	0.82[abcd]	0.87[bc]
GR5	0.48[a]	0.57[ab]	0.86[a]	0.96[a]	1.01[ab]
GR6	0.46[ab]	0.60[a]	0.85[a]	0.96[a]	1.08[a]
GR7	0.42[b]	0.48[c]	0.57[d]	0.65[de]	0.71[cd]
GR8	0.47[ab]	0.54[abc]	0.61[cd]	0.67[cde]	0.70[cd]
GR9	0.42[b]	0.48[c]	0.59[cd]	0.62[e]	0.63[d]
GR10	0.42[b]	0.54[abc]	0.71[abcd]	0.81[abcd]	0.89[abc]
GR11	0.43[ab]	0.56[ab]	0.66[bcd]	0.75[bcde]	0.85[bc]
GR12	0.46[ab]	0.59[a]	0.72[abcd]	0.84[abc]	0.91[abc]
Significance	S	S	S	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Among the different media (Table 24), M4 (coir pith compost) recorded the highest internodal length (1.16 cm) at three month stage. M3 (vermicompost) recorded the highest values at all other stages with an internodal length of 3.46 cm at the end of the study. At twelve and fifteen month stages, M3 was significantly superior to all other media.

At the initial three months period where interaction was not significant (Table 25), GR9 (GA 300 ppm) and GR8 (GA 200 ppm) were the two superior treatments recording an internodal length of 1.23 cm and 1.14 cm respectively.

At fifteen month stage (Table 26), among the media, M3 recorded the maximum internodal length (3.46 cm) which was superior to all other media. M1 (1.03 cm), M2 (1.04 cm) and M4 (1.05 cm) were homogeneous at this period.

Effect of growth regulators varied widely among the media at all the stages where media – growth regulator interaction was significant. Towards the end of the study at fifteen months, in M1 medium, GR6 (IBA 450 ppm) recorded the highest internodal length (2.86 cm) which was homogeneous with all other treatments except control (0.90 cm). In M2 and M3 medium, GR8 (GA 200 ppm) recorded the highest internodal length of 3.56 cm and 4.78 cm respectively. In M2, GR8 was superior to all other treatments except GR1, GR3, GR4 and GR7 while in M3, it was on par with all other treatments except GR1 and GR5. In the medium M4, GR7 (GA 100 ppm) had the maximum internodal length (3.72 cm) which differed significantly with GR1, GR4, GR8, GR12 and control.

Table 24. Internodal length for each medium averaged over all growth regulators (cm)

Media	3 months[@]
M1	0.99[b]
M2	0.71[c]
M3	1.11[a]
M4	1.16[a]
Significance	S

Table 25. Internodal length for each growth regulator averaged over all media (cm)

Growth Regulators	3 months[@]
GR1	0.96[b]
GR2	0.95[b]
GR3	0.99[b]
GR4	0.91[b]
GR5	0.97[b]
GR6	0.94[b]
GR7	0.97[b]
GR8	1.14[a]
GR9	1.23[a]
GR10	0.93[b]
GR11	0.95[b]
GR12	1.01[b]
Significance	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 26. Influence of media and growth regulators on internodal length (cm)

Growth Regulators	6 months				9 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	1.09^{aA}	0.81^{aA}	0.98^{eA}	1.23^{abcdA}	1.41^{abA}	1.03^{bA}	1.25^{eA}	1.47^{bcdA}
GR2	0.94^{aA}	0.75^{aA}	1.19^{cdeA}	1.18^{abcdA}	1.08^{abA}	0.83^{bA}	1.55^{deA}	1.41^{bcdA}
GR3	1.19^{aA}	0.68^{aB}	1.19^{cdeA}	1.23^{abcdAB}	1.41^{abA}	0.93^{bA}	1.64^{cdeA}	1.34^{bcdA}
GR4	0.96^{aA}	0.91^{aA}	1.16^{cdeA}	1.03^{cdA}	1.18^{abA}	1.08^{bA}	1.38^{eA}	1.13^{cdA}
GR5	1.34^{aA}	0.78^{aB}	1.12^{deAB}	1.11^{bcdAB}	1.72^{abA}	0.91^{bA}	1.43^{eA}	1.34^{bcdA}
GR6	1.19^{aAB}	0.72^{aB}	1.14^{cdeAB}	1.38^{abcA}	1.28^{abA}	0.80^{bA}	1.42^{eA}	1.53^{abcdA}
GR7	0.99^{aBC}	0.86^{aC}	1.58^{bcA}	1.39^{abcAB}	1.27^{abB}	1.27^{abB}	2.54^{abA}	1.99^{abAB}
GR8	1.09^{aB}	1.01^{aB}	1.74^{abA}	1.50^{abA}	1.76^{aAB}	1.97^{aAB}	2.56^{abA}	1.67^{abcdB}
GR9	1.25^{aBC}	1.04^{aC}	2.19^{aA}	1.61^{aB}	1.39^{abB}	0.91^{bB}	2.91^{aA}	2.33^{aA}
GR10	0.97^{aA}	0.90^{aA}	1.07^{deA}	1.19^{abcdA}	1.28^{abA}	1.20^{abA}	1.60^{cdeA}	1.26^{bcA}
GR11	1.14^{aAB}	0.69^{aB}	1.26^{ceA}	1.50^{abA}	1.18^{abBC}	0.83^{bC}	2.34^{abcdA}	1.95^{abcAB}
GR12	1.04^{aBC}	0.89^{aC}	1.51^{bcdA}	1.26^{abcdAB}	1.17^{abB}	1.12^{bB}	2.44^{abcA}	1.44^{bcdB}
Control	1.00^{aA}	0.81^{aA}	1.22^{cdeA}	0.85^{dA}	0.90^{bB}	1.00^{bAB}	1.79^{bcdeA}	0.92^{dB}
Significance	S				S			
CV (%)	24.7				35.4			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 26. continued

Growth Regulators	12 months				15 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	1.57^{abA}	1.96^{abcA}	1.81^{dA}	1.68^{abcdA}	1.82^{abA}	2.62^{abcA}	2.56^{cA}	2.03^{bcA}
GR2	1.87^{abA}	1.23^{bcA}	2.27^{bcdA}	2.33^{abcA}	2.33^{ab}	1.96^{bcA}	3.39^{abcA}	3.16^{abA}
GR3	1.75^{abA}	1.76^{abcA}	2.81^{abcdA}	2.17^{abcA}	2.07^{abB}	2.55^{abcB}	4.71^{aA}	2.98^{abB}
GR4	1.73^{abA}	2.18^{abA}	2.47^{abcdA}	1.50^{cdA}	2.23^{abB}	3.01^{abAB}	4.19^{abA}	1.92^{bcB}
GR5	1.96^{abA}	1.15^{bcA}	2.04^{cdA}	1.98^{abcdA}	2.36^{abAB}	1.49^{bcB}	3.09^{bcA}	2.77^{abAB}
GR6	2.12^{aA}	1.01^{cB}	2.77^{acdA}	1.83^{abcdAB}	2.86^{aB}	1.45^{bcB}	4.59^{abA}	2.24^{abcB}
GR7	1.67^{abB}	1.83^{abcB}	3.22^{aA}	2.72^{aAB}	2.06^{abC}	2.43^{abBC}	4.03^{abcA}	3.72^{aAB}
GR8	2.13^{aBC}	2.85^{aAB}	3.48^{aA}	1.66^{abcdC}	2.52^{abBC}	3.56^{aAB}	4.78^{aA}	1.62^{bcC}
GR9	1.94^{abBC}	1.26^{bcC}	3.49^{aA}	2.67^{abAB}	2.33^{abB}	1.68^{bB}	4.41^{abA}	3.17^{abAB}
GR10	1.73^{abA}	1.34^{bcA}	2.17^{bcdA}	1.60^{bcdA}	2.09^{abAB}	1.53^{bcB}	3.44^{abA}	2.16^{abcAB}
GR11	1.51^{abBC}	0.93^{cC}	3.13^{abcA}	2.15^{abcAB}	1.92^{abB}	1.08^{cB}	4.01^{abcA}	2.31^{abcB}
GR12	1.95^{abB}	1.30^{bcB}	3.27^{abA}	1.73^{abcdB}	2.45^{abB}	1.63^{bcB}	4.11^{abcA}	2.07^{bcB}
Control	0.93^{bB}	1.04^{cB}	2.49^{abcdA}	0.98^{dB}	1.03^{bB}	1.04^{cB}	3.46^{abcA}	1.05^{cB}
Significance	S				S			
CV (%)	34.0				37.0			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.11 Number of branches

Production of branches started after six months of commencement of the study. The interaction between media and growth regulator was found to be significant at nine month stage only. At this stage, among the media, M2 (poultry manure) recorded the maximum number of branches (1.20) which was on par with M1 (0.20) and significantly superior to M3 (0.00) and M4 (0.00). M1, M3 and M4 were homogeneous at this period.

In M1, GR11 (BA 200 ppm) and in M2 both GR11 and GR12 (BA 300 ppm) recorded the maximum number of 1.60 and 3.60 branches respectively at nine month stage. In M3, all the treatments were homogeneous and the maximum number of branches (1.20) was recorded in GR11. In the medium M4 also treatments did not show any significant difference and both GR11 and GR12 had the maximum number of branches (0.40).

During the later stages of study at twelve and fifteen month periods, the interaction between media and growth regulator was found to be non significant. But significant difference was observed among the media and among the growth regulators.

Among the media, M1 (well rotten cow dung) recorded the highest number of branches both at twelve (1.33) and fifteen (1.45) month stages and number of branches was lowest (0.33 and 0.40 respectively) in M4 at these stages (Table 27). Among the growth regulators (Table 28), GR11 (BA 200 ppm) recorded the highest number of branches both at twelve (2.35) and fifteen (2.45) months.

Table 27. Number of branches for each medium averaged over all growth regulators

Media	12 months[@]	15 months
M1	1.33 (1.46)[a]	1.45 (1.50)[a]
M2	0.77 (1.28)[bc]	0.87 (1.31)[ab]
M3	1.07 (1.36)[ab]	1.17 (1.39)[a]
M4	0.33 (1.11)[c]	0.40 (1.14)[b]
Significance	S	S

Table 28. Number of branches for each growth regulator averaged over all media

Growth Regulators	12 months[@]	15 months
GR1	0.75 (1.25)[c]	0.90 (1.31)[c]
GR2	0.75 (1.27)[c]	0.85 (1.30)[c]
GR3	0.50 (1.18)[c]	0.55 (1.19)[c]
GR4	1.05 (1.37)[bc]	1.15 (1.40)[bc]
GR5	0.35 (1.13)[c]	0.40 (1.15)[c]
GR6	0.55 (1.21)[c]	0.65 (1.24)[c]
GR7	0.75 (1.28)[b]	0.80 (1.30)[c]
GR8	0.80 (1.31)[c]	0.90 (1.34)[c]
GR9	0.45 (1.17)[c]	0.50 (1.19)[c]
GR10	0.45 (1.16)[c]	0.50 (1.18)[c]
GR11	2.35 (1.72)[a]	2.45 (1.76)[a]
GR12	1.75 (1.59)[ab]	2.00 (1.67)[ab]
Significance	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous
Values in the parenthesis are mean of square root transformed values

4.1.1.1.12 Leaf thickness

The interaction between media and growth regulator was found to be significant at all the stages.

Among the different media (Table 29), M3 (vermicompost) recorded the highest leaf thickness during the entire period. Influence of other media varied between different periods. But in general, M1 (well rotten cow dung) was the next superior medium during most of the periods followed by M4 (coir pith compost) and M2 (poultry manure). At fifteen month stage, among the media, M3 recorded the highest leaf thickness (0.379 mm) which was on par with M1 (0.365 mm) and significantly superior to M2 (0.318 mm) and M4 (0.325 mm).

Effect of growth regulators varied widely among the media at all the stages where media – growth regulator interaction was significant. Towards the end of the study at fifteen months, in the M3 medium, GR11 (BA 200 ppm) recorded the highest leaf thickness (0.390 mm) while control recorded the maximum leaf thickness (0.365 mm) in M1. GR1 (IAA 150 ppm) recorded the maximum leaf thickness (0.350 mm) in M2 and in the medium M4, GR10 (BA 100 ppm) had the maximum leaf thickness (0.357 mm).

4.1.1.1.13 Leaf duration

On emergence of new leaves, these leaves were tagged to find out the leaf duration (period from emergence to senescence). These newly produced leaves did not fall and remained on the plant during the entire experimental period of fifteen months. Hence duration of leaves could not be ascertained.

Table 29. Influence of media and growth regulators on leaf thickness (mm)

Growth Regulators	3 months[@]				6 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.326^{aA}	0.318^{abA}	0.303^{aA}	0.272^{bcdB}	0.336^{abA}	0.347^{aA}	0.355^{abcdA}	0.318^{abA}
GR2	0.314^{aA}	0.323^{aA}	0.308^{aA}	0.246^{dB}	0.338^{abA}	0.342^{aA}	0.357^{abcdA}	0.323^{abA}
GR3	0.304^{aA}	0.320^{aA}	0.300^{aA}	0.266^{bcdB}	0.335^{abA}	0.337^{abA}	0.356^{abcdA}	0.322^{abA}
GR4	0.313^{aA}	0.316^{abA}	0.309^{aA}	0.274^{bcdB}	0.322^{bA}	0.316^{abA}	0.328^{dA}	0.329^{abA}
GR5	0.313^{aA}	0.319^{aA}	0.313^{aA}	0.261^{cdA}	0.359^{abA}	0.316^{abB}	0.344^{dAB}	0.314^{bB}
GR6	0.325^{aA}	0.326^{aA}	0.323^{aA}	0.282^{abcB}	0.345^{abA}	0.302^{bB}	0.342^{cdA}	0.331^{abAB}
GR7	0.322^{aA}	0.321^{aA}	0.299^{aAB}	0.273^{bcdB}	0.329^{abA}	0.339^{abA}	0.348^{bcdA}	0.316^{abA}
GR8	0.307^{aA}	0.317^{abA}	0.311^{aA}	0.270^{bcdB}	0.340^{abA}	0.314^{abA}	0.344^{dA}	0.336^{abA}
GR9	0.316^{aAB}	0.333^{aA}	0.307^{aAB}	0.290^{abcB}	0.331^{abA}	0.310^{abA}	0.346^{cdA}	0.314^{bA}
GR10	0.309^{aA}	0.308^{abA}	0.315^{aA}	0.303^{abA}	0.345^{abBC}	0.316^{abC}	0.384^{abA}	0.354^{aAB}
GR11	0.313^{aAB}	0.327^{aA}	0.316^{aAB}	0.296^{abB}	0.337^{abB}	0.312^{abB}	0.384^{abA}	0.336^{abB}
GR12	0.311^{aA}	0.304^{abA}	0.326^{aA}	0.305^{aA}	0.348^{abB}	0.342^{aB}	0.387^{aA}	0.334^{abB}
Control	0.301^{aA}	0.288^{bA}	0.306^{aA}	0.312^{aA}	0.361^{aA}	0.315^{abB}	0.382^{acA}	0.310^{bB}
Significance	S				S			
CV (%)	5.670				6.800			

@ Experiment was initiated with six month old polybag plants

Means with same lower case letter as superscript within a column are homogeneous

Means with same upper case letter as superscript within a row are homogeneous for a period

Table 29. continued

Growth Regulators	9 months				12 months				15 months			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.341^{abA}	0.351^{aA}	0.358^{abcA}	0.322^{abA}	0.341^{abA}	0.350^{aA}	0.357^{abcA}	0.322^{abA}	0.340^{abA}	0.350^{aA}	0.357^{abcA}	0.321^{abA}
GR2	0.344^{abA}	0.343^{abA}	0.357^{abcA}	0.327^{abA}	0.344^{abA}	0.341^{abcA}	0.358^{abcA}	0.329^{abA}	0.341^{abA}	0.338^{abcA}	0.358^{abcA}	0.333^{abA}
GR3	0.337^{abA}	0.338^{abcA}	0.358^{abcA}	0.324^{abA}	0.337^{abA}	0.336^{abcdA}	0.358^{abcA}	0.326^{abA}	0.336^{abA}	0.334^{abcA}	0.356^{abcA}	0.326^{abA}
GR4	0.323^{bA}	0.310^{bcdA}	0.336^{dA}	0.320^{abA}	0.324^{bA}	0.312^{bcdA}	0.336^{cA}	0.321^{abA}	0.322^{bA}	0.316^{abcdA}	0.336^{cA}	0.322^{abA}
GR5	0.362^{aA}	0.317^{abcdB}	0.348^{cdA}	0.314^{bB}	0.362^{aA}	0.319^{abcdB}	0.348^{bcAB}	0.314^{bB}	0.362^{aA}	0.322^{abcdB}	0.348^{bcAB}	0.313^{bB}
GR6	0.348^{abA}	0.296^{dB}	0.342^{dA}	0.336^{abA}	0.349^{abA}	0.300^{dB}	0.342^{cA}	0.334^{abAB}	0.349^{abB}	0.297^{dA}	0.341^{cB}	0.333^{abB}
GR7	0.331^{abAB}	0.338^{abcAB}	0.349^{cdA}	0.310^{bB}	0.325^{bA}	0.342^{abcA}	0.348^{bcA}	0.315^{bA}	0.321^{bA}	0.342^{abcA}	0.346^{bcA}	0.320^{abA}
GR8	0.345^{abA}	0.318^{abcdA}	0.347^{cdA}	0.340^{abA}	0.345^{abA}	0.320^{abcdA}	0.347^{bcA}	0.342^{abA}	0.347^{abA}	0.323^{abcdA}	0.346^{bcA}	0.343^{abA}
GR9	0.334^{abAB}	0.304^{cdB}	0.350^{bcdA}	0.315^{bAB}	0.332^{abAB}	0.306^{cdB}	0.349^{bcA}	0.320^{bAB}	0.330^{abAB}	0.307^{cdB}	0.348^{bcA}	0.325^{abAB}
GR10	0.346^{abBC}	0.316^{abcdC}	0.385^{abcA}	0.358^{aAB}	0.346^{abAB}	0.314^{abcdB}	0.382^{abA}	0.357^{aA}	0.346^{abA}	0.312^{bcdB}	0.378^{abA}	0.357^{aA}
GR11	0.340^{abB}	0.319^{abcB}	0.388^{abA}	0.333^{abB}	0.339^{abB}	0.319^{abcdB}	0.389^{aA}	0.336^{abB}	0.338^{abB}	0.319^{abcdB}	0.391^{aA}	0.336^{abB}
GR12	0.343^{abB}	0.345^{abB}	0.390^{aA}	0.333^{abB}	0.344^{abB}	0.345^{abB}	0.390^{aA}	0.331^{abB}	0.346^{abB}	0.345^{abB}	0.390^{aA}	0.330^{abB}
Control	0.368^{aA}	0.314^{abcdB}	0.382^{abcA}	0.322^{abB}	0.365^{aA}	0.316^{abcdB}	0.380^{abA}	0.324^{abB}	0.365^{aA}	0.318^{abcdB}	0.379^{abA}	0.325^{abB}
Significance	S				S				S			
CV (%)	6.602				6.596				6.512			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.14 Colour development of leaf at different stages

Young emerging leaves of mangosteen seedlings are purple, light brick red or pink in colour. Later they turn light green and to dark green on maturity. No variation was observed between treatments in leaf colour development.

4.1.1.1.15 Fresh and dry weight of plant parts

Interaction between media and growth regulator was found to be significant with respect to fresh and dry weight of all the plant parts both at six and twelve month stages.

4.1.1.1.15.1 Fresh weight of whole plant

At six months (Table 30), among the media, M3 (vermicompost) recorded the highest fresh weight (28.46 g) which was significantly superior to all other media *viz.* M1 (5.76 g), M2 (8.90 g) and M4 (11.46 g). In the M3 medium, GR4 (IBA 150 ppm) recorded the highest fresh weight (40.58 g) which was significantly superior to all other treatments except GR3, GR11, GR12 and control. In the medium M1, GR12 (BA 300 ppm) recorded the maximum fresh weight (16.94 g) which was on par with all other treatments. GR3 (IAA 450 ppm) recorded the maximum fresh weight (24.57 g) in M2 which differed significantly with GR7, GR8, GR11 and control. In the medium M4 also, GR3 had the highest fresh weight (31.30 g) which was significantly superior to GR4, GR7, GR8, GR9, GR11 and control.

At twelve month stage also (Table 30), among the media, M3 (vermicompost) recorded the highest fresh weight (50.82 g) which was significantly superior to all other media, *viz.*, M1 (13.72 g), M2 (10.20 g) and M4 (18.27 g). In the M3 medium, GR10 (BA 100 ppm) recorded the highest fresh weight (76.30 g) which was significantly superior to all other treatments except GR11. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum fresh weight (33.07 g) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the maximum fresh weight (40.88

g) in M2 which differed significantly with GR6, GR7, GR8, GR9, GR11 and control. In the medium M4, GR1 (IAA 150 ppm) had the highest fresh weight (51.71 g) which was significantly superior to GR7, GR8, GR9, GR11, GR12 and control.

4.1.1.1.15.2 Fresh weight of shoot

At six months (Table 31), among the media, M3 (vermicompost) recorded the highest shoot fresh weight (24.16 g) which was significantly superior to all other media *viz.* M1 (4.55 g), M2 (7.04 g) and M4 (7.96 g). In the M3 medium, GR4 (IBA 150 ppm) recorded the highest shoot fresh weight (35.87 g) which was significantly superior to all other treatments except GR12. In the medium M1, GR12 (BA 300 ppm) recorded the maximum shoot fresh weight (14.94 g) which was on par with all other treatments except control. GR3 (IAA 450 ppm) recorded the maximum shoot fresh weight (19.81 g) in M2 which differed significantly with GR4, GR7, GR8, GR11 and control. In the medium M4, GR1 (IAA 150 ppm) had the highest shoot fresh weight (26.40 g) which was significantly superior to GR4, GR7, GR8, GR9, GR10, GR11 and control.

A similar trend was observed at twelve months also (Table 31). Among the media, M3 (vermicompost) recorded the highest fresh weight (42.90 g) which was significantly superior to all other media *viz.* M1 (11.84 g), M2 (8.33 g) and M4 (14.65 g). In the M3 medium, GR10 (BA 100 ppm) recorded the highest shoot fresh weight (63.48 g) which was significantly superior to all other treatments except GR4, GR6, GR10, GR11 and GR12. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum shoot fresh weight (29.72 g) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the maximum shoot fresh weight (33.04 g) in M2 which differed significantly with GR8, GR9 and control. In the medium M4, GR1 (IAA 150 ppm) had the highest shoot fresh weight (43.35 g) which was significantly superior to GR7, GR8, GR9, GR11, GR12 and control.

Table 30. Influence of media and growth regulators on fresh weight of whole plant (g)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	15.72^{aB}	13.00^{aB}	20.47^{bcdAB}	31.02^{aA}	27.08^{aB}	28.84^{abAB}	21.75^{dB}	51.71^{aA}
GR2	10.46^{aB}	18.60^{aAB}	16.19^{cdAB}	26.74^{abcA}	16.37^{aB}	26.69^{abAB}	40.80^{bcdA}	31.41^{abAB}
GR3	7.03^{aB}	24.57^{aA}	28.87^{aA}	31.30^{aA}	19.01^{aA}	32.17^{abA}	40.82^{bcdA}	34.55^{abA}
GR4	15.40^{aB}	12.24^{aB}	40.58^{aA}	15.54^{cdeB}	21.77^{aB}	24.42^{abB}	52.23^{bA}	35.08^{abAB}
GR5	11.14^{aB}	15.36^{aAB}	23.73^{bcdAB}	25.51^{abcdA}	24.97^{aA}	40.88^{aA}	32.64^{bcdA}	42.53^{abA}
GR6	12.24^{aA}	16.77^{aA}	22.39^{bcdA}	20.93^{abcdeA}	33.07^{aAB}	17.62^{bB}	49.28^{bcA}	40.67^{abAB}
GR7	14.44^{aA}	10.83^{aA}	17.32^{bcdA}	14.73^{cdeA}	26.40^{aA}	17.53^{bA}	22.74^{dA}	18.26^{bA}
GR8	7.33^{aA}	9.20^{aA}	10.48^{eA}	12.21^{eA}	20.26^{aA}	13.38^{bA}	26.40^{cdA}	27.27^{bA}
GR9	10.92^{aA}	14.08^{aA}	13.94^{deA}	13.56^{deA}	18.81^{aA}	15.39^{bA}	23.21^{dA}	25.90^{bA}
GR10	7.70^{aB}	14.60^{aAB}	25.49^{bdA}	20.62^{abcdeA}	13.65^{aC}	19.77^{abC}	76.30^{aA}	41.37^{abB}
GR11	14.12^{aB}	8.93^{aB}	28.58^{abcA}	18.10^{bcdeAB}	19.37^{aB}	17.22^{bB}	55.78^{abA}	26.63^{bB}
GR12	16.94^{aB}	16.33^{aB}	32.77^{abA}	24.92^{abcdAB}	21.80^{aB}	26.02^{abB}	52.90^{bA}	26.49^{bB}
Control	5.76^{aB}	8.90^{aB}	28.46^{abcA}	11.46^{eB}	13.72^{aB}	10.20^{bB}	50.82^{bA}	18.27^{bB}
Significance	S				S			
CV (%)	32.88				37.68			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 31. Influence of growth regulators and media on fresh weight of shoot (g)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	13.16^{abC}	10.93^{abC}	16.16^{cdeB}	26.40^{aA}	24.50^{aAB}	24.41^{abAB}	17.21^{dB}	43.35^{aA}
GR2	8.67^{abB}	15.58^{abAB}	12.59^{deAB}	22.18^{abcA}	14.17^{aA}	23.37^{abA}	33.90^{bcdA}	26.45^{abcA}
GR3	4.60^{abB}	19.81^{aA}	23.36^{bcA}	25.06^{abA}	15.27^{aB}	26.82^{abAB}	35.26^{bcdA}	26.55^{abcAB}
GR4	12.37^{abB}	9.40^{bB}	35.87^{aA}	10.98^{cdeB}	17.73^{aB}	19.55^{abB}	47.50^{abcA}	28.80^{abcAB}
GR5	8.90^{abB}	13.05^{abAB}	19.68^{bcdeA}	18.66^{abcdAB}	20.97^{aA}	33.04^{aA}	28.56^{cdA}	35.47^{abA}
GR6	9.88^{abA}	13.57^{abA}	17.85^{cdeA}	16.99^{abcdeA}	29.72^{aAB}	14.37^{abB}	44.14^{abcA}	32.59^{abAB}
GR7	10.81^{abA}	8.73^{bA}	15.08^{cdeA}	10.30^{cdeA}	22.35^{aA}	14.33^{abA}	19.75^{dA}	12.86^{cA}
GR8	5.59^{abA}	7.67^{bA}	7.63^{eA}	9.11^{deA}	16.59^{aA}	10.64^{bA}	22.32^{dA}	20.22^{bcA}
GR9	9.05^{abA}	10.45^{abA}	12.17^{deA}	8.56^{deA}	15.32^{aA}	11.71^{bA}	20.15^{dA}	19.84^{bcA}
GR10	5.87^{abB}	12.37^{abAB}	22.47^{bdA}	15.20^{bcdeAB}	11.80^{aC}	16.88^{abBC}	63.48^{aA}	34.87^{abB}
GR11	11.50^{abB}	6.53^{bB}	25.47^{bcA}	16.12^{bcdeAB}	16.06^{aB}	14.75^{abB}	50.05^{abA}	20.75^{bcB}
GR12	14.94^{aB}	13.91^{abB}	28.45^{abA}	20.81^{abcAB}	19.70^{aB}	23.56^{abB}	44.78^{abcA}	22.15^{bcB}
Control	4.55^{bB}	7.04^{bB}	24.16^{bcA}	7.96^{eB}	11.84^{aB}	8.33^{bB}	42.90^{bcA}	14.65^{bcB}
Significance	S				S			
CV (%)	32.87				37.78			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.15.3 Fresh weight of leaves

Among the media, M3 (vermicompost) recorded the highest leaf fresh weight (16.39 g) at six months (Table 32) which was significantly superior to all other media *viz.* M1 (2.30 g), M2 (4.33 g) and M4 (5.51 g). In the M3 medium, GR4 (IBA 150 ppm) recorded the highest leaf fresh weight (22.74 g) which was significantly superior to all other treatments except GR11 and GR12. In the medium M1, GR12 (BA 300 ppm) recorded the maximum leaf fresh weight (7.84 g) which was on par with all other treatments except GR3 and GR8. GR3 (IAA 450 ppm) recorded the maximum leaf fresh weight (13.86 g) in M2 which differed significantly with all other treatments except GR2, GR5 and GR6. In the medium M4, GR1 (IAA 150 ppm) had the highest leaf fresh weight (17.03 g) which was significantly superior to all other treatments except GR2, GR3, GR5, GR7 and GR12.

At twelve months also (Table 32), among the media, M3 (vermicompost) recorded the highest leaf fresh weight (26.58 g) which was significantly superior to all other media, *viz.*, M1 (7.72 g), M2 (4.60 g) and M4 (9.58 g). In the M3 medium, GR10 (BA 100 ppm) recorded the highest leaf fresh weight (44.40 g) which was significantly superior to all other treatments except GR4. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum leaf fresh weight (19.10 g) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the maximum leaf fresh weight (19.74 g) in M2 which differed significantly with GR8, GR9 and control. In the medium M4, GR1 (IAA 150 ppm) had the highest leaf fresh weight (30.47 g) which was significantly superior to GR2, GR7, GR8, GR9, GR11, GR12 and control.

Table 32. Influence of growth regulators and media on fresh weight of leaves (g)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	6.22^{abB}	6.09^{bcB}	11.22^{cdB}	17.03^{aA}	14.81^{aB}	16.93^{abB}	12.55^{deB}	30.47^{aA}
GR2	5.96^{abB}	9.71^{abAB}	8.74^{deAB}	13.03^{abcA}	8.59^{aB}	14.53^{abcAB}	23.74^{bcdA}	15.84^{bcAB}
GR3	1.88^{bB}	13.86^{aA}	16.03^{bA}	14.94^{abA}	9.89^{aB}	18.60^{aAB}	23.65^{bcdA}	18.02^{abcAB}
GR4	7.79^{aB}	5.73^{bcB}	22.74^{aA}	7.57^{cdeB}	12.51^{aB}	12.22^{abcB}	31.57^{abA}	18.98^{abcB}
GR5	5.38^{abB}	8.40^{abcAB}	12.79^{cdA}	12.72^{abcA}	12.11^{aA}	19.74^{aA}	20.20^{bcdA}	22.60^{abA}
GR6	5.79^{abB}	8.21^{abcAB}	11.56^{cdA}	10.98^{bcdAB}	19.10^{aAB}	9.23^{abcB}	27.59^{bcA}	22.67^{abA}
GR7	4.08^{abA}	5.18^{bcA}	9.72^{cdA}	6.13^{cdeA}	11.61^{aA}	8.29^{abcA}	12.26^{deA}	7.66^{cA}
GR8	1.63^{bA}	3.05^{cA}	4.54^{deA}	4.12^{eA}	6.97^{aA}	3.32^{cA}	14.76^{deA}	9.17^{cA}
GR9	2.31^{abA}	2.86^{cA}	3.18^{eA}	4.23^{deA}	6.77^{aA}	3.21^{cA}	6.40^{eA}	7.05^{cA}
GR10	3.12^{abC}	7.62^{bcBC}	15.70^{bcA}	9.39^{bcdeB}	7.79^{aC}	10.22^{abcC}	44.40^{aA}	24.28^{abB}
GR11	6.21^{abBC}	3.38^{cC}	18.14^{abA}	9.55^{bcdeB}	10.45^{aB}	9.47^{abcB}	30.26^{bA}	13.47^{bcB}
GR12	7.84^{aB}	7.54^{bcB}	19.40^{abA}	13.00^{abcA}	13.06^{aB}	16.61^{abAB}	27.56^{bcA}	13.59^{bcB}
Control	2.30^{abB}	4.33^{bcB}	16.39^{bcA}	5.51^{deB}	7.72^{aB}	4.60^{bcB}	26.58^{bcA}	9.58^{cB}
Significance	S				S			
CV (%)	30.07				38.8			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.15.4 Fresh weight of roots

At six month period (Table 33), among the media, M3 (vermicompost) recorded the highest root fresh weight (4.30 g) which was on par with M2 (1.86 g) and M4 (3.50 g) and significantly superior to M1 (1.21 g). In the M3 medium, GR3 (IAA 450 ppm) recorded the highest root fresh weight (5.51 g) which was significantly superior to GR7 and GR9. In the medium M1, GR7 (GA 100 ppm) recorded the maximum root fresh weight (3.63 g) which was on par with all other treatments. GR3 (IAA 450 ppm) recorded the highest root fresh weight (4.77 g) in M2 which differed significantly with all other treatments except GR8 and control. In the medium M4, GR5 (IBA 300 ppm) had the highest root fresh weight (6.85 g) which was significantly superior to GR6, GR8, GR11, GR12 and control.

Among the media, M3 (vermicompost) recorded the highest root fresh weight (7.92 g) at twelve months (Table 33) which was significantly superior to all other media *viz.* M1 (1.88 g), M2 (1.87 g) and M4 (3.62 g). M1, M2 and M4 were homogeneous at this period. In the medium M3, GR10 (BA 100 ppm) had the highest root fresh weight (12.82 g) which was significantly superior to all other treatments. In the medium M1, GR7 (GA 100 ppm) recorded the maximum root fresh weight (4.05 g) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the maximum root fresh weight (7.84 g) in M2 which differed significantly with all other treatments except GR1, GR3, GR4 and GR9. In the M4 medium, GR1 (IAA 150 ppm) recorded the highest root fresh weight (8.37 g) which was significantly superior to only control.

Table 33. Influence of media and growth regulators on fresh weight of roots (g)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	2.56^{aA}	2.07^{abA}	4.31^{abA}	4.63^{abcdA}	2.58^{aB}	4.43^{abAB}	4.54^{bcAB}	8.37^{aA}
GR2	1.79^{aA}	3.03^{abA}	3.60^{abA}	4.56^{abcdA}	2.20^{aB}	3.32^{bAB}	6.90^{bcA}	4.96^{abAB}
GR3	2.44^{aB}	4.77^{aAB}	5.51^{aA}	6.24^{abcA}	3.74^{aB}	5.35^{abAB}	5.56^{bcAB}	8.00^{aA}
GR4	3.03^{aA}	2.84^{abA}	4.71^{aA}	4.56^{abcdA}	4.04^{aA}	4.87^{abA}	4.73^{bcA}	6.27^{abA}
GR5	2.24^{aB}	2.31^{abB}	4.05^{abAB}	6.85^{aA}	4.00^{aA}	7.84^{aA}	4.08^{bcA}	7.06^{abA}
GR6	2.36^{aA}	3.20^{abA}	4.53^{aA}	3.94^{cdA}	3.35^{aB}	3.26^{bB}	5.14^{bcAB}	8.08^{aA}
GR7	3.63^{aA}	2.10^{abA}	2.24^{bA}	4.43^{abcdA}	4.05^{aA}	3.20^{bA}	2.99^{cA}	5.40^{abA}
GR8	1.75^{aA}	1.52^{bA}	2.85^{abA}	3.10^{cdA}	3.68^{aAB}	2.74^{bB}	4.08^{bcAB}	7.06^{abA}
GR9	1.86^{aB}	3.64^{abAB}	1.77^{bB}	5.00^{abcA}	3.49^{aA}	3.68^{abA}	3.06^{cA}	6.07^{abA}
GR10	1.82^{aB}	2.24^{abB}	3.02^{abAB}	5.41^{abcA}	1.85^{aB}	2.88^{bB}	12.82^{aAB}	6.50^{abB}
GR11	2.62^{aA}	2.40^{abA}	3.11^{abA}	1.98^{dA}	3.32^{aA}	2.47^{bA}	5.73^{bcA}	5.88^{abA}
GR12	2.00^{aA}	2.42^{abA}	4.31^{abA}	4.11^{cdA}	2.10^{aB}	2.46^{bB}	8.11^{bA}	4.34^{abAB}
Control	1.21^{aB}	1.86^{bAB}	4.30^{abA}	3.50^{cdAB}	1.88^{aB}	1.87^{bB}	7.92^{bA}	3.62^{bB}
Significance	S				S			
CV (%)	37.0				44.7			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.15.5 Dry weight of whole plant

Among the media, M3 (vermicompost) recorded the highest dry weight (11.39 g) at six month stage (Table 34) which was on par with M4 (5.87 g) and significantly superior to M1 (3.18 g) and M2 (3.60 g). M1, M2 and M4 were homogeneous at this period. In the M3 medium, GR4 (IBA 150 ppm) recorded the highest dry weight (20.29 g) which was significantly superior to all other treatments. In the medium M1, GR1 (IAA 150 ppm) recorded the maximum dry weight (11.07 g) which was on par with all other treatments except GR3, GR10 and control. GR3 (IAA 450 ppm) recorded the maximum dry weight (8.48 g) in M2 which was on par with all other treatments. In the medium M4, GR1 had the highest dry weight (14.54 g) which was significantly superior to GR4, GR7, GR8, GR9, GR11 and control.

At twelve months (Table 34), among the media, M3 (vermicompost) recorded the highest dry weight (22.52 g) which was significantly superior to all other media *viz.* M1 (6.82 g), M2 (5.04 g) and M4 (8.54 g). In the medium M3, GR10 (BA 100 ppm) had the highest dry weight (28.83 g) which was significantly superior to GR1, GR5, GR7, GR8 and GR9. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum dry weight (16.30 g) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the maximum dry weight (17.44 g) in M2 which differed significantly with control treatment only. In the M4 also, GR5 recorded the highest dry weight (21.89 g) which was significantly superior to GR7, GR12 and control.

4.1.1.1.15.6 Dry weight of shoot

At six month stage (Table 35), among the media, M3 (vermicompost) recorded the highest shoot dry weight (9.51 g) which was significantly superior to all other media *viz.* M1 (2.54 g), M2 (2.87 g) and M4 (3.95 g). In the M3 medium, GR4 (IBA 150 ppm) recorded the highest shoot dry weight (17.78 g) which was significantly superior to all other treatments. In the medium M1, GR1 (IAA 150 ppm) recorded the maximum shoot dry weight (9.23 g) which was on par with all

other treatments except GR3, GR8, GR10 and control. GR3 (IAA 450 ppm) recorded the highest shoot dry weight (6.82 g) in M2 which was on par with all other treatments. In the medium M4, GR1 (IAA 150 ppm) had the highest shoot dry weight (11.86 g) which was significantly superior to GR4, GR7, GR8, GR9, GR10, GR11 and control.

A similar trend was noticed during twelve month period (Table 35) and among the media, M3 (vermicompost) recorded the highest dry weight (18.54 g) which was significantly superior to all other media *viz.* M1 (5.84 g), M2 (4.15 g) and M4 (6.60 g). In the M3 medium, GR10 (BA 100 ppm) recorded the highest shoot dry weight (22.35 g) which differed significantly with GR1, GR5, GR7, GR8 and GR9. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum shoot dry weight (14.58 g) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the maximum shoot dry weight (13.88 g) in M2 which differed significantly with only control. In the medium M4 also, GR5 had the highest shoot dry weight (18.24 g) which was significantly superior to GR7 and control.

4.1.1.1.15.7 Dry weight of leaves

Among the media, M3 (vermicompost) recorded the highest leaf dry weight (6.35 g) at six months (Table 36) which was significantly superior to all other media *viz.* M1 (1.28 g), M2 (1.65 g) and M4 (2.73 g). In the M3 medium, GR4 (IBA 150 ppm) recorded the highest leaf dry weight (10.97 g) which was significantly superior to all other treatments. In the medium M1, GR12 (BA 300 ppm) recorded the maximum leaf dry weight (4.79 g) which was on par with all other treatments except GR3, GR8, GR9 and control. GR3 (IAA 450 ppm) recorded the maximum leaf dry weight (4.64 g) in M2 which differed significantly with GR8, GR9, GR11 and control. In the medium M4, GR1 (IAA 150 ppm) had the highest leaf dry weight (7.33 g) which was significantly superior to GR4, GR7, GR8, GR9, GR10, GR11 and control.

Table 34. Influence of media and growth regulators on dry weight of whole plant (g)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	11.07^{aAB}	5.21^{aB}	9.37^{bcAB}	14.54^{aA}	12.91^{aA}	10.65^{abA}	10.49^{eA}	20.89^{abA}
GR2	5.52^{abcB}	7.94^{aAB}	7.94^{bcAB}	11.68^{abcA}	8.87^{aB}	12.00^{abB}	23.67^{abA}	14.74^{abcAB}
GR3	4.78^{bcB}	8.48^{aAB}	11.61^{bcA}	13.84^{aA}	9.41^{aA}	12.19^{abA}	17.72^{abcdeA}	16.11^{abcA}
GR4	8.46^{abcB}	4.76^{aB}	20.29^{aA}	7.41^{bcB}	9.89^{aB}	10.83^{abAB}	21.78^{abcdA}	16.52^{abcAB}
GR5	6.36^{abcAB}	5.61^{aB}	12.16^{bA}	11.72^{abcA}	13.72^{aA}	17.44^{aA}	13.30^{cdeA}	21.89^{aA}
GR6	7.57^{abcA}	6.66^{aA}	11.33^{bcA}	9.16^{abcA}	16.30^{aAB}	6.71^{abB}	24.52^{abA}	20.30^{abA}
GR7	10.34^{abA}	4.13^{aB}	8.79^{bcAB}	6.89^{bcAB}	12.96^{aA}	6.93^{abA}	11.60^{cdeA}	8.61^{cA}
GR8	5.38^{abcA}	3.61^{aA}	5.01^{cA}	5.84^{cA}	8.87^{aA}	6.43^{abA}	10.71^{deA}	14.67^{abcA}
GR9	6.04^{abcA}	5.63^{aA}	5.97^{cA}	6.50^{cA}	9.17^{aA}	6.80^{abA}	12.62^{cdeA}	13.04^{abcA}
GR10	4.76^{bcA}	6.22^{aA}	9.65^{bcA}	9.24^{abcA}	6.89^{aC}	8.52^{abBC}	28.83^{aA}	18.48^{abcAB}
GR11	8.65^{abcAB}	3.34^{aB}	10.17^{bcA}	7.42^{bcAB}	10.18^{aB}	6.85^{abB}	23.49^{abA}	14.21^{abcAB}
GR12	9.46^{abAB}	6.49^{aB}	13.22^{bA}	10.61^{abcAB}	10.20^{aB}	9.17^{abB}	23.20^{abA}	10.67^{bcB}
Control	3.18^{cB}	3.60^{aB}	11.39^{bcA}	5.87^{cAB}	6.82^{aB}	5.04^{bB}	22.52^{abA}	8.54^{cB}
Significance	S				S			
CV (%)	34.04				39.64			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 35. Influence of media and growth regulators on dry weight of shoot (g)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	9.23^{aAB}	4.51^{aB}	7.22^{bcdeAB}	11.86^{aA}	11.03^{aA}	8.72^{abA}	8.12^{dA}	16.89^{abA}
GR2	4.51^{abcdA}	6.55^{aA}	5.96^{cdeA}	9.12^{abcA}	7.66^{aB}	10.38^{abAB}	19.08^{abcA}	11.85^{abAB}
GR3	3.17^{cdB}	6.82^{aAB}	9.16^{bcdA}	11.34^{abA}	7.74^{aA}	9.94^{abA}	15.26^{abcdA}	11.90^{abA}
GR4	6.79^{abcdB}	3.76^{aB}	17.78^{aA}	4.93^{cdB}	7.76^{aB}	8.52^{abB}	19.26^{abcA}	13.40^{abAB}
GR5	5.13^{abcdB}	4.80^{aB}	10.18^{bcA}	8.35^{abcdAB}	11.28^{aA}	13.88^{aA}	11.24^{cdA}	18.24^{aA}
GR6	6.02^{abcdA}	5.50^{aA}	9.01^{bcdA}	7.32^{abcdA}	14.58^{aAB}	5.43^{abB}	22.00^{abA}	15.51^{abA}
GR7	7.75^{abcA}	3.36^{aA}	7.69^{bcdeA}	4.49^{cdA}	10.53^{aA}	5.59^{abA}	10.23^{cdA}	5.64^{bA}
GR8	4.18^{bcdA}	2.99^{aA}	3.27^{eA}	3.91^{dA}	7.13^{aA}	5.08^{abA}	9.22^{cdA}	10.88^{abA}
GR9	5.04^{abcdA}	4.23^{aA}	5.13^{deA}	3.95^{dA}	7.17^{aA}	5.37^{abA}	10.74^{cdA}	9.79^{abA}
GR10	3.63^{cdB}	5.21^{aAB}	8.43^{bcdA}	6.68^{bcdAB}	5.75^{aC}	7.34^{abBC}	22.35^{aA}	15.14^{abAB}
GR11	6.83^{abcdAB}	2.54^{aB}	8.90^{bcdA}	6.54^{cdAB}	8.21^{aB}	5.95^{abB}	20.55^{abA}	11.13^{abAB}
GR12	8.51^{abAB}	5.41^{aB}	11.02^{bcA}	8.64^{abcAB}	9.22^{aB}	8.08^{abB}	19.14^{abcA}	9.12^{abB}
Control	2.54^{dB}	2.87^{aB}	9.51^{bcdA}	3.95^{dB}	5.84^{aB}	4.15^{bB}	18.54^{abcA}	6.60^{bB}
Significance	S				S			
CV (%)	33.79				39.49			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 36. Influence of media and growth regulators on dry weight of leaves (g)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	4.59^{aAB}	2.46^{abB}	4.81^{bAB}	7.33^{aA}	7.06^{abA}	6.01^{abA}	6.04^{deA}	11.67^{aA}
GR2	3.16^{abA}	3.81^{abA}	3.95^{bcA}	5.35^{abcdA}	4.51^{abB}	6.29^{abB}	13.40^{abcA}	7.91^{abcAB}
GR3	1.48^{bB}	4.64^{aA}	6.15^{bA}	6.15^{abA}	5.17^{abA}	7.47^{aA}	10.09^{bcdA}	7.80^{abcA}
GR4	4.39^{aB}	2.08^{abB}	10.97^{aA}	3.30^{cdeB}	5.37^{abB}	5.01^{abB}	12.88^{abcA}	8.45^{abcAB}
GR5	3.24^{abB}	2.87^{abB}	6.35^{bA}	5.40^{abcdAB}	6.25^{abA}	7.45^{aA}	7.71^{cdeA}	10.54^{abA}
GR6	3.69^{abA}	2.91^{abA}	5.63^{bA}	4.65^{abcdA}	9.29^{aA}	3.36^{abB}	13.64^{abA}	10.35^{abA}
GR7	3.32^{abAB}	1.95^{abB}	4.76^{bA}	2.53^{deAB}	5.14^{abA}	3.43^{abA}	6.26^{deA}	3.18^{dA}
GR8	1.45^{bA}	1.08^{bA}	1.85^{cA}	2.21^{eA}	2.85^{bA}	1.45^{bA}	5.42^{deA}	4.48^{cdA}
GR9	1.26^{bA}	1.01^{bA}	1.25^{cA}	1.78^{eA}	3.10^{bA}	1.12^{bA}	3.17^{eA}	3.32^{dA}
GR10	2.06^{abB}	2.95^{abB}	5.78^{bA}	3.84^{bcdeAB}	3.92^{abC}	4.30^{abC}	17.18^{aA}	10.08^{abcB}
GR11	3.79^{abAB}	1.37^{bB}	6.11^{bA}	3.79^{bcdeAB}	5.17^{abB}	3.67^{abB}	13.13^{abcA}	7.07^{abcdB}
GR12	4.79^{aAB}	2.73^{abB}	7.14^{bA}	5.40^{abcdA}	6.01^{abB}	5.72^{abB}	12.35^{abcA}	5.43^{bcdB}
Control	1.28^{bB}	1.65^{bB}	6.35^{bA}	2.73^{deB}	3.60^{bB}	2.03^{abB}	11.81^{abcA}	4.25^{dB}
Significance	S				S			
CV (%)	34.2				40.3			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

At twelve months (Table 36), among the media, M3 (vermicompost) recorded the highest leaf dry weight (11.81 g) which was significantly superior to all other media *viz.,* M1 (3.60 g), M2 (2.03 g) and M4 (4.25 g). In M3, GR10 (BA 100 ppm) recorded the highest leaf dry weight (17.18 g) which was significantly superior to all other treatments except GR2, GR4, GR6, GR11, GR12 and control. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum leaf dry weight (9.29 g) which was significantly superior to GR8, GR9 and control. GR3 (IAA 450 ppm) recorded the maximum leaf dry weight (7.47 g) in M2 which differed significantly with GR8 and GR9. In the medium M4, GR1 (IAA 150 ppm) had the highest leaf dry weight (11.67 g) which was significantly superior to GR7, GR8, GR9, GR12 and control.

4.1.1.1.15.8 Dry weight of roots

At six months (Table 37), among the media, M4 (coir pith compost) recorded the highest root dry weight (1.92 g) which was on par with all other media *viz.,* M1 (0.64 g) and M2 (0.72 g) and M3 (1.88 g). In the medium M4, GR5 (IBA 300 ppm) had the highest root dry weight (3.36 g) which was significantly superior to GR6, GR8, GR11, GR12 and control. In the medium M1, GR7 (GA 100 ppm) recorded the maximum root dry weight (2.42 g) which was on par with all other treatments except GR9, GR12 and control. GR3 (IAA 450 ppm) recorded the highest root dry weight (1.66 g) in M2 which was on par with all other treatments. In the M3 medium, GR4 (IBA 150 ppm) recorded the highest root dry weight (2.51 g) which was significantly superior to only GR9.

Table 37. Influence of media and growth regulators on dry weight of roots (g)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	1.83^{abAB}	0.70^{aB}	2.15^{abA}	2.67^{abA}	1.88^{aA}	1.92^{abA}	2.37^{abcA}	4.01^{abA}
GR2	1.02^{abB}	1.39^{aAB}	1.98^{abAB}	2.56^{abA}	1.21^{aB}	1.61^{abB}	4.59^{abA}	2.89^{abcAB}
GR3	1.60^{abA}	1.66^{aA}	2.45^{aA}	2.51^{abA}	1.67^{aB}	2.24^{abAB}	2.46^{bcAB}	4.21^{abA}
GR4	1.66^{abAB}	1.01^{aB}	2.51^{aA}	2.49^{abAB}	2.13^{aA}	2.31^{abA}	2.52^{bcA}	3.12^{abcA}
GR5	1.22^{abB}	0.80^{aB}	1.98^{abAB}	3.36^{aA}	2.44^{aA}	3.56^{aA}	2.06^{cA}	3.66^{abcA}
GR6	1.54^{abA}	1.17^{aA}	2.33^{aA}	1.84^{bcA}	1.71^{aB}	1.28^{abB}	2.52^{bcAB}	4.79^{aA}
GR7	2.42^{aA}	0.77^{aB}	1.10^{abAB}	2.40^{abA}	2.43^{aA}	1.34^{abA}	1.37^{cA}	2.97^{abcA}
GR8	1.21^{abA}	0.62^{aA}	1.74^{abA}	1.93^{bcA}	1.74^{aAB}	1.36^{abB}	1.49^{cAB}	3.79^{abcA}
GR9	0.99^{bB}	1.41^{aAB}	0.84^{bB}	2.56^{abA}	2.00^{aA}	1.43^{abA}	1.88^{cA}	3.25^{abcA}
GR10	1.13^{abB}	1.01^{aB}	1.22^{abAB}	2.56^{abA}	1.14^{aB}	1.18^{bB}	6.48^{aA}	3.34^{abcB}
GR11	1.82^{abA}	0.81^{aA}	1.28^{abA}	0.87^{cA}	1.96^{aA}	0.90^{bA}	2.94^{bcA}	3.08^{abcA}
GR12	0.95^{bA}	1.08^{aA}	2.20^{abA}	1.52^{cA}	0.98^{aB}	1.09^{bB}	4.07^{bA}	1.54^{cB}
Control	0.64^{bA}	0.72^{aA}	1.88^{abA}	1.92^{cA}	0.98^{aB}	0.90^{bB}	3.98^{bA}	1.94^{bcAB}
Significance	S				S			
CV (%)	39.41				47.80			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Among the media, M3 (vermicompost) recorded the highest root dry weight (3.98 g) at twelve months (Table 37), which was on par with M4 (1.94 g) and significantly superior to M1 (0.98 g) and M2 (0.90 g). M1, M2 and M4 were homogeneous at this period. In the medium M3, GR10 (BA 100 ppm) had the highest root dry weight (6.48 g) which was significantly superior to all treatments except GR2. In the medium M1, GR5 (IBA 300 ppm) recorded the maximum root dry weight (2.44 g) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the maximum root dry weight (3.56 g) in M2 also which differed significantly with GR10, GR11, GR12 and control. In the M4 medium, GR6 (IBA 450 ppm) recorded the highest root dry weight (4.79 g) which was significantly superior to GR12 and control.

4.1.1.1.16 Root parameters

4.1.1.1.16.1 Number of roots

The interaction between media and growth regulator was found to be significant with respect to number of roots both at six and twelve month stages.

4.1.1.1.16.1.1 Number of primary roots

Among the media, M3 (vermicompost) recorded the highest number of primary roots (18.00) which was on par with M4 (16.33) and significantly superior to M1 (8.67) and M2 (12.33) at six month stage (Table 38). At this period, GR3 (IAA 450 ppm) recorded the highest number of primary roots in M2 (26.00), M3 (27.00) and M4 (28.33) media, while GR4 (IBA 150 ppm) was the most superior treatment in M1 (21.67).

At twelve months stage also (Table 38), among the media, M3 (vermicompost) recorded maximum number of primary roots (25.67) which was on par with M4 (20.00) and significantly superior to M1 (14.67) and M2 (13.00). During this period also, GR3 (IAA 450 ppm) recorded the highest number of primary roots in

M2 (29.00) and M4 (33.67) media, while GR7 (GA 100 ppm) and GR12 (BA 300 ppm) were the most superior treatments in M1 and M3 recording values of 29.33 and 40.67 respectively.

4.1.1.1.16.1.2 Number of secondary roots

The highest number of secondary roots (42.00) among the media was recorded in M3 (vermicompost) which was on par with M4 (36.67) and significantly superior to M1 (18.67) and M2 (23.00) at six month stage (Table 39). At this period, GR4 (IBA 150 ppm), GR3 (IBA 450 ppm), GR11 (BA 200 ppm) and GR10 (BA 100 ppm) were the most superior treatments in M1, M2, M3 and M4 media recording 85.33, 62.67, 63.67 and 94.00 secondary roots respectively.

With respect to number of secondary roots at twelve month stage (Table 39), among the media, M3 (vermicompost) recorded the highest value (50.00) which was on par with M4 (40.00) and significantly superior to M1 (24.67) and M2 (26.33). At this period, GR10 (BA 100 ppm) recorded the highest values in M3 (81.00) and M4 (99.33) while GR4 (IBA 150 ppm) and GR3 (IAA 450 ppm) showed the highest values in M1 (118.67) and M2 (73.00) media.

4.1.1.1.16.1.3 Number of tertiary roots

Among the media, M2 (poultry manure) recorded the highest number of tertiary roots (10.67) which was on par with M4 (9.33) and significantly superior to M1 (1.67) and M3 (3.67) at six month stage (Table 40). At this period, GR2 (IAA 300 ppm), GR3 (IAA 450 ppm), GR1 (IAA 150 ppm) and GR5 (IBA 300 ppm) were the most superior treatments in M1, M2, M3 and M4 media recording 65.67, 40.33, 40.00 and 81.00 tertiary roots respectively.

At twelve months stage (Table 40), among the media, M3 (vermicompost) recorded maximum number of tertiary roots (22.33) which was on par with M2

Table 38. Influence of media and growth regulators on number of primary roots

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	14.67 (3.95)^{bC}	12.00 (3.60)^{cdC}	19.67 (4.54)^{bcB}	28.00 (5.38)^{aA}	15.67 (4.08)^{cdeC}	18.67 (4.43)^{cdeBC}	21.67 (4.76)^{cdB}	33.33 (5.86)^{aA}
GR2	14.67 (3.92)^{bB}	21.00 (4.68)^{abA}	21.00 (4.68)^{abcA}	25.67 (5.16)^{abcA}	18.67 (4.43)^{cdB}	23.67 (4.95)^{abcAB}	30.00 (5.55)^{bA}	28.00 (5.38)^{abcA}
GR3	14.33 (3.91)^{bB}	26.00 (5.19)^{aA}	27.00 (5.28)^{aA}	28.33 (5.4)^{aA}	17.33 (4.27)^{cdB}	29.00 (5.47)^{aA}	28.33 (5.41)^{bA}	33.67 (5.89)^{aA}
GR4	21.67 (4.75)^{aA}	20.67 (4.65)^{abA}	20.67 (4.65)^{abcA}	25.33 (5.13)^{abcA}	24.67 (5.06)^{abAB}	25.00 (5.09)^{abAB}	21.33 (4.72)^{cdB}	30.33 (5.60)^{abA}
GR5	13.33 (3.78)^{bB}	14.00 (3.87)^{cdB}	20.33 (4.62)^{abcA}	25.33 (5.12)^{abcA}	19.33 (4.51)^{bcdC}	26.00 (5.19)^{aA}	21.00 (4.69)^{cdBC}	28.67 (5.44)^{abcA}
GR6	13.00 (3.74)^{bcC}	17.33 (4.28)^{bcBC}	26.33 (5.23)^{abA}	19.00 (4.47)^{cB}	19.00 (4.47)^{bcdB}	19.33 (4.51)^{bcdB}	29.00 (5.47)^{bA}	31.33 (5.68)^{aA}
GR7	21.33 (4.72)^{aA}	12.67 (3.69)^{cdB}	13.67 (3.83)^{dB}	20.67 (4.65)^{bcdA}	29.33 (5.50)^{aA}	19.67 (4.53)^{bcdB}	15.33 (4.04)^{eB}	27.67 (5.35)^{abcA}
GR8	11.67 (3.54)^{bcdB}	10.00 (3.30)^{dB}	13.67 (3.81)^{dB}	22.33 (4.82)^{abcdA}	20.00 (4.58)^{bcB}	16.33 (4.16)^{defB}	18.67 (4.43)^{deB}	28.67 (5.44)^{abcA}
GR9	8.33 (3.01)^{dC}	21.67 (4.76)^{abA}	13.67 (3.81)^{dB}	26.00 (5.20)^{abA}	17.00 (4.23)^{cdC}	23.00 (4.89)^{abcdAB}	18.67 (4.43)^{deBC}	29.00 (5.47)^{abcA}
GR10	10.00 (3.31)^{cdC}	13.00 (3.74)^{cdC}	20.67 (4.65)^{abcB}	28.00 (5.38)^{abA}	11.33 (3.51)^{eC}	14.00 (3.87)^{efC}	26.33 (5.22)^{bcB}	33.00 (5.83)^{aA}
GR11	15.67 (4.08)^{abAB}	13.67 (3.83)^{cdBC}	21.33 (4.72)^{abcA}	9.33 (3.17)^{eC}	20.00 (4.58)^{bcB}	15.33 (4.04)^{defC}	28.67 (5.44)^{bA}	23.00 (4.90)^{cdAB}
GR12	15.67 (4.08)^{abA}	16.67 (4.20)^{bA}	21.33 (4.72)^{abcA}	22.00 (4.79)^{abcdA}	16.33 (4.16)^{cdC}	17.33 (4.28)^{defC}	40.67 (6.45)^{aA}	24.00 (5.00)^{bcdB}
Control	8.67 (3.09)^{cdC}	12.33 (3.64)^{cdBC}	18.00 (4.35)^{cdA}	16.33 (4.15)^{dAB}	14.67 (3.95)^{deB}	13.00 (3.73)^{fB}	25.67 (5.15)^{bcA}	20.00 (4.57)^{dA}
Significance	S				S			
CV (%)	7.66				5.82			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for period

Table 39. Influence of media and growth regulators on number of secondary roots

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	49.33 (7.09)^{cdA}	24.33 (5.03)^{deB}	61.33 (7.84)^{abA}	64.67 (8.07)^{bA}	55.67 (7.52)^{cdA}	56.33 (7.56)^{abA}	65.67 (8.11)^{abcA}	78.00 (8.86)^{abcdA}
GR2	63.33 (7.95)^{abcA}	51.00 (7.21)^{abA}	23.67 (4.91)^{efgB}	70.00 (8.40)^{abA}	68.67 (8.29)^{bcAB}	55.33 (7.5)^{abB}	29.67 (5.51)^{fC}	82.33 (9.08)^{abcA}
GR3	57.00 (7.60)^{bcA}	62.67 (7.96)^{aA}	37.00 (6.14)^{deB}	48.00 (6.99)^{bcdeAB}	65.33 (8.13)^{bcA}	73.00 (8.59)^{aA}	41.33 (6.48)^{defB}	56.67 (7.59)^{defAB}
GR4	85.33 (9.28)^{aA}	54.00 (7.39)^{abB}	26.33 (5.23)^{dB}	30.67 (5.62)^{efB}	118.67 (10.91)^{aA}	59.00 (7.72)^{abB}	32.00 (5.74)^{efC}	96.33 (9.82)^{abA}
GR5	58.00 (7.59)^{bcAB}	37.33 (6.18)^{bcdeB}	59.33 (7.74)^{abcA}	54.67 (7.45)^{bcdAB}	76.00 (8.76)^{bcA}	43.00 (6.63)^{bcB}	63.33 (8.00)^{abAB}	62.33 (7.95)^{cdeAB}
GR6	78.67 (8.92)^{abA}	40.67 (6.45)^{bcB}	42.67 (6.6)^{bcdB}	36.00 (6.07)^{defB}	88.67 (9.47)^{bA}	47.33 (6.95)^{bcB}	48.33 (7.02)^{cdeB}	43.33 (6.65)^{efgB}
GR7	18.67 (4.41)^{fgC}	53.00 (7.22)^{abAB}	38.67 (6.29)^{deB}	59.33 (7.76)^{bcA}	35.33 (6.00)^{fC}	59.67 (7.67)^{abAB}	41.67 (6.51)^{dfB}	70.33 (8.44)^{bcdA}
GR8	2.00 (1.73)^{hB}	28.00 (5.37)^{cdeA}	39.67 (6.37)^{cdA}	40.00 (6.4)^{cdefA}	25.33 (5.13)^{fC}	31.67 (5.71)^{cdC}	50.00 (7.14)^{cdB}	76.00 (8.77)^{abcdA}
GR9	12.67 (3.68)^{gC}	39.67 (6.37)^{bcdA}	16.67 (4.19)^{gBC}	27.00 (5.29)^{fAB}	26.33 (5.21)^{fB}	44.00 (6.71)^{bcA}	29.00 (5.47)^{fAB}	33.33 (5.85)^{gAB}
GR10	28.67 (5.40)^{efB}	43.33 (6.63)^{abcB}	38.67 (6.29)^{deB}	94.00 (9.72)^{aA}	31.00 (5.59)^{efC}	48.67 (7.03)^{bcB}	81.00 (9.05)^{aA}	99.33 (9.98)^{aA}
GR11	34.67 (5.95)^{deB}	37.33 (6.18)^{bcdeB}	63.67 (8.03)^{aA}	36.00 (6.08)^{defB}	38.67 (6.27)^{defB}	40.67 (6.45)^{bcdB}	72.00 (8.52)^{abA}	92.00 (9.64)^{abA}
GR12	37.33 (6.11)^{deAB}	37.67 (6.21)^{bcdeA}	21.33 (4.72)^{fgB}	50.00 (7.14)^{bcdA}	43.67 (6.64)^{deB}	41.00 (6.47)^{bcdB}	56.00 (7.55)^{bcdAB}	65.33 (8.14)^{cdA}
Control	18.67 (4.43)^{fgC}	23.00 (4.92)^{eBC}	42.00 (6.53)^{bcdA}	36.67 (6.10)^{defAB}	24.67 (5.06)^{fB}	26.33 (5.21)^{dB}	50.00 (7.13)^{cdeA}	40.00 (6.36)^{fgAB}
Significance	S				S			
CV (%)	10.28				9.02			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 40. Influence of media and growth regulators on number of tertiary roots

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	24.67 (5.03)^{dB}	17.67 (4.31)^{cdB}	40.00 (6.39)^{aA}	17.00 (4.22)^{cdB}	30.33 (5.57)^{eB}	26.00 (5.19)^{bcdB}	47.00 (6.92)^{abA}	62.00 (7.93)^{bcA}
GR2	65.67 (8.16)^{aA}	13.33 (3.78)^{dC}	4.33 (2.22)^{efD}	41.67 (6.53)^{bB}	72.67 (8.57)^{aA}	19.00 (4.46)^{deC}	25.67 (5.12)^{deC}	45.33 (6.8)^{dB}
GR3	24.33 (5.03)^{dB}	40.33 (6.41)^{aA}	6.67 (2.75)^{deC}	25.67 (5.16)^{cB}	32.33 (5.77)^{deB}	48.33 (7.01)^{aA}	18.33 (4.38)^{eC}	29.33 (5.51)^{eB}
GR4	47.33 (6.93)^{bcA}	4.33 (2.27)^{fC}	12.67 (3.68)^{dB}	3.33 (2.07)^{gC}	53.33 (7.36)^{bcB}	7.67 (2.92)^{fgC}	49.67 (7.11)^{aB}	76.00 (8.74)^{abA}
GR5	24.67 (5.03)^{dB}	11.33 (3.50)^{deC}	28.00 (5.38)^{bcB}	81.00 (9.05)^{aA}	31.00 (5.64)^{eBC}	21.33 (4.68)^{cdeC}	33.00 (5.82)^{bcdB}	91.00 (9.58)^{aA}
GR6	61.33 (7.89)^{abA}	13.33 (3.78)^{dC}	23.00 (4.89)^{cB}	5.67 (2.56)^{fgD}	70.67 (8.46)^{abA}	20.33 (4.62)^{cdeC}	29.00 (5.47)^{deB}	24.00 (5.00)^{eB}
GR7	37.67 (6.21)^{cA}	33.33 (5.81)^{abA}	3.33 (2.06)^{efB}	8.33 (3.03)^{efgB}	46.00 (6.85)^{cdA}	38.67 (6.23)^{abAB}	28.67 (5.41)^{deB}	29.00 (5.46)^{eB}
GR8	1.67 (1.63)^{fB}	17.67 (4.31)^{cA}	3.67 (2.15)^{efB}	15.00 (3.98)^{deA}	13.33 (3.78)^{fC}	22.33 (4.82)^{cdeBC}	26.00 (5.19)^{deB}	64.67 (8.09)^{bA}
GR9	7.00 (2.82)^{eA}	4.00 (2.22)^{fAB}	1.33 (1.52)^{fB}	4.67 (2.37)^{fgB}	24 .00 (4.97)^{eB}	6.33 (2.70)^{gC}	43.33 (6.65)^{abcA}	15.67 (4.07)^{fB}
GR10	22.00 (4.77)^{dB}	23.00 (4.89)^{bcB}	38.33 (6.27)^{abA}	37.33 (6.19)^{bA}	25.00 (5.08)^{eB}	26.67 (5.24)^{bcdB}	52.67 (7.32)^{aA}	48.67 (7.04)^{cdA}
GR11	44.00 (6.67)^{cA}	6.00 (2.61)^{ef}	3.00 (1.99)^{ef}	25.00 (5.09)^{cB}	52.33 (7.26)^{cB}	9.33 (3.20)^{gfD}	30.33 (5.59)^{cC}	85.00 (9.26)^{aA}
GR12	35.33 (6.00)^{cAB}	29.33 (5.50)^{abB}	26.67 (5.25)^{cB}	45.33 (6.80)^{bA}	70.67 (8.43)^{abA}	32.00 (5.73)^{bcC}	31.33 (5.68)^{cC}	51.33 (7.22)^{cdB}
Control	1.67 (1.63)^{fC}	10.67 (3.40)^{deA}	3.67 (2.16)^{efC}	9.33 (3.20)^{efA}	8.00 (2.99)^{fB}	14.67 (3.91)^{efAB}	22.33 (4.82)^{deA}	14.67 (3.93)^{fAB}
Significance	S				S			
CV (%)	10.5				9.0			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

(14.67) and M4 (14.67) and significantly superior to M1 (8.00). During this period also, GR2 (IAA 300 ppm), GR3 (IAA 450 ppm) and GR5 (IBA 300 ppm) were the most superior treatments in M1, M2 and M4 media recording 72.67, 48.33 and 91.00 tertiary roots respectively. The maximum number of tertiary roots in M3 (52.67) at this stage was noted in GR10 (BA 100 ppm) at this period.

4.1.1.1.16.1.4 Number of total roots

The highest number of total roots (63.67) among the media was recorded in M3 (vermicompost) which was on par with M4 (62.33) and significantly superior to M1 (29.00) and M2 (46.33) at six month stage (Table 41). At this period, GR4 (IBA 150 ppm), GR3 (IAA 450 ppm), GR1 (IAA 150 ppm) and GR5 (IBA 300 ppm) were the most superior treatments in M1, M2, M3 and M4 media recording 154.33, 129.00, 121.00 and 161.00 total roots respectively.

With respect to number of total roots at twelve month stage also (Table 41), among the media, M3 (vermicompost) recorded the highest value (98.00) which was on par with M4 (74.67) and significantly superior to M1 (47.33) and M2 (54.00). As in the case of six months period, GR4 (IBA 150 ppm) and GR3 (IAA 450 ppm) recorded the highest values in M1 (196.67) and M2 (150.33) respectively at this stage also, while GR10 (BA 100 ppm) and GR11 (BA 200 ppm) showed the highest values in M3 (160.00) and M4 (200.00) media (Plate 2 to 5).

4.1.1.1.16.2. Root length and spread

The interaction between media and growth regulator was found to be significant with respect to root length and breadth both at six and twelve month stages (Tables 42 and 43).

Table 41. Influence of media and growth regulators on total number of roots

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	88.67 (9.46)^{bB}	54.00 (7.40)^{efC}	121.00 (11.01)^{aA}	109.67 (10.51)^{bcAB}	101.67 (10.12)^{cC}	101.00 (10.09)^{bcC}	134.33 (11.59)^{abB}	173.33 (13.2)^{abA}
GR2	143.67 (12)^{aA}	85.33 (9.29)^{bB}	49.00 (7.05)^{efC}	137.33 (11.74)^{abA}	160.00 (12.67)^{abA}	98.00 (9.94)^{bcB}	85.33 (9.29)^{eB}	155.67 (12.49)^{bA}
GR3	95.67 (9.82)^{bBC}	129.00 (11.38)^{aA}	70.67 (8.44)^{cdeC}	102.00 (10.15)^{cdAB}	115.00 (10.76)^{cBC}	150.33 (12.29)^{aA}	88.00 (9.41)^{deC}	119.67 (10.98)^{deAB}
GR4	154.33 (12.44)^{aA}	79.00 (8.92)^{bcdB}	59.67 (7.79)^{eB}	59.33 (7.76)^{eB}	196.67 (14.05)^{aA}	91.67 (9.61)^{bcdB}	103.00 (10.19)^{bcdeB}	202.67 (14.24)^{aA}
GR5	96.00 (9.77)^{bB}	62.67 (7.97)^{cdefC}	107.67 (10.4)^{abB}	161.00 (12.73)^{aA}	126.33 (11.27)^{bcB}	90.33 (9.55)^{bcdC}	117.33 (10.86)^{bcdB}	182.00 (13.52)^{abA}
GR6	153.00 (12.41)^{aA}	71.33 (8.50)^{bcdeB}	92.00 (9.64)^{abcB}	60.67 (7.85)^{eB}	178.33 (13.39)^{aA}	87.00 (9.38)^{bcdB}	106.33 (10.36)^{bcdeB}	98.67 (9.98)^{efB}
GR7	77.67 (8.86)^{bcAB}	99.00 (9.90)^{bA}	55.67 (7.52)^{eB}	88.33 (9.44)^{cdA}	110.67 (10.56)^{cAB}	118.00 (10.79)^{bA}	85.67 (9.28)^{eB}	127.00 (11.31)^{deA}
GR8	16.00 (4.10)^{dB}	55.67 (7.51)^{defA}	57.00 (7.61)^{eA}	77.33 (8.85)^{deA}	58.67 (7.72)^{dC}	70.33 (8.44)^{deBC}	94.67 (9.78)^{deB}	169.33 (13.05)^{abcA}
GR9	28.00 (5.37)^{dB}	65.33 (8.14)^{cdefA}	31.67 (5.70)^{fB}	57.67 (7.66)^{eA}	67.33 (8.23)^{dA}	73.33 (8.62)^{cdeA}	91.00 (9.59)^{deA}	78.00 (8.88)^{fA}
GR10	60.67 (7.81)^{cC}	79.33 (8.95)^{bcdBC}	97.67 (9.93)^{abB}	159.33 (12.64)^{aA}	67.33 (8.23)^{dB}	89.33 (9.48)^{bcdB}	160.00 (12.68)^{aA}	181.00 (13.47)^{abA}
GR11	94.33 (9.73)^{bA}	57.00 (7.60)^{defB}	88.00 (9.42)^{bcdeA}	70.33 (8.44)^{eAB}	111.00 (10.55)^{cB}	65.33 (8.14)^{deC}	131.00 (11.47)^{abB}	200.00 (14.17)^{aA}
GR12	88.33 (9.4)^{bB}	83.67 (9.20)^{bcB}	69.33 (8.38)^{cdeB}	117.33 (10.87)^{bcA}	130.67 (11.43)^{bcA}	90.33 (9.55)^{bcdB}	128.00 (11.36)^{abcA}	140.67 (11.9)^{cdA}
Control	29.00 (5.47)^{dC}	46.33 (6.86)^{fBC}	63.67 (8.02)^{deA}	62.33 (7.93)^{eAB}	47.33 (6.95)^{dC}	54.00 (7.40)^{eBC}	98.00 (9.94)^{cdeA}	74.67 (8.68)^{fAB}
Significance	S				S			
CV (%)	7.82				6.5			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

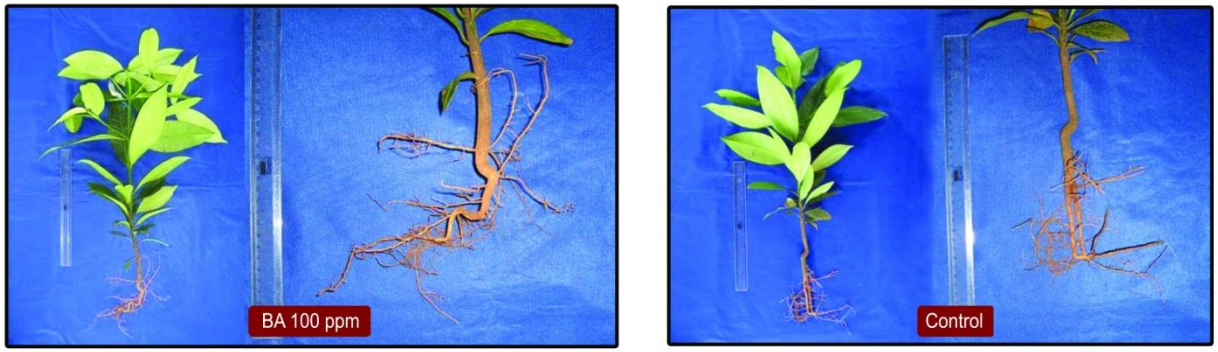


Plate 2. Influence of growth regulators on total number of roots in vermicompost medium at twelve month stage

Plate 3. Influence of growth regulators on total number of roots in coir pith compost medium at twelve month stage

Plate 4. Influence of growth regulators on total number of roots in poultry manure medium at twelve month stage

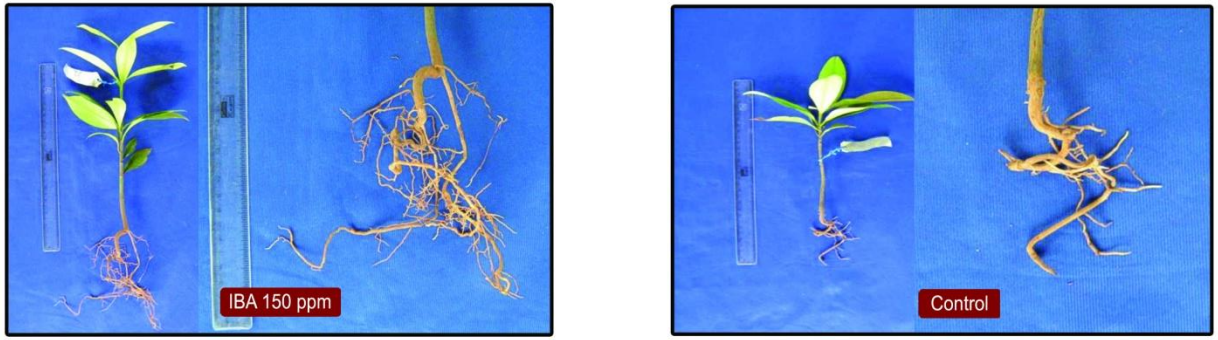


Plate 5. Influence of growth regulators on total number of roots in cow dung medium at twelve month stage

Among the media, M3 (vermicompost) recorded the highest root length (23.33 cm) which was on par with M4 (18.27 cm) and significantly superior to M1 (14.03 cm) and M2 (13.43 cm) at six month stage (Table 42). At this period, GR11 (BA 200 ppm), GR12 (BA 300 ppm), GR4 (IBA 150 ppm) and GR5 (IBA 300 ppm) were the most superior treatments in M1, M2, M3 and M4 media recording a root length of 36.63 cm, 26.73 cm, 31.23 cm and 30.83 cm respectively.

At twelve months stage also (Table 42), among the media, M3 (vermicompost) recorded the highest root length (29.30 cm) which was on par with M4 (21.53 cm) and significantly superior to M1 (19.83 cm) and M2 (19.40 cm). At this period, GR5 (IBA 300 ppm) recorded the highest values in M1 (40.97 cm) and M4 (32.20 cm) while GR6 (IBA 450 ppm) and GR4 (IBA 150 ppm) were the most superior treatments in M2, and M3 media recording a root length of 31.23 cm and 33.67 cm respectively.

With respect to root spread at six month stage (Table 43), among the media, M3 (vermicompost) recorded the highest value (23.53 cm) which was significantly superior to all other media, *viz.,* M1 (7.33 cm), M2 (11.50 cm) and M4 (15.17 cm). At this period, GR1 (IAA 150 ppm) recorded the highest root spread in M1 (29.50 cm) and M3 (35.03 cm) while GR7 (GA 100 ppm) showed the highest values in M2 (27.23) and M4 (30.97 cm) media.

The highest root spread (25.50 cm) among the media was recorded in M3 (vermicompost) which was on par with M2 (20.57 cm) and significantly superior to M1 (17.53 cm) and M4 (18.63 cm) at twelve month stage (Table 43). At this period, GR6 (IBA 450 ppm), GR5 (IBA 300 ppm), GR10 (BA 100 ppm) and GR8 (GA 200 ppm) were the most superior treatments in M1, M2, M3 and M4 media recording a root spread of 31.47 cm, 32.10 cm, 39.03 cm and 43.63 cm respectively. Control recorded the lowest root spread in M4 media both at six and twelve moth stages.

Table 42. Influence of media and growth regulators on root length (cm)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	21.13^{bcdAB}	26.67^{aA}	16.80^{bB}	17.03^{bcdeB}	23.87^{defgA}	28.33^{abcA}	26.63^{abA}	31.13^{aA}
GR2	19.30^{bcdB}	21.70^{abcdAB}	18.97^{bB}	28.43^{abA}	21.83^{efgA}	24.07^{abcdA}	26.20^{abcA}	30.20^{abA}
GR3	16.97^{deA}	23.27^{abcA}	15.43^{bA}	23.13^{abcdA}	35.73^{abcA}	23.90^{abcdB}	18.00^{cB}	24.27^{abcdB}
GR4	27.47^{bAB}	15.27^{cdefC}	31.23^{aA}	20.90^{bcdBC}	31.33^{bcdA}	29.87^{abA}	33.67^{aA}	25.93^{abcA}
GR5	37.47^{aA}	17.10^{bcdefC}	23.87^{abBC}	30.83^{aAB}	40.97^{aA}	24.43^{abcdB}	25.43^{abcB}	32.20^{aAB}
GR6	18.33^{cdAB}	24.77^{abA}	24.13^{abA}	14.80^{deB}	30.07^{cdeA}	31.23^{aA}	26.13^{abcA}	16.10^{deB}
GR7	17.57^{cdeAB}	21.67^{abcdA}	18.33^{bAB}	11.47^{eB}	20.27^{fgAB}	24.47^{abcdA}	26.87^{abcA}	12.93^{eB}
GR8	8.83^{eB}	11.57^{fAB}	17.33^{bA}	10.03^{eAB}	10.93^{hB}	26.60^{abcdA}	21.83^{bcA}	26.30^{abcA}
GR9	13.27^{deB}	20.40^{abcdeAB}	18.70^{bAB}	23.13^{abcdA}	15.33^{ghB}	21.40^{bcdAB}	21.33^{bcAB}	24.00^{abcdA}
GR10	9.30^{eB}	12.03^{defB}	23.00^{abA}	25.07^{abcA}	19.43^{fgA}	25.27^{abcdA}	24.67^{abcA}	25.67^{abcA}
GR11	36.63^{aA}	15.07^{cdefB}	16.50^{bB}	18.17^{cdeB}	39.63^{abA}	16.80^{dB}	23.77^{bcB}	19.83^{cdeB}
GR12	25.87^{bcA}	26.73^{aA}	21.53^{abA}	24.80^{abcA}	28.07^{cdefA}	29.33^{abA}	28.00^{abA}	28.43^{abcA}
Control	14.03^{deB}	13.43^{defB}	23.33^{abA}	18.27^{cdeAB}	19.83^{fghB}	19.40^{cdB}	29.30^{abA}	21.53^{bcdeAB}
Significance	S				S			
CV (%)	20.71				17.37			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 43. Influence of media and growth regulators on root spread (cm)

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	29.50^{aB}	13.80^{cdeC}	35.03^{aA}	29.07^{abB}	31.27^{aBC}	27.17^{abcC}	36.77^{abAB}	40.07^{abA}
GR2	22.63^{bcB}	17.43^{bcBC}	13.33^{efC}	29.23^{abA}	24.70^{bcdB}	24.27^{bcdB}	19.27^{fgB}	31.60^{cdeA}
GR3	21.20^{bcdAB}	16.30^{bcdBC}	13.43^{efC}	22.90^{cdA}	22.17^{bcdeAB}	16.93^{efB}	20.33^{fAB}	24.73^{fgA}
GR4	17.00^{defB}	21.00^{bAB}	24.27^{cdA}	19.60^{defAB}	23.67^{bcdeC}	22.20^{bcdeC}	31.10^{bcdB}	38.73^{abA}
GR5	17.10^{defB}	20.80^{bAB}	20.93^{dAB}	25.50^{bcA}	25.00^{bcB}	32.10^{aA}	27.60^{cdeAB}	27.33^{defAB}
GR6	23.40^{bcB}	16.73^{bcdC}	31.13^{abA}	20.00^{defBC}	31.47^{aA}	21.57^{cdeB}	32.67^{bcA}	22.50^{fgB}
GR7	12.30^{fghB}	27.23^{aA}	14.90^{eB}	30.97^{aA}	26.03^{abB}	28.33^{abAB}	21.70^{efC}	32.43^{cdA}
GR8	5.50^{iB}	17.03^{bcA}	9.50^{efB}	18.17^{defA}	19.40^{cdeC}	19.10^{defC}	33.47^{abcB}	43.63^{aA}
GR9	11.03^{ghB}	10.33^{eB}	8.83^{fB}	19.13^{defA}	26.17^{abA}	14.00^{fB}	13.33^{gB}	25.47^{efA}
GR10	14.50^{efgB}	17.47^{bcB}	28.13^{bcA}	31.97^{aA}	18.67^{deB}	19.87^{defB}	39.03^{aA}	34.50^{bcA}
GR11	18.30^{cdeA}	20.50^{bA}	11.90^{efB}	16.80^{efAB}	22.37^{bcdeAB}	21.93^{cdeB}	28.47^{cdA}	26.23^{defAB}
GR12	26.83^{abA}	19.40^{bB}	29.83^{abA}	21.27^{cdeB}	28.33^{abAB}	21.97^{cdeC}	31.93^{bA}	24.50^{fgBC}
Control	7.33^{hiC}	11.50^{deBC}	23.53^{cdA}	15.17^{fB}	17.53^{eB}	20.57^{deAB}	25.50^{defA}	18.63^{gB}
Significance	S				S			
CV (%)	13.10				11.36			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.17 Physiological parameters

4.1.1.1.17.1 Leaf Area Index (LAI)

The interaction between media and growth regulator was found to be significant at twelfth and fifteenth months. But during other periods also, significant difference was observed among the growth regulators and also among the media.

Among the different media (Table 44), M4 (coir pith compost) recorded the highest LAI up to sixth month. Thereafter, M2 (poultry manure) recorded the highest values in all the stages with a LAI of 2.171 at the end of the study.

The treatments did not show any definite pattern of influence on LAI during the entire period. In the initial periods when interaction was not significant (Table 45), GR1 (IAA 150 ppm), GR12 (BA 300 ppm) and GR11 (BA 200 ppm) recorded the highest values of 1.157, 1.284 and 1.655 respectively during third, sixth and ninth months.

At fifteen month stage (Table 46), among the media, M2 (poultry manure) recorded the highest LAI (2.171) which was on par with all other media *viz.* M1 (1.723), M3 (1.808) and M4 (1.435).

Effect of growth regulators varied widely among the media at all the stages when media – growth regulator interaction was significant. Towards the end of the study at fifteen months, in M2 medium, GR12 (BA 300 ppm) recorded the highest LAI (2.233) which was significantly superior to GR8 and GR9. In the medium M1, GR11 (BA 200 ppm) recorded the maximum LAI (2.725), which was superior to GR2, GR3, GR5, GR7, GR8, GR9 and control. GR6 (IBA 450 ppm) recorded the maximum LAI (3.410) in M3 which was significantly superior to all other treatments. In the medium M4, GR5 (IBA 300 ppm) had the maximum LAI (2.668) which was significantly superior to GR2, GR3, GR7, GR8, GR9 and control.

Table 44. Leaf Area Index for each medium averaged over all growth regulators

Media	3 months[@]	6 months	9 months
M1	1.093[a]	0.970[b]	1.091[b]
M2	0.932[b]	0.974[b]	1.181[b]
M3	1.132[a]	1.291[a]	1.528[a]
M4	1.208[a]	1.326[a]	1.402[a]
Significance	S	S	S

Table 45. Leaf Area Index for each growth regulator averaged over all media

Growth Regulators	3 months[@]	6 months	9 months
GR1	1.157	1.199[a]	1.402[ab]
GR2	1.120	1.194[a]	1.324[ab]
GR3	1.080	1.203[a]	1.373[ab]
GR4	1.151	1.243[a]	1.451[ab]
GR5	1.066	1.267[a]	1.544[ab]
GR6	1.142	1.279[a]	1.542[ab]
GR7	1.128	1.069[ab]	0.957[cd]
GR8	0.962	0.877[bc]	0.840[d]
GR9	0.944	0.743[c]	0.658[d]
GR10	1.088	1.049[ac]	1.277[bc]
GR11	1.103	1.277[a]	1.655[a]
GR12	1.154	1.284[a]	1.582[ab]
Significance	NS	S	S

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 46. Influence of media and growth regulators on Leaf Area Index

Growth regulators	12 months				15 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	1.710^{bcdA}	2.031^{abA}	1.702^{bcA}	2.015^{abA}	2.330^{abA}	2.023^{aA}	2.027^{bA}	2.253^{abA}
GR2	1.150^{cdeA}	1.713^{abcA}	1.796^{bcA}	1.501^{abcA}	1.319^{deA}	1.841^{aA}	1.773^{bA}	1.376^{deA}
GR3	1.433^{bcdeA}	1.632^{abcA}	1.665^{bcA}	1.687^{abcA}	1.574^{bcdeA}	1.936^{aA}	1.797^{bA}	1.782^{bcdeA}
GR4	1.894^{bcA}	1.541^{abcdA}	2.054^{abcA}	1.697^{abcA}	1.905^{abcdeA}	1.990^{aA}	2.257^{bA}	2.049^{abcdA}
GR5	1.525^{bcdeA}	1.596^{abcdA}	2.025^{abcA}	2.286^{aA}	1.737^{bcdeB}	1.716^{abB}	2.194^{bAB}	2.668^{aA}
GR6	2.091^{bAB}	1.768^{abcB}	2.651^{aA}	1.863^{abcAB}	2.384^{abB}	1.879^{aB}	3.410^{aA}	2.247^{abB}
GR7	1.060^{deA}	1.110^{cdeA}	1.561^{bcA}	1.222^{cA}	1.465^{cdeA}	1.398^{abcA}	2.129^{bA}	1.353^{deA}
GR8	1.019^{deA}	0.830^{deA}	1.512^{cA}	1.132^{cA}	1.452^{cdeAB}	0.982^{bcB}	2.075^{bA}	1.120^{eB}
GR9	0.754^{eA}	0.625^{eA}	0.693^{dA}	1.181^{cA}	1.082^{eA}	0.811^{cA}	0.664^{cA}	1.405^{cdeA}
GR10	1.564^{bcdA}	1.483^{bcdA}	2.040^{abcA}	2.167^{aA}	2.095^{abcdA}	1.805^{aA}	2.357^{bA}	2.183^{abcdA}
GR11	2.888^{aA}	1.765^{abcB}	2.177^{abcAB}	1.873^{abcB}	2.725^{aA}	1.748^{abB}	2.004^{bAB}	2.093^{abcdAB}
GR12	1.891^{bcA}	2.297^{aA}	2.305^{abA}	2.060^{aA}	2.241^{abcA}	2.233^{aA}	2.517^{bA}	2.224^{abcA}
Control	1.566^{bcdAB}	2.302^{aA}	1.885^{abcAB}	1.271^{bcB}	1.723^{bcdeA}	2.171^{aA}	1.808^{bA}	1.435^{bcdeA}
Significance	S				S			
CV (%)	28.76				27.40			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.17.2 Specific Leaf Weight (SLW)

The interaction between media and growth regulator was found to be significant with respect to SLW both at six and twelve month stages (Table 47).

At six months, among the media, M4 (coir pith compost) recorded the highest SLW (72.24 g m^{-2}) which was on par with M1 (52.27 g m^{-2}) and M2 (60.95 g m^{-2}) and significantly superior to M3 (40.93 g m^{-2}). In the medium M4, GR8 (GA 200 ppm) had the highest SLW (94.97 g m^{-2}) which was significantly superior to all other treatments. In the medium M1 and M2, GR9 (GA 300 ppm) recorded the maximum SLW of 154.60 g m^{-2} and 114.30 g m^{-2} respectively which were significantly superior to all other treatments. Similar trend was observed in M3 medium also, with GR9 recording the highest SLW (92.95 g m^{-2}) which was significantly superior to all other treatments except GR7 and GR8.

Among the media, M4 (coir pith compost) recorded the highest SLW (63.64 g m^{-2}) at twelfth month which was on par with all other media *viz.*, M1 (56.58 g m^{-2}) and M2 (61.18 g m^{-2}) and M3 (49.53 g m^{-2}). During this period, GR9 (GA 300 ppm) recorded the highest SLW in all the media which was significantly superior to all other treatments.

4.1.1.1.17.3 Relative Growth Rate (RGR)

The interaction between media and growth regulator was found to be non significant. But there was significant difference among the growth regulators and also among the media.

Among the media (Table 48), RGR was highest (0.00659) in M3 (vermicompost) which was on par with M4 (0.00578). M1 (0.00366) was homogeneous with M2 (0.00444) and both these media had significantly lower values compared to the other two.

Table 47. Influence of media and growth regulators on Specific Leaf Weight (g m^{-2})

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	61.61^{deA}	51.16^{cdAB}	49.19^{cdAB}	37.66^{eB}	56.14^{bcdA}	36.20^{dA}	42.99^{bcA}	50.22^{cdA}
GR2	51.93^{eAB}	67.58^{cA}	43.81^{dAB}	47.01^{eB}	40.85^{cdA}	52.73^{cdA}	52.16^{bcA}	52.04^{cdA}
GR3	71.38^{cdeA}	41.86^{dB}	52.62^{cdAB}	56.93^{bcdeAB}	52.47^{bcdA}	51.82^{cdA}	48.39^{bcA}	41.98^{dA}
GR4	62.59^{deA}	54.80^{cdA}	47.08^{dA}	52.48^{bcdeA}	54.02^{bcdAB}	66.35^{bcA}	49.43^{bcAB}	43.92^{cdB}
GR5	65.36^{cdeA}	50.48^{cdAB}	39.88^{dB}	48.23^{deAB}	60.32^{bcB}	80.81^{bA}	34.78^{cC}	47.92^{cdBC}
GR6	55.00^{eA}	55.64^{cdA}	51.51^{cdA}	48.04^{deA}	69.84^{bA}	39.98^{dB}	50.31^{bcAB}	48.60^{cdB}
GR7	109.87^{bA}	64.09^{cB}	67.49^{abcB}	69.40^{bcB}	57.27^{bcdA}	63.18^{bcA}	45.47^{bcA}	50.29^{cdA}
GR8	101.20^{bA}	91.72^{bAB}	73.97^{abB}	94.97^{aA}	65.04^{bA}	74.60^{bA}	39.01^{bcB}	74.67^{bA}
GR9	154.60^{aA}	114.30^{aB}	92.95^{aC}	67.21^{bcdD}	121.12^{aA}	108.35^{aAB}	92.09^{aB}	125.66^{aA}
GR10	83.22^{cA}	54.20^{cdB}	44.31^{dB}	46.66^{eB}	61.39^{bA}	50.50^{cdA}	59.92^{bA}	51.51^{cdA}
GR11	57.75^{eA}	55.59^{cdAB}	37.08^{dB}	51.68^{cdeAB}	39.24^{dA}	49.63^{cdA}	52.74^{bcA}	54.23^{cdA}
GR12	80.26^{cdA}	65.24^{cAB}	45.32^{dB}	46.15^{eB}	54.27^{bcdA}	40.17^{dA}	44.30^{bcA}	38.11^{dA}
Control	52.97^{eAB}	60.95^{cdAB}	40.93^{dB}	72.24^{bA}	56.58^{bA}	61.18^{bA}	49.53^{bcA}	63.64^{bcA}
Significance	S				S			
CV (%)	15.29				16.66			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 48. RGR for each medium averaged over all growth regulators

Media	RGR
M1	0.00366[b]
M2	0.00444[b]
M3	0.00659[a]
M4	0.00578[a]
Significance	S

Table 49. RGR for each growth regulator averaged over all media

Growth regulators	RGR
GR1	0.00430[b]
GR2	0.00548[ab]
GR3	0.00548[ab]
GR4	0.00579[ab]
GR5	0.00605[ab]
GR6	0.00502[ab]
GR7	0.00375[b]
GR8	0.00407[b]
GR9	0.00447[ab]
GR10	0.00674[a]
GR11	0.00506[ab]
GR12	0.00523[ab]
Significance	S

Means with same letter as superscript are homogeneous

Comparison of RGR in each growth regulator averaged over all media (Table 49) revealed that GR10 (BA 100 ppm) recorded the highest RGR (0.00674 cm) which was homogeneous with all other treatments except GR1, GR7 and GR8. The lowest RGR (0.00375) was noted in GR7 (GA 100 ppm).

4.1.1.17.4 Shoot – root ratio

The interaction between media and growth regulator was found to be significant with respect to shoot – root ratio both at six and twelve month stages (Table 50).

At six month period, among the media, M3 (vermicompost) recorded the highest shoot – root ratio (5.83) which was significantly superior to all other media *viz.* M1 (3.82), M2 (3.72) and M4 (2.30). In the M3 medium, GR11 (BA 200 ppm) recorded the highest shoot – root ratio (8.49) which was significantly superior to all other treatments except GR4 and GR10. In the medium M1, GR12 (BA 300 ppm) recorded the maximum shoot – root ratio (7.68) which was significantly superior to all other treatments. GR10 (BA 100 ppm) recorded the maximum shoot – root ratio (5.68) in M2 which differed significantly with GR4, GR9, GR11 and control. In the medium M4, GR11 had the highest shoot – root ratio (8.44) which was significantly superior to all other treatments.

Among the media, M1 (well rotten cow dung) recorded the highest shoot – root ratio (6.30) at twelfth month which was homogeneous with all other media *viz.*, M2 (4.50), M3 (5.42) and M4 (4.05). During this period, in the medium M1, GR1 (IAA 150 ppm) recorded the maximum shoot – root ratio (9.50) which was significantly superior to all other treatments except GR6 and GR12. GR12 recorded the maximum shoot – root ratio (9.42) in M2 which differed significantly with all other treatments except GR2, GR10 and GR11. In the M3 medium, GR4 (IBA 150 ppm) recorded the highest shoot – root ratio (10.81) which was on par with GR3, GR4, GR5, GR6 and GR11. In M4, GR12 recorded the highest shoot – root ratio (6.70) which was significantly superior to GR7 and GR8.

Table 50. Influence of media and growth regulators on shoot - root ratio

Growth regulators	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	5.23^{bA}	5.37^{abA}	3.40^{fgB}	5.69^{bA}	9.50^{aA}	5.64^{bcB}	6.91^{bcdAB}	5.16^{abcB}
GR2	4.97^{bcAB}	5.38^{abA}	3.51^{fgBC}	2.89^{cdeBC}	6.64^{abcA}	7.12^{abcA}	5.26^{cdA}	5.49^{abcA}
GR3	1.88^{eB}	4.14^{abcdA}	4.32^{efgA}	3.99^{bcdA}	4.40^{cB}	5.03^{bcB}	10.32^{abA}	3.33^{abcB}
GR4	4.08^{bcdB}	3.28^{cdB}	7.98^{abA}	2.39^{deB}	4.35^{cB}	3.97^{cB}	10.81^{aA}	4.77^{abcB}
GR5	3.91^{bcdBC}	5.66^{aA}	4.87^{defAB}	2.71^{cdeC}	5.03^{cB}	4.32^{cB}	9.17^{abA}	6.01^{abAB}
GR6	4.21^{bcdA}	4.26^{abcdA}	4.02^{fgA}	4.40^{bcA}	8.49^{abcA}	4.43^{cB}	8.74^{abcA}	3.95^{abcB}
GR7	2.89^{deBC}	4.14^{abcdB}	6.73^{bcA}	2.35^{deC}	5.60^{cAB}	4.52^{cAB}	6.60^{cdA}	2.38^{cB}
GR8	3.25^{cdeAB}	4.97^{abcA}	2.68^{gB}	2.94^{cdeB}	4.45^{cA}	3.97^{cA}	5.47^{cdA}	2.84^{bcA}
GR9	5.32^{bA}	2.88^{dB}	6.56^{bcdA}	1.70^{eB}	4.45^{cA}	3.51^{cA}	6.71^{bcdA}	3.31^{abcA}
GR10	3.17^{deC}	5.68^{aB}	7.68^{abA}	2.81^{cdeC}	8.12^{abcA}	5.85^{abcA}	4.95^{dA}	5.33^{abcA}
GR11	4.45^{bcdB}	2.76^{dB}	8.49^{aA}	8.44^{aA}	4.83^{cB}	8.49^{abA}	9.58^{abA}	3.46^{abcB}
GR12	7.68^{aA}	4.83^{abcBC}	6.56^{bcdAB}	4.77^{bC}	9.38^{abA}	9.42^{aA}	5.52^{cdB}	6.70^{aAB}
Control	3.82^{bcdB}	3.72^{bcdB}	5.83^{cdeA}	2.30^{deB}	6.30^{abcA}	4.50^{cA}	5.42^{cdA}	4.05^{abcA}
Significance	S				S			
CV (%)	18.2				27.93			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.1.17.5 Chlorophyll content

The interaction between media and growth regulator was found to be significant with respect to chlorophyll ‘a’ ‘b’ and total chlorophyll content at four, eight and twelve month stages.

Among the different media (Tables 51 to 53), M3 (vermicompost) was the most significantly superior medium recording the highest content of chlorophyll ‘a’, ‘b’ and total content during all the stages, except for chlorophyll ‘a’ and total content at four month stage and chlorophyll ‘b’ at eight month stage. During the end of the study, M3 recorded the values 1.012, 0.510 and 1.552 mg g^{-1} for chlorophyll ‘a’, ‘b’ and total content respectively.

Effect of growth regulators varied widely among the media at all the stages and a definite pattern of influence could not be observed. In general, it was found that BA treatments had some positive response towards chlorophyll content compared to other growth regulator treatments.

4.1.1.1.18 Nutrient content

Interaction between media and growth regulators was found to be significant with respect to content of all macro and micro nutrients both at six and twelve month stages.

4.1.1.18.1 Nitrogen content

Among the media, M2 (poultry manure) recorded the highest nitrogen content (1.7500 %) at six month stage (Table 54) which was on par with all other media *viz.* M1 (1.6267 %), M3 (1.6833 %) and M4 (1.5517 %). By twelfth month (Table 54), M1 (well rotten cow dung) recorded the highest nitrogen content (1.5467 %) which was on par with M4 (1.4767 %) and significantly superior to M2 (1.3683 %) and M3 (1.2533 %).

Table 51. Influence of media and growth regulators on chlorophyll 'a' content of leaves (mg g $^{-1}$)

Growth regulators	4 months				8 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.077^{fgB}	0.046^{gC}	0.155^{fB}	0.401^{aA}	0.809^{dB}	0.890^{bA}	0.490^{cD}	0.798^{aC}	0.370^{eC}	0.351^{eD}	0.735^{iB}	0.783^{aA}
GR2	0.095^{eC}	0.041^{gD}	0.131^{gB}	0.334^{cdA}	0.399^{lB}	0.525^{iA}	0.345^{iD}	0.372^{jC}	0.279^{ijC}	0.222^{gD}	0.849^{fA}	0.389^{jB}
GR3	0.059^{iD}	0.214^{bB}	0.199^{bcdC}	0.375^{bA}	1.083^{aA}	0.565^{gC}	0.433^{fD}	0.625^{bB}	0.296^{hD}	0.475^{bC}	0.751^{hA}	0.492^{gB}
GR4	0.073^{ghC}	0.304^{aA}	0.163^{fB}	0.316^{eA}	0.628^{gC}	0.678^{dB}	0.380^{hD}	0.804^{aA}	0.059^{kD}	0.291^{fC}	0.895^{eA}	0.508^{fB}
GR5	0.074^{ghC}	0.063^{efC}	0.182^{eB}	0.343^{cA}	0.935^{bA}	0.464^{kB}	0.343^{iD}	0.386^{iC}	0.454^{cD}	0.469^{bC}	0.645^{jA}	0.540^{dB}
GR6	0.301^{bB}	0.067^{eD}	0.109^{hC}	0.324^{deA}	0.887^{cA}	0.406^{lC}	0.388^{ghD}	0.418^{hB}	0.319^{gD}	0.430^{cC}	0.918^{dA}	0.596^{cB}
GR7	0.399^{aA}	0.073^{deC}	0.186^{dB}	0.177^{iB}	0.608^{hA}	0.549^{hB}	0.454^{eC}	0.608^{cA}	0.270^{jD}	0.589^{aB}	0.918^{dA}	0.521^{eC}
GR8	0.233^{cA}	0.054^{fgD}	0.165^{fC}	0.215^{gB}	0.494^{jB}	0.643^{eA}	0.316^{jC}	0.490^{fB}	0.346^{fB}	0.127^{hD}	0.928^{cA}	0.307^{kC}
GR9	0.050^{iC}	0.054^{fgC}	0.194^{cdeB}	0.214^{gA}	0.686^{eA}	0.596^{fB}	0.480^{dD}	0.544^{eC}	0.321^{gC}	0.226^{gD}	0.636^{jA}	0.625^{bB}
GR10	0.103^{eC}	0.074^{cdeD}	0.239^{aA}	0.166^{iB}	0.590^{iB}	0.829^{cA}	0.396^{gC}	0.392^{iC}	0.469^{bB}	0.415^{dC}	1.126^{aA}	0.461^{hB}
GR11	0.062^{hiC}	0.063^{efC}	0.236^{aA}	0.195^{hB}	0.689^{eB}	1.026^{aA}	0.687^{bB}	0.374^{jC}	0.645^{aB}	0.299^{fC}	1.128^{aA}	0.180^{lD}
GR12	0.090^{efC}	0.081^{cdC}	0.205^{bcB}	0.219^{gA}	0.459^{kC}	1.033^{aA}	0.411^{gD}	0.581^{dB}	0.398^{dC}	0.230^{gD}	0.797^{gA}	0.414^{iB}
Control	0.162^{dC}	0.087^{cD}	0.209^{bB}	0.237^{fA}	0.652^{fB}	0.495^{jC}	0.721^{aA}	0.465^{gD}	0.282^{iD}	0.351^{eC}	1.012^{bA}	0.398^{jB}
Significance	S				S				S			
CV (%)	3.70				0.77				0.88			

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Table 52. Influence of media and growth regulators on chlorophyll ‘b’ content of leaves (mg g $^{-1}$)

Growth regulators	4 months				8 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.047^{deC}	0.036^{dC}	0.076^{efgB}	0.222^{aA}	0.399^{dC}	0.448^{bA}	0.241^{bD}	0.422^{aB}	0.192^{dB}	0.186^{eB}	0.403^{fA}	0.392^{aA}
GR2	0.065^{dB}	0.037^{dC}	0.074^{fgB}	0.161^{cA}	0.238^{iB}	0.275^{gA}	0.152^{eD}	0.187^{gC}	0.158^{gC}	0.120^{ghD}	0.363^{gA}	0.207^{fB}
GR3	0.032^{eC}	0.125^{bB}	0.113^{bcdB}	0.188^{bA}	0.667^{aA}	0.301^{fB}	0.207^{cC}	0.317^{cB}	0.155^{gC}	0.249^{cB}	0.325^{iA}	0.244^{eB}
GR4	0.052^{deC}	0.184^{aA}	0.099^{defB}	0.162^{bcA}	0.325^{gB}	0.392^{cA}	0.219^{cC}	0.376^{bA}	0.039^{iD}	0.156^{fC}	0.472^{dA}	0.268^{dB}
GR5	0.049^{deC}	0.037^{dC}	0.106^{cdB}	0.175^{bcA}	0.508^{bA}	0.204^{iB}	0.207^{cB}	0.202^{fgB}	0.260^{bC}	0.270^{bC}	0.343^{hA}	0.287^{cB}
GR6	0.172^{bA}	0.037^{dB}	0.061^{gB}	0.165^{bcA}	0.465^{cA}	0.211^{iB}	0.180^{dC}	0.203^{fgB}	0.191^{deD}	0.255^{cC}	0.466^{dA}	0.286^{cB}
GR7	0.239^{aA}	0.043^{dC}	0.127^{abB}	0.039^{gC}	0.294^{hAB}	0.277^{gB}	0.223^{cC}	0.307^{cA}	0.139^{hD}	0.320^{aB}	0.491^{cA}	0.283^{cC}
GR8	0.150^{bA}	0.026^{dC}	0.103^{dB}	0.097^{deB}	0.296^{hB}	0.372^{dA}	0.165^{deD}	0.258^{eC}	0.191^{deB}	0.104^{hD}	0.419^{eA}	0.172^{gC}
GR9	0.037^{eC}	0.073^{cB}	0.131^{abcA}	0.066^{fB}	0.379^{efA}	0.339^{eB}	0.255^{bD}	0.282^{dC}	0.178^{efC}	0.131^{gD}	0.363^{gA}	0.344^{bB}
GR10	0.068^{dBC}	0.043^{dC}	0.135^{abA}	0.085^{efB}	0.327^{gB}	0.435^{bA}	0.208^{cC}	0.207^{fC}	0.259^{bB}	0.212^{dD}	0.686^{aA}	0.237^{eC}
GR11	0.051^{deC}	0.029^{dC}	0.142^{aA}	0.079^{efB}	0.388^{deB}	0.560^{aA}	0.338^{aB}	0.206^{fC}	0.350^{aB}	0.176^{eC}	0.699^{aA}	0.126^{hD}
GR12	0.047^{deB}	0.044^{dB}	0.101^{deA}	0.114^{dA}	0.283^{hB}	0.569^{aA}	0.215^{cC}	0.282^{dB}	0.229^{cB}	0.157^{fC}	0.393^{fA}	0.237^{eB}
Control	0.111^{cA}	0.038^{dB}	0.109^{bcdA}	0.119^{dA}	0.362^{fA}	0.251^{hC}	0.333^{aB}	0.253^{eC}	0.171^{fD}	0.212^{dC}	0.510^{bA}	0.245^{eB}
Significance	S				S				S			
CV (%)	13.11				3.25				1.94			

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Table 53. Influence of media and growth regulators on total chlorophyll content of leaves (mg g $^{-1}$)

Growth regulators	4 months				8 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.125^{gC}	0.083^{eD}	0.231^{eB}	0.623^{aA}	1.208^{dB}	1.338^{bA}	0.731^{cC}	1.221^{aB}	0.562^{dC}	0.537^{fD}	1.138^{gB}	1.175^{aA}
GR2	0.160^{efC}	0.079^{eD}	0.205^{eB}	0.495^{cdA}	0.637^{kB}	0.800^{iA}	0.497^{iD}	0.559^{hC}	0.438^{gC}	0.342^{jD}	1.212^{fA}	0.596^{kB}
GR3	0.091^{hiC}	0.339^{bB}	0.312^{bcB}	0.562^{bA}	1.750^{aA}	0.866^{gC}	0.640^{eD}	0.943^{cB}	0.450^{gC}	0.723^{bB}	1.076^{hA}	0.736^{hB}
GR4	0.125^{gC}	0.488^{aA}	0.262^{dB}	0.479^{dA}	0.953^{gC}	1.070^{dB}	0.600^{gD}	1.181^{bA}	0.099^{iC}	0.447^{hB}	1.367^{cdA}	0.776^{gB}
GR5	0.123^{ghC}	0.100^{cdeD}	0.288^{cdB}	0.517^{cA}	1.443^{bA}	0.667^{kB}	0.550^{hD}	0.588^{jC}	0.714^{bD}	0.739^{bC}	0.988^{iA}	0.827^{dB}
GR6	0.473^{bA}	0.104^{cdeC}	0.170^{fB}	0.488^{dA}	1.353^{cA}	0.617^{lB}	0.569^{hC}	0.621^{iB}	0.509^{fD}	0.685^{cC}	1.385^{cA}	0.882^{cB}
GR7	0.637^{aA}	0.116^{cdD}	0.313^{bcB}	0.215^{iC}	0.902^{hB}	0.827^{hC}	0.677^{dD}	0.915^{dA}	0.408^{hD}	0.909^{aB}	1.410^{eA}	0.804^{fC}
GR8	0.383^{cA}	0.080^{eD}	0.269^{dC}	0.313^{fB}	0.790^{iB}	1.015^{eA}	0.481^{iD}	0.748^{gC}	0.537^{eB}	0.231^{jD}	1.347^{dA}	0.479^{lC}
GR9	0.087^{iD}	0.127^{cC}	0.325^{bA}	0.280^{gB}	1.065^{eA}	0.935^{fB}	0.735^{cD}	0.826^{fC}	0.499^{fC}	0.358^{jD}	0.999^{iA}	0.968^{bB}
GR10	0.171^{eC}	0.117^{cdD}	0.375^{aA}	0.251^{hB}	0.917^{hB}	1.264^{cA}	0.604^{fgC}	0.600^{iC}	0.727^{bB}	0.627^{dD}	1.811^{aA}	0.698^{iC}
GR11	0.113^{ghiC}	0.091^{deD}	0.378^{aA}	0.274^{ghB}	1.077^{eB}	1.586^{aA}	1.025^{bC}	0.580^{jhD}	0.995^{aB}	0.474^{gC}	1.827^{aA}	0.306^{mD}
GR12	0.137^{fgC}	0.125^{cC}	0.306^{bcB}	0.332^{efA}	0.742^{jC}	1.603^{aA}	0.626^{efD}	0.863^{eB}	0.627^{cC}	0.387^{iD}	1.190^{fA}	0.651^{jB}
Control	0.272^{dC}	0.125^{cD}	0.318^{bB}	0.356^{eA}	1.013^{fB}	0.747^{jC}	1.053^{aA}	0.718^{hD}	0.453^{gD}	0.563^{eC}	1.522^{bA}	0.644^{jB}
Significance	S				S				S			
CV (%)	5.30				1.12				1.27			

Means with same lower case letter as superscript within a column are homogeneous
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Table 54. Influence of media and growth regulators on nitrogen content (%)

Growth regulators	N content (%)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	1.4300^{dB}	1.4100^{eB}	1.6450^{bcdA}	1.5433^{bcdAB}	1.5817^{aA}	1.3650^{cdeB}	1.3550^{cdB}	1.3600^{bcB}
GR2	1.6383^{bcdB}	1.6200^{bcdeB}	1.7500^{abAB}	1.9400^{aA}	1.4350^{bcdB}	1.3267^{defB}	1.6517^{aA}	1.5617^{aA}
GR3	1.7517^{bA}	1.4100^{eB}	1.7183^{bcA}	1.7433^{abA}	1.4200^{cdB}	1.4167^{dB}	1.5717^{abA}	1.4317^{bB}
GR4	1.6517^{bcB}	1.9367^{aA}	1.3617^{eC}	1.3933^{dC}	1.2700^{efgB}	1.3367^{defAB}	1.4383^{cA}	1.2883^{deB}
GR5	2.1033^{aA}	1.1833^{fC}	1.9350^{aA}	1.4633^{dB}	1.4383^{bcdB}	1.2317^{fC}	1.5750^{abA}	1.4700^{abAB}
GR6	1.5817^{bcdAB}	1.6517^{bcdA}	1.4100^{eB}	1.5467^{bcAB}	1.3650^{cdeB}	1.5367^{abA}	1.4767^{bcAB}	1.3800^{bcdB}
GR7	1.6050^{bcdA}	1.5367^{cdeA}	1.4633^{deA}	1.4417^{dA}	1.2000^{gC}	1.5750^{aA}	1.4283^{cB}	1.2283^{eC}
GR8	1.1900^{cB}	1.7283^{abcA}	1.6583^{bcdA}	1.5150^{cdA}	1.3400^{defA}	1.2883^{efA}	1.2817^{dA}	1.2917^{cdeA}
GR9	1.0500^{cB}	1.5783^{bcdeA}	1.6833^{bcA}	1.6583^{bA}	1.5433^{abA}	1.2217^{fC}	1.5117^{bcAB}	1.4067^{bcB}
GR10	1.0833^{cC}	1.5150^{deB}	1.9350^{aA}	1.6983^{bcB}	1.4767^{abcA}	1.4700^{abcA}	1.5333^{bcA}	1.2850^{deB}
GR11	1.1883^{cB}	1.7083^{bcA}	1.5150^{cdeA}	1.1333^{eB}	1.2217^{gB}	1.4417^{bcdA}	1.5217^{bcA}	1.2417^{eB}
GR12	1.4967^{cdBC}	1.7533^{abA}	1.6850^{bcAB}	1.3017^{deC}	1.2350^{fgC}	1.4283^{bcdB}	1.5467^{abA}	1.2817^{deC}
Control	1.6267^{bcdA}	1.7500^{abcA}	1.6833^{bcA}	1.5517^{bcdA}	1.5467^{abA}	1.3683^{cdeBC}	1.2533^{dC}	1.4767^{abAB}
Significance	S				S			
CV (%)	6.38				3.91			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Effect of growth regulators varied widely among the media at both the stages and a definite pattern of influence could not be observed. Towards the end at twelve months, in the M1 medium, GR1 (IAA 150 ppm) recorded the highest nitrogen content (1.5817 %) which was significantly superior to all other treatments except GR9, GR10 and control. In the medium M2, GR7 (GA 100 ppm) recorded the maximum nitrogen content (1.5750 %) which differed significantly with all other treatments except GR6 and GR10. GR2 (IAA 300 ppm) recorded the maximum nitrogen content (1.6517 %) in M3 which differed significantly with all other treatments except GR3, GR5 and GR12. In the medium M4 also, GR2 (IAA 300 ppm) had the maximum nitrogen content (1.5617 %) which was significantly superior to all other treatments except GR5 and control.

4.1.1.18.2 Phosphorous content

At six months (Table 55), among the media, M3 (vermicompost) recorded the highest phosphorous content (0.1247 %) which was on par with all other media *viz.* M1 (0.0823 %), M2 (0.1159 %) and M4 (0.0937 %). At twelve months (Table 55), M1 (well rotten cow dung) recorded the highest phosphorous content (0.2430 %) which was significantly superior to all other media *viz.* M2 (0.1527 %), M3 (0.1489 %) and M4 (0.1045 %).

Effect of growth regulators varied widely among the media at both the stages and a definite pattern of influence could not be observed. At the end of study at twelve months, in the M1 medium, GR6 (IBA 450 ppm) recorded the highest phosphorous content (0.2888 %) which was significantly superior to all other treatments except GR2, GR5, GR8, GR10 and control. In the medium M2, GR10 (BA 100 ppm) recorded the maximum phosphorous content (0.2894 %) which differed significantly with all other treatments except GR2, GR3, GR4 and GR11. GR11 (BA 200 ppm) recorded the maximum phosphorous content (0.1785 %) in M3 which was homogeneous with all other treatments. In the medium M4, GR1 (IAA

150 ppm) had the maximum phosphorous content (0.2357 %) which was significantly superior to all other treatments except GR2.

4.1.1.18.3 Potassium content

Among the media, M3 (vermicompost) recorded the highest potassium content (1.7667 %) at six month stage (Table 56) which was on par with M4 (1.5083 %) and significantly superior to both M1 (0.8417 %) and M2 (1.2167 %). At twelve months (Table 56), M3 (vermicompost) recorded the highest potassium content (1.5583 %) which was on par with M2 (1.5333 %) and M4 (1.4000 %) and significantly superior to M1 (0.8333 %).

Influence of growth regulators varied widely among the media at both the stages and a definite pattern of influence could not be observed. At the end of twelve months, in the M3 medium, control recorded the highest potassium content (1.5583 %) which was significantly superior to GR3, GR9 and GR11. In the medium M1, GR11 (BA 200 ppm) recorded the maximum potassium content (1.3250 %) which differed significantly with all other treatments except GR1, GR2, GR4, GR8 and GR121. GR7 (GA 100 ppm) recorded the maximum potassium content (1.6000 %) in M2 which was significantly superior to all other treatments except GR6 and control. In the medium M4, GR12 (BA 450 ppm) had the maximum potassium content (1.6333 %) which was significantly superior to all other treatments except GR3 and GR10.

4.1.1.18.4 Calcium content

All the media differed significantly among themselves in calcium content of plants at six months (Table 57) and among the media, M2 (poultry manure) recorded the highest calcium content (1.2258 %) followed by M4 (0.9400 %), M3 (0.5492 %) and M1 (0.4042 %). At twelve months (Table 57), M3 (vermicompost) recorded the highest calcium content (1.1308 %) which was on par with M2 (1.0525 %) and M4 (1.1133 %) and all these treatments were significantly superior to M1 (0.8167 %).

Table 55. Influence of media and growth regulators on phosphorous content (%)

Growth regulators	P content (%)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.1124^{bcdA}	0.1500^{abcdeA}	0.1148^{bA}	0.1263^{abA}	0.1869^{bcdAB}	0.2085^{bcAB}	0.1500^{aB}	0.2357^{aA}
GR2	0.3498^{aA}	0.2041^{abB}	0.1113^{bB}	0.1552^{abBC}	0.2168^{abcAB}	0.2577^{abA}	0.1380^{aB}	0.1759^{abB}
GR3	0.1826^{bA}	0.1851^{acA}	0.1097^{bA}	0.1240^{abA}	0.1486^{cdB}	0.2348^{abcA}	0.1545^{aAB}	0.1467^{bB}
GR4	0.1395^{bcdA}	0.1351^{bcdefA}	0.1031^{bA}	0.1525^{abA}	0.1920^{bcdAB}	0.2336^{abcA}	0.1250^{aB}	0.1538^{bAB}
GR5	0.1836^{bAB}	0.2210^{aA}	0.1297^{bB}	0.1795^{aAB}	0.2323^{abA}	0.1544^{cAB}	0.1489^{aB}	0.1193^{bB}
GR6	0.1493^{bcA}	0.1064^{defA}	0.0881^{bA}	0.1401^{abA}	0.2888^{aA}	0.1959^{bcB}	0.1638^{aBC}	0.1136^{bC}
GR7	0.1435^{bcdA}	0.1516^{abcdeA}	0.0909^{bA}	0.1094^{abA}	0.1864^{bcdA}	0.1901^{bcA}	0.1555^{aA}	0.1270^{bA}
GR8	0.1438^{bcdA}	0.0593^{fB}	0.0701^{bAB}	0.1282^{abAB}	0.2229^{abcA}	0.1552^{cAB}	0.1355^{aB}	0.0985^{bC}
GR9	0.0674^{dB}	0.0838^{efB}	0.2175^{aA}	0.1230^{abB}	0.1126^{dA}	0.1687^{cA}	0.1384^{aA}	0.1214^{bA}
GR10	0.1576^{bcA}	0.1642^{abcdA}	0.1313^{bA}	0.1156^{abA}	0.2116^{acAB}	0.2894^{aA}	0.1438^{aBC}	0.1262^{bC}
GR11	0.1549^{bcA}	0.1224^{cdefA}	0.1253^{bA}	0.1142^{abA}	0.1667^{bcdB}	0.2518^{abA}	0.1785^{aAB}	0.1078^{bB}
GR12	0.1363^{bcdA}	0.1232^{cdefA}	0.1197^{bA}	0.1195^{abA}	0.2048^{bcA}	0.1576^{cA}	0.1646^{aA}	0.1428^{bA}
Control	0.0823^{cdA}	0.1159^{cdefA}	0.1247^{bA}	0.0937^{bA}	0.2430^{abA}	0.1527^{cB}	0.1489^{aB}	0.1045^{bB}
Significance	S				S			
CV (%)	23.35				18.31			

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Table 56. Influence of media and growth regulators on potassium content (%)

Growth regulators	K content (%)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	1.7000^{bA}	1.2083^{eB}	1.5917^{bA}	1.5917^{aA}	1.1667^{abcdB}	1.1167^{fB}	1.4667^{abA}	1.4000^{bcdA}
GR2	3.7500^{aA}	1.2667^{cdeC}	1.8917^{aB}	1.3250^{bcC}	1.1917^{abcdC}	1.1583^{eA}	1.4750^{abAB}	1.3000^{cdB}
GR3	1.2250^{defB}	1.4750^{cdAB}	1.5833^{bA}	1.6667^{aA}	1.0583^{bcdeC}	0.7392^{gD}	1.2667^{bcB}	1.5250^{abA}
GR4	1.2667^{cdeAB}	1.2000^{eB}	1.3167^{cdAB}	1.4750^{abA}	1.1917^{abcdAB}	1.1750^{defB}	1.3917^{abA}	1.0417^{efB}
GR5	1.4000^{cdAB}	1.5417^{bcA}	1.2333^{dB}	1.5667^{abA}	1.0250^{defB}	1.1750^{defB}	1.4333^{abA}	1.4250^{bA}
GR6	1.2417^{defB}	1.3917^{cdeAB}	1.5917^{bA}	1.5250^{abA}	0.8833^{efB}	1.4083^{abcA}	1.4083^{abA}	1.3417^{bcdA}
GR7	1.5250^{bcAB}	1.7833^{abA}	1.2583^{dB}	1.5667^{abA}	1.0083^{defC}	1.6000^{aA}	1.3667^{abB}	1.3500^{bcdB}
GR8	1.4167^{cdA}	0.8000^{fB}	1.5583^{bcA}	1.5583^{aA}	1.2500^{abAB}	1.2333^{cdefAB}	1.4250^{abA}	1.2167^{deB}
GR9	0.6917^{hC}	1.6000^{bA}	1.3125^{cdB}	1.2083^{cBC}	0.9500^{efB}	1.3417^{bcdeA}	1.1583^{cAB}	0.9500^{fB}
GR10	1.1750^{dfB}	1.4917^{cA}	1.6333^{abA}	1.4417^{acA}	1.0417^{cdeB}	1.1667^{defB}	1.4417^{abA}	1.4333^{abcA}
GR11	1.0025^{fgC}	1.5333^{bB}	1.8167^{abA}	1.6083^{aAB}	1.3250^{aA}	1.1750^{defA}	1.2667^{bcA}	1.3083^{cdA}
GR12	1.0917^{efgB}	1.9000^{aA}	1.6583^{abA}	1.6917^{aA}	1.2333^{abcB}	1.3667^{bcdB}	1.3667^{abB}	1.6333^{aA}
Control	0.8417^{ghC}	1.2167^{deB}	1.7667^{abA}	1.5083^{abA}	0.8333^{fB}	1.5333^{abA}	1.5583^{aA}	1.4000^{bcdA}
Significance	S				S			
CV (%)	8.30				7.50			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 57. Influence of media and growth regulators on calcium content (%)

Growth regulators	Ca content (%)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.8250^{cB}	0.6150^{deC}	0.6750^{cdefC}	0.9867^{aA}	0.6408^{fghB}	0.6792^{fgA}	0.7792^{bAB}	0.6792^{fA}
GR2	1.5492^{aA}	0.6842^{cdB}	0.7225^{cdB}	0.7825^{cB}	0.8692^{deA}	0.7292^{efAB}	0.5842^{bC}	0.6917^{fBC}
GR3	0.8425^{cA}	0.7208^{cdAB}	0.7058^{cdeB}	0.8175^{bcAB}	1.2525^{aA}	0.7033^{efB}	0.7325^{bB}	0.6983^{fB}
GR4	0.6558^{dC}	0.7908^{cB}	1.3450^{aA}	0.4925^{eD}	0.7575^{efgB}	0.7892^{dfB}	0.7792^{bB}	1.0575^{eA}
GR5	0.6217^{dB}	1.0350^{bA}	0.9275^{bA}	0.9825^{aA}	0.7758^{efB}	0.5475^{gC}	0.7600^{bB}	2.1100^{cA}
GR6	1.0500^{bA}	1.0425^{bA}	0.9225^{bA}	0.7792^{cB}	0.5758^{hD}	0.8892^{dB}	0.7183^{bC}	3.0558^{bA}
GR7	0.7100^{dB}	1.0483^{bA}	0.5975^{defgB}	0.6183^{deB}	0.8175^{eA}	0.8375^{deA}	0.6792^{bB}	0.7325^{fAB}
GR8	1.0467^{bA}	0.3125^{fC}	0.7492^{cB}	0.8108^{bcB}	0.9900^{cdC}	1.2292^{aB}	0.5192^{bD}	1.5275^{dA}
GR9	0.4558^{efB}	0.5067^{eB}	0.5796^{efgB}	0.8600^{abcA}	0.6250^{ghC}	1.0342^{bcB}	1.0675^{aB}	3.7283^{aA}
GR10	1.5200^{aA}	1.0700^{bB}	0.6908^{cefC}	0.5025^{eC}	1.0683^{cA}	0.8992^{cdB}	0.6767^{bC}	1.1358^{eA}
GR11	0.5567^{deAB}	0.6775^{cdA}	0.5408^{gB}	0.5025^{eB}	1.1133^{bcB}	0.5558^{gC}	0.6675^{bC}	1.1858^{eA}
GR12	0.6100^{dC}	0.9567^{bA}	0.5883^{efgC}	0.7458^{cdB}	1.2375^{abA}	0.7758^{dB}	0.7267^{bB}	0.7292^{fB}
Control	0.4042^{fD}	1.2258^{aA}	0.5492^{fgC}	0.9400^{abB}	0.8167^{eB}	1.0525^{bA}	1.1308^{aA}	1.1133^{eA}
Significance	S				S			
CV (%)	8.03				6.50			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Effect of growth regulators varied widely among the media at both the stages and a definite pattern of influence could not be observed. Towards the end of the experiment, in the M3 medium, control recorded the highest calcium content (1.1308 %) which was significantly superior to all other treatments except GR9. In the medium M1, GR3 (IAA 450 ppm) recorded the maximum calcium content (1.2525 %) which differed significantly with all other treatments except GR12. GR8 (GA 200 ppm) recorded the maximum calcium content (1.2292 %) in M2 which was superior to all other treatments. In the medium M4, GR9 (GA 300 ppm) had the maximum calcium content (3.7283 %) which was significantly superior to all other treatments.

4.1.1.18.5 Magnesium content

At six months (Table 58), among the media, M2 (poultry manure) recorded the highest magnesium content (0.3050 %) which was significantly superior to all other media *viz.*, M1 (0.1708 %), M3 (0.1442 %) and M4 (0.1825 %). At twelve months (Table 58), M1 (well rotten cow dung) recorded the highest magnesium content (0.3650 %) which differed significantly with all other media *viz.*, M2 (0.3042 %), M3 (0.2142 %) and M4 (0.2467 %).

Influence of growth regulators varied widely among the media at both the stages and a definite pattern of effect could not be observed. At the end of the study, in M1 medium, both GR5 (IBA 300 ppm) and GR10 (BA 100 ppm) recorded the highest magnesium content (0.3725 %) which was on par with all other treatments except GR6 and GR12. In the medium M2, GR9 (GA 300 ppm) recorded the maximum magnesium content (0.5067 %) which differed significantly with all other treatments. GR3 (IAA 450 ppm) recorded the maximum magnesium content (0.2300 %) in M3 which was significantly superior to GR6, GR10 and GR12. In the medium M4, GR11 (BA 300 ppm) had the maximum magnesium content (0.2875 %) which was significantly superior to all other treatments except control.

Table 58. Influence of media and growth regulators on magnesium content (%)

Growth regulators	Mg content (%)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.2058^{efghB}	0.2992^{cA}	0.2067^{bcB}	0.1850^{eC}	0.2792^{abA}	0.2108^{deB}	0.2067^{abB}	0.1775^{cdeB}
GR2	0.6225^{aA}	0.2750^{cdB}	0.2242^{bC}	0.1550^{efD}	0.2617^{abcA}	0.1633^{efB}	0.2092^{abB}	0.2017^{bcdeB}
GR3	0.2825^{bcA}	0.2283^{defB}	0.1992^{bcB}	0.2283^{bcdeB}	0.2908^{aA}	0.1558^{fC}	0.2300^{aB}	0.1825^{cdeC}
GR4	0.2192^{defgA}	0.2375^{deA}	0.2067^{bcA}	0.1967^{eA}	0.2592^{abcAB}	0.2642^{bcA}	0.2033^{abC}	0.2100^{bcdBC}
GR5	0.2417^{cdefAB}	0.2633^{cdeA}	0.2192^{bAB}	0.2133^{cdeB}	0.3725^{aA}	0.2450^{cdB}	0.2075^{abB}	0.2125^{bcdB}
GR6	0.2983^{bA}	0.1767^{gB}	0.2200^{bB}	0.2092^{cdeB}	0.2292^{bcAB}	0.2525^{bcdA}	0.1525^{cC}	0.2092^{bcdBC}
GR7	0.2158^{defgB}	0.1750^{gB}	0.1683^{cdB}	0.2542^{bcA}	0.2550^{abcA}	0.2458^{cdAB}	0.1992^{abcBC}	0.1683^{deC}
GR8	0.1933^{fghB}	0.1692^{gB}	0.3633^{aA}	0.3317^{aA}	0.3533^{a}	0.2425^{cd}	0.1958^{abc}	0.2242^{b}
GR9	0.1650^{hC}	0.5883^{aA}	0.3242^{aB}	0.1292^{fC}	0.2708^{abB}	0.5067^{aA}	0.2025^{abcC}	0.1525^{eC}
GR10	0.4433^{bA}	0.2142^{efgC}	0.1250^{dD}	0.2767^{bB}	0.3725^{aA}	0.2633^{cB}	0.1767^{bcC}	0.1767^{cdeC}
GR11	0.2650^{bcdB}	0.3650^{bA}	0.1308^{dC}	0.2533^{bcdB}	0.2808^{abA}	0.2450^{cdAB}	0.2133^{abB}	0.2875^{aA}
GR12	0.2550^{bcdeA}	0.1950^{fgB}	0.2067^{bcB}	0.2042^{deB}	0.2125^{cAB}	0.2208^{cdA}	0.1658^{bcB}	0.2208^{bcA}
Control	0.1708^{ghB}	0.3050^{bcA}	0.1442^{dB}	0.1825^{eB}	0.3650^{aA}	0.3042^{bB}	0.2142^{abC}	0.2467^{abC}
Significance	S				S			
CV (%)	13.02				13.30			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.18.6 Iron content

Among the media, M4 (coir pith compost) recorded the highest iron content (1458.33 mg kg $^{-1}$) at six month stage (Table 59) which differed significantly with all other media. Both M2 (676.33 mg kg $^{-1}$) and M3 (468.67 mg kg $^{-1}$) were homogeneous and M1 (340.67 mg kg $^{-1}$) recorded the lowest value. At twelve months (Table 59), M4 (coir pith compost) recorded the highest iron content (557.67 mg kg $^{-1}$) which was on par with all other media except M1. Media M1 (368.33 mg kg $^{-1}$), M2 (447.67 mg kg $^{-1}$) and M3 (452.00 mg kg $^{-1}$) were also uniform.

Effect of growth regulators varied widely among the media at both the stages and a definite pattern of influence could not be observed. At the end of the study, in the M4 medium, GR11 (BA 200 ppm) recorded the highest iron content (632.67 mg kg $^{-1}$) which was significantly superior to GR1, GR3, GR4, GR6 and GR8. In the medium M1, GR10 (BA 100 ppm) recorded the maximum iron content (666.00 mg kg $^{-1}$) which differed significantly with all other treatments except GR8. GR8 (GA 200 ppm) recorded the maximum iron content (726.00 mg kg $^{-1}$) in M2 which was superior to all other treatments except GR1, GR9, GR10 and GR11. In the medium M3, GR1 (IAA 150 ppm) had the maximum iron content (768.67 mg kg $^{-1}$) which was significantly superior to all other treatments except GR4 and GR5.

4.1.1.18.7 Copper content

At six months (Table 60), among the media, M4 (coir pith compost) recorded the highest copper content (12.47 mg kg $^{-1}$) which was on par with M3 (9.83 mg kg $^{-1}$) and both M3 and M4 were significantly superior to M1 (2.13 mg kg $^{-1}$) and M2 (6.47 mg kg $^{-1}$). At twelve months (Table 60), M3 (vermicompost) recorded the highest copper content (10.60 mg kg $^{-1}$) which differed significantly with all other media *viz.*, M1 (5.50 mg kg $^{-1}$), M2 (4.67 mg kg $^{-1}$) and M4 (4.47 mg kg $^{-1}$).

Table 59. Influence of media and growth regulators on iron content (mg kg $^{-1}$)

Growth regulators	Fe content (mg kg $^{-1}$)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	546.00^{defB}	642.67^{abB}	871.00^{aA}	1083.00^{bcdA}	477.33^{bB}	562.33^{abB}	768.67^{aA}	442.67^{bcdB}
GR2	1154.50^{bA}	524.33^{abcB}	676.67^{abcB}	1131.33^{bcA}	457.00^{bAB}	373.00^{cB}	558.00^{bcdA}	550.67^{abAB}
GR3	649.67^{cdeB}	360.33^{cC}	676.33^{abcB}	980.67^{cdA}	449.33^{bAB}	357.00^{cB}	568.33^{bcA}	457.33^{bcdAB}
GR4	479.33^{efB}	550.00^{abcB}	676.00^{abcB}	1065.67^{bcdA}	448.67^{bB}	445.67^{bcB}	645.33^{abA}	346.67^{dB}
GR5	465.00^{efBC}	445.33^{bcdC}	674.00^{abcB}	1134.00^{bcdA}	253.00^{cC}	439.33^{bcB}	754.00^{aA}	559.33^{abB}
GR6	546.00^{defBC}	383.00^{cC}	647.33^{abcB}	1055.00^{cdA}	347.00^{bcA}	421.00^{bcA}	474.33^{bcdeA}	382.00^{cdA}
GR7	652.67^{cdeB}	456.67^{bcB}	569.00^{bcB}	941.00^{dA}	374.00^{bcB}	372.67^{cB}	452.67^{cdeAB}	569.67^{abA}
GR8	843.33^{cB}	237.00^{dC}	469.00^{cC}	1163.00^{bcA}	657.00^{aA}	726.00^{aA}	386.00^{defB}	437.67^{bcdB}
GR9	349.00^{fC}	659.67^{abB}	744.67^{abB}	1057.33^{cdA}	367.00^{bcA}	459.67^{abcA}	451.33^{cdeA}	468.00^{abcdA}
GR10	2074.00^{aA}	559.67^{abcC}	577.67^{bcC}	1471.00^{aB}	666.00^{aA}	464.00^{abcB}	255.67^{fC}	460.00^{abcdB}
GR11	652.00^{cdeB}	651.67^{abB}	554.33^{bcB}	1470.33^{aA}	464.33^{bAB}	525.67^{abcAB}	357.33^{efB}	632.67^{aA}
GR12	739.00^{cdB}	653.33^{abB}	660.33^{abcB}	1278.33^{abA}	475.33^{bA}	440.00^{bcA}	257.67^{fC}	535.67^{abcA}
Control	340.67^{fC}	676.33^{aB}	468.67^{cBC}	1458.33^{aA}	368.33^{bcB}	447.67^{bcAB}	452.00^{cdeAB}	557.67^{abA}
Significance	S				S			
CV (%)	13.30				17.60			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 60. Influence of media and growth regulators on copper content (mg kg $^{-1}$)

Growth regulators	Cu content (mg kg $^{-1}$)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	6.60^{deC}	9.23^{bBC}	24.13^{aA}	11.57^{cdeB}	5.43^{bB}	6.50^{cdeB}	10.13^{abA}	6.83^{abcdB}
GR2	24.65^{aA}	10.53^{abBC}	9.50^{bcdC}	12.67^{bcB}	5.37^{bB}	9.93^{aA}	8.67^{abcdA}	8.23^{abAB}
GR3	6.60^{deA}	8.40^{bA}	8.57^{cdeA}	9.67^{cdefA}	5.33^{bA}	6.93^{abcdeA}	6.33^{deA}	6.67^{abcdA}
GR4	7.60^{deC}	12.40^{aAB}	11.77^{bB}	15.13^{abA}	6.57^{bB}	9.83^{aA}	8.90^{abcdAB}	9.33^{aAB}
GR5	9.40^{cdA}	10.57^{abA}	8.53^{cdeA}	10.67^{cdefA}	6.67^{abA}	7.57^{abcdeA}	7.30^{bcdeA}	7.27^{abcdA}
GR6	6.57^{deAB}	7.13^{cAB}	6.13^{eB}	9.47^{defA}	7.53^{abA}	8.50^{abcdA}	9.73^{abcA}	7.03^{abcdA}
GR7	6.50^{deB}	6.47^{cB}	6.37^{eB}	17.80^{aA}	6.27^{bA}	8.73^{abcA}	8.73^{abcdA}	6.73^{abcdA}
GR8	13.80^{bA}	1.63^{dD}	6.77^{deC}	10.57^{cdfB}	6.90^{abA}	5.50^{deA}	6.77^{cdeA}	4.47^{dA}
GR9	1.77^{fC}	4.82^{cBC}	5.58^{eB}	8.67^{efA}	4.53^{bA}	6.70^{bcdeA}	4.57^{eA}	4.97^{cdA}
GR10	10.77^{bcA}	7.83^{bcA}	8.37^{cdeA}	9.37^{efA}	9.70^{aA}	9.53^{abcA}	7.40^{bcdeA}	7.33^{abcdA}
GR11	8.33^{cA}	1.87^{dB}	10.47^{bcA}	8.27^{fA}	6.57^{bC}	9.77^{abAB}	10.77^{aA}	7.67^{abcBC}
GR12	5.70^{eBC}	4.70^{cC}	9.53^{bcdA}	8.57^{efAB}	6.43^{bB}	9.97^{aA}	8.23^{abcdAB}	5.50^{bcdB}
Control	2.13^{fC}	6.47^{cB}	9.83^{bcdA}	12.47^{bcdA}	5.50^{bB}	4.67^{eB}	10.60^{aA}	4.47^{dB}
Significance	S				S			
CV (%)	15.94				20.20			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Influence of growth regulators varied widely among the media at both the stages and a definite pattern of effect could not be observed. At the end, in the M3 medium, GR11 (BA 200 ppm) recorded the highest copper content (10.77 mg kg $^{-1}$) which differed significantly with GR3, GR5, GR8, GR9 and GR10. In the medium M1, GR10 (BA 100 ppm) recorded the maximum copper content (9.70 mg kg $^{-1}$) which differed significantly with all other treatments except GR5, GR6 and GR8. GR12 (BA 300 ppm) recorded the maximum copper content (9.97 mg kg $^{-1}$) in M2 which was significantly superior to GR1, GR8, GR9 and control. In the medium M4, GR4 (IBA 150 ppm) had the maximum copper content (9.33 mg kg $^{-1}$) which was significantly superior to GR8, GR9, GR12 and control.

4.1.1.18.8 Manganese content

Among the media, M4 (coir pith compost) recorded the highest manganese content (113.53 mg kg $^{-1}$) at six months (Table 61) which differed significantly with all other media. Both M2 (74.99 mg kg $^{-1}$) and M3 (84.40 mg kg $^{-1}$) were homogeneous and M1 (34.17 mg kg $^{-1}$) recorded the lowest value which was significantly lower compared to all other media. At twelve months (Table 61), M3 (vermicompost) recorded the highest manganese content (182.63 mg kg $^{-1}$) which differed significantly with all other media. Both M1 (77.97 mg kg $^{-1}$) and M2 (81.10 mg kg $^{-1}$) were homogeneous and both recorded significantly lower values compared to M4 (102.93 mg kg $^{-1}$) also.

Effect of growth regulators varied widely among the media at both the stages and a definite pattern of influence could not be observed. Towards the end of the experiment, in the M3 medium, control recorded the highest manganese content (182.63 mg kg $^{-1}$). In the medium M1, GR10 (BA 100 ppm) recorded the maximum manganese content (144.57 mg kg $^{-1}$) while in M2, GR8 (GA 200 ppm) showed the

highest manganese content (134.60 mg kg $^{-1}$). In all the three media, the respective growth regulators were superior to all other treatments. In the medium M4, GR9 (GA 300 ppm) had the maximum manganese content (183.37 mg kg $^{-1}$) which was significantly superior to all other treatments except GR1.

4.1.1.18.9 Zn content

At six months (Table 62), among the media, M4 (coir pith compost) recorded the highest zinc content (19.30 mg kg $^{-1}$) which was on par with M2 (12.10 mg kg $^{-1}$) and M3 (14.23 mg kg $^{-1}$) and significantly superior to M1 (3.20 mg kg $^{-1}$). During twelve month stage (Table 62), M3 (vermicompost) recorded the highest zinc content (16.43 mg kg $^{-1}$) which was on par with all other media *viz.*, M1 (11.73 mg kg $^{-1}$), M2 (9.30 mg kg $^{-1}$) and M4 (6.93 mg kg $^{-1}$).

Influence of growth regulators varied widely among the media at both the stages and a definite pattern of effect could not be observed. Towards the end of the study, in the M3 medium, GR3 (IAA 150 ppm) recorded the highest zinc content (27.97 mg kg $^{-1}$) which differed significantly with GR2, GR4 and GR8. In the medium M1, GR7 (GA 100 ppm) recorded the maximum zinc content (27.17 mg kg $^{-1}$) which differed significantly with GR4, GR11 and control. GR2 (IAA 150 ppm) recorded the maximum zinc content (20.53 mg kg $^{-1}$) in M2 which was significantly superior to only GR3. In the medium M4, GR5 (IBA 300 ppm) had the maximum zinc content (56.20 mg kg $^{-1}$) which was significantly superior to all other treatments.

4.1.1.19 Nutrient uptake

The interaction between media and growth regulators was found to be significant with respect to uptake of N, P, K, Ca, Cu, Mn and Zn after one year of study. In the case of Mg and Fe, even though interaction was non significant, significant difference was observed among the growth regulators and also among the media during this period.

Table 61. Influence of media and growth regulators on manganese content (mg kg $^{-1}$)

Growth regulators	Mn content (mg kg $^{-1}$)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	76.30^{deB}	88.77^{abB}	114.50^{bA}	124.27^{dA}	86.93^{bcB}	66.53^{cdC}	98.30^{dfB}	168.87^{aA}
GR2	208.27^{aA}	54.00^{eD}	86.33^{cdC}	174.53^{aB}	94.73^{bB}	73.77^{bcC}	73.43^{ghC}	133.30^{bcA}
GR3	47.07^{fgC}	57.60^{deC}	77.00^{dB}	113.40^{deA}	95.73^{bB}	53.70^{dC}	68.63^{hC}	148.17^{bA}
GR4	86.47^{cdB}	98.77^{aB}	96.20^{cB}	127.90^{cdA}	64.80^{deB}	51.97^{dB}	93.10^{defA}	88.23^{efA}
GR5	63.07^{efC}	72.77^{bcdBC}	144.20^{aA}	83.97^{fgB}	83.93^{bcB}	64.87^{cdC}	85.23^{fgB}	101.93^{deA}
GR6	93.10^{cdB}	76.40^{bcB}	85.73^{cdB}	169.80^{aA}	86.27^{bcB}	53.90^{dC}	125.50^{cA}	117.97^{cdA}
GR7	120.60^{bA}	73.47^{bcdC}	83.70^{cdBC}	97.87^{efB}	95.03^{bA}	75.43^{bcB}	87.57^{fgAB}	100.87^{eA}
GR8	125.43^{bA}	34.03^{fD}	105.43^{bcB}	96.03^{eC}	53.67^{eC}	134.60^{aA}	89.70^{efgB}	98.10^{eB}
GR9	38.43^{gB}	76.23^{bcA}	78.82^{cdA}	76.13^{gA}	88.67^{bcC}	85.83^{bC}	104.27^{deB}	183.37^{aA}
GR10	135.93^{bA}	64.80^{ceB}	71.77^{dB}	146.83^{bA}	144.57^{aA}	52.37^{dC}	145.53^{bA}	95.17^{eB}
GR11	96.30^{cB}	54.50^{eC}	82.70^{cdB}	145.33^{bcA}	86.53^{bcB}	76.70^{bcB}	108.13^{dA}	87.87^{efB}
GR12	122.67^{bA}	65.57^{cdeC}	107.53^{bcAB}	104.30^{eB}	54.13^{eB}	52.33^{dB}	86.63^{fgA}	74.87^{fA}
Control	34.17^{gC}	74.99^{bcdB}	84.40^{cdB}	113.53^{deA}	77.97^{cdC}	81.10^{bcC}	182.63^{aA}	102.93^{deB}
Significance	S				S			
CV (%)	9.03				8.30			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

Table 62. Influence of media and growth regulators on zinc content (mg kg $^{-1}$)

Growth regulators	Zn content (mg kg $^{-1}$)							
	6 months				12 months			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	13.93^{bcdA}	13.57^{bcA}	22.17^{aA}	24.47^{abA}	17.97^{abA}	18.30^{aA}	13.47^{aA}	10.47^{bA}
GR2	37.58^{aA}	16.07^{bcB}	15.57^{aB}	20.87^{abB}	16.17^{abA}	20.53^{aA}	12.73^{bA}	12.60^{bA}
GR3	14.17^{bcdA}	14.77^{bcA}	16.50^{aA}	20.63^{abA}	13.43^{abB}	3.33^{bB}	27.97^{aA}	12.60^{bB}
GR4	12.20^{cdB}	17.77^{bcAB}	16.27^{aAB}	28.47^{aA}	12.70^{bA}	15.93^{abA}	9.30^{bA}	16.83^{bA}
GR5	14.57^{bcdB}	29.70^{aA}	18.70^{aAB}	16.67^{abB}	17.57^{abB}	10.93^{abB}	17.47^{abB}	56.20^{aA}
GR6	9.77^{cdB}	13.83^{bcB}	26.90^{aA}	13.37^{bB}	19.63^{abA}	17.23^{aA}	18.47^{abA}	10.97^{bA}
GR7	15.87^{bcdB}	24.57^{abA}	19.53^{aB}	17.70^{abB}	27.17^{aA}	16.13^{abAB}	20.57^{abAB}	11.57^{bB}
GR8	21.20^{bcA}	10.13^{cA}	15.63^{aA}	15.33^{bA}	20.27^{abA}	9.80^{abAB}	11.57^{bAB}	6.93^{bB}
GR9	6.70^{dB}	24.37^{abA}	21.27^{aA}	24.27^{abA}	16.40^{abA}	12.43^{abA}	16.67^{abA}	8.27^{bA}
GR10	25.33^{abA}	12.83^{bcA}	14.67^{aA}	24.53^{abA}	22.03^{abA}	18.83^{aA}	15.87^{abA}	9.80^{bA}
GR11	13.27^{bcdA}	21.87^{abcA}	16.20^{aA}	13.83^{bA}	11.90^{bA}	15.83^{abA}	15.63^{abA}	12.03^{bA}
GR12	13.90^{bcdA}	15.10^{bcA}	16.73^{aA}	21.70^{abA}	21.30^{abA}	12.63^{abA}	16.40^{abA}	12.13^{bA}
Control	3.20^{dB}	12.10^{bcAB}	14.23^{aAB}	19.30^{abA}	11.73^{bA}	9.30^{abA}	16.43^{abA}	6.93^{bA}
Significance	S				S			
CV (%)	33.9				39.3			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous for a period

4.1.1.19.1 Nitrogen

Among the media, M3 (vermicompost) recorded the highest uptake of nitrogen (246.32 mg plant^{-1}) which was significantly superior to all other media *viz.* M1 (86.22 mg plant^{-1}), M2 (42.79 mg plant^{-1}) and M4 (97.23 mg plant^{-1}) (Table 63).

Influence of growth regulators varied among the growth regulators without following a definite pattern. In the M3 medium, GR10 (BA 100 ppm) recorded the highest uptake of nitrogen (333.11 mg plant^{-1}) which was significantly superior to all other treatments except GR2, GR4, GR6, GR11, GR12 and control. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum uptake of nitrogen (173.70 mg plant^{-1}) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the maximum uptake of nitrogen (140.89 mg plant^{-1}) in M2 which was on par with all other treatments. In the medium M4 also, GR5 (IBA 300 ppm) had the maximum uptake of nitrogen (211.74 mg plant^{-1}) which was significantly superior to GR7, GR8, GR9, GR12 and control.

4.1.1.19.2 Phosphorous

Among the media, M3 (vermicompost) recorded the highest uptake of phosphorous (30.01 mg plant^{-1}) which was on par with M1 (14.54 mg plant^{-1}) and significantly superior to M2 (7.47 mg plant^{-1}) and M4 (11.00 mg plant^{-1}) (Table 63).

Effect of growth regulators varied among the media and a definite pattern of influence could not be observed. In the M3 medium, GR11 (BA 200 ppm) recorded the highest uptake of phosphorous (38.82 mg plant^{-1}) which was significantly superior to GR1, GR5, GR7, GR8 and GR9. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum uptake of phosphorous (41.53 mg plant^{-1}) which differed significantly with all other treatments except GR5. GR5 (IBA 300 ppm) recorded the maximum uptake of phosphorous (26.90 mg plant^{-1}) in M2 which was on par with all

other treatments except GR8 and control. In the medium M4, GR1 (IAA 150 ppm) had the maximum uptake of phosphorous (38.81 mg plant^{-1}) which was significantly superior to all the treatments except GR2, GR4 and GR5.

4.1.1.19.3 Potassium

M3 (vermicompost) recorded the highest uptake of potassium (245.24 mg plant^{-1}) among the media (Table 63) which was significantly superior to all other media *viz.,* M1 (50.98 mg plant^{-1}), M2 (43.08 mg plant^{-1}) and M4 (80.77 mg plant^{-1}).

Growth regulator treatments did not show a definite pattern of influence among the media in the uptake of potassium also. In the M3 medium, GR10 (BA 100 ppm) recorded the highest uptake of potassium (330.60 mg plant^{-1}) which was significantly superior to all other treatments except GR2, GR6, GR12 and control. GR6 (IBA 450 ppm) and GR5 (IBA 300 ppm) recorded the maximum uptake of potassium in M1 (110.63 mg plant^{-1}) and M2 (123.81 mg plant^{-1}) respectively which were on par with all other treatments. In the medium M4 GR1 (IAA 150 ppm) had the maximum uptake of potassium (195.27 mg plant^{-1}) which was significantly superior to GR7, GR8, GR9 and control.

4.1.2.19.4 Calcium

Among the media (Table 64), M3 (vermicompost) recorded the highest uptake of calcium (132.00 mg plant^{-1}) which was on par with all other media *viz.* M1 (96.92 mg plant^{-1}), M2 (48.03 mg plant^{-1}) and M4 (108.95 mg plant^{-1}).

With respect to the influence of growth regulators, in the medium M3, GR10 (BA 150 ppm) had the maximum uptake of calcium (294.33 mg plant^{-1}) which was significantly superior to all other treatments except GR2 and GR11. GR5 (IBA 300 ppm) recorded the maximum uptake of calcium in both M1 (145.41 mg plant^{-1}) and M2 (134.33 mg plant^{-1}) media which was on par with all other treatments. In the M4

medium, GR6 (IBA 450 ppm) recorded the highest uptake of calcium (364.25 mg plant^{-1}) which was significantly superior to all other treatments except GR5, and GR9.

4.1.2.19.5 Magnesium

Unlike uptake of other nutrients, interaction between media and growth regulators was found to be non significant. But there was significant difference among growth regulators and also among media.

Highest uptake of magnesium (29.81 mg plant^{-1}) was noted (Table 65) in M3 (vermicompost) which was on par with M4 (26.29 mg plant^{-1}). The lowest uptake was noted in M2 (16.87 mg plant^{-1}) which was on par with M1 (22.55 mg plant^{-1}).

Among the growth regulators (Table 66), GR5 (IBA 300 ppm) recorded the highest uptake (31.86 mg plant^{-1}) which was homogeneous with all other treatments except GR7, GR8 and GR9. The lowest value of 15.89 mg plant^{-1} was noted in GR7.

4.1.2.19.6 Iron

Similar to uptake of magnesium, interaction between media and growth regulators was found to be non significant. But significant difference was observed among the media and among the growth regulators.

Uptake of iron was highest (6.62 mg plant^{-1}) in M3 (vermicompost) which was on par with M4 (5.83 mg plant^{-1}) and both differed significantly with other two media (Table 65). The lowest value for uptake was recorded in M2 (3.28 mg plant^{-1}) which was homogeneous with M1 (4.04 mg plant^{-1}).

Table 63. Influence of media and growth regulators on uptake of N, P and K (mg per plant)

Growth regulators	Uptake at 12 months (mg plant^{-1})											
	N				P				K			
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4
GR1	154.00^{aA}	104.78^{aA}	105.06^{deA}	208.63^{abA}	23.47^{bcAB}	18.50^{abcB}	14.97^{cdeB}	38.81^{aA}	110.53^{aAB}	87.86^{aB}	113.43^{eAB}	195.27^{aA}
GR2	103.80^{aB}	114.44^{aB}	258.21^{abcA}	164.51^{abcAB}	16.66^{bcA}	26.55^{aA}	26.65^{abcdeA}	21.16^{abA}	68.12^{aB}	96.01^{aB}	239.92^{abcA}	132.77^{abcdB}
GR3	102.40^{aA}	125.85^{aA}	195.89^{bcdA}	147.39^{abcA}	12.77^{bcA}	25.03^{abA}	26.15^{abcdeA}	15.92^{bA}	72.15^{aB}	72.00^{aB}	169.35^{bcdeA}	149.67^{abcdAB}
GR4	91.00^{aB}	94.89^{aB}	245.54^{abcA}	149.62^{abcAB}	16.69^{bAc}	19.52^{abcA}	30.35^{abcdA}	23.31^{abA}	77.24^{aB}	76.17^{aB}	226.22^{bcdA}	115.38^{abcdB}
GR5	135.13^{aA}	140.89^{aA}	154.30^{cdeA}	211.74^{aA}	28.84^{abA}	26.90^{aA}	19.31^{bcdeA}	21.80^{abA}	82.63^{aB}	123.81^{aAB}	139.38^{deAB}	194.68^{aA}
GR6	173.70^{aAB}	67.66^{aB}	279.01^{abA}	183.22^{abA}	41.53^{aA}	11.44^{abcC}	32.74^{abcAB}	17.96^{bBC}	110.63^{aBC}	60.39^{aC}	251.71^{abA}	171.85^{abcAB}
GR7	110.43^{aA}	75.26^{aA}	87.75^{eA}	59.62^{cA}	18.69^{bcA}	9.28^{abcA}	13.89^{deA}	7.66^{bA}	78.65^{aA}	68.59^{aA}	92.88^{eA}	62.87^{dA}
GR8	85.54^{aA}	44.49^{aA}	89.15^{eA}	102.78^{bcA}	15.58^{bcA}	6.74^{cA}	10.70^{eA}	10.28^{bA}	52.48^{aA}	39.59^{aA}	106.17^{eA}	96.93^{bcdA}
GR9	73.06^{aA}	38.57^{aA}	84.74^{eA}	82.91^{cA}	10.43^{cA}	8.89^{abcA}	11.60^{eA}	8.59^{bA}	53.88^{aA}	40.53^{aA}	84.77^{eA}	88.87^{bcdA}
GR10	71.38^{aB}	84.80^{aB}	333.11^{aA}	159.27^{abcB}	13.81^{bcB}	21.35^{abcAB}	37.25^{abA}	18.46^{bB}	52.43^{aC}	72.04^{aC}	330.60^{aA}	179.28^{abB}
GR11	89.29^{aB}	66.29^{aB}	262.02^{abA}	116.79^{abcB}	14.98^{bcB}	19.37^{abcB}	38.82^{aA}	13.35^{bB}	84.38^{aB}	62.02^{aB}	230.53^{bcdA}	119.68^{abcdB}
GR12	90.13^{aB}	93.54^{aB}	251.94^{abcA}	99.69^{cB}	18.17^{bcAB}	13.52^{abcAB}	31.11^{abcdA}	12.05^{bB}	85.31^{aB}	95.65^{aB}	248.57^{abA}	119.22^{abcdB}
Control	86.22^{aB}	42.79^{aB}	246.32^{abcA}	97.23^{cB}	14.54^{bcAB}	7.47^{bcB}	30.01^{abcdA}	11.00^{bB}	50.98^{aB}	43.08^{aB}	245.24^{abA}	80.77^{cdB}
Significance	S				S				S			
CV (%)	38.91				44.87				38.4			

Means with same lower case letter as superscript within a column are homogeneous, means with same upper case letter as superscript within a row are homogeneous

Table 64. Influence of media and growth regulators on uptake of Ca and Cu (mg per plant)

Growth regulators	Uptake at 12 months (mg plant^{-1})							
	Ca				Cu			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	78.71^{aA}	72.38^{aA}	63.84^{dA}	143.87^{defA}	0.1114^{bcA}	0.0633^{aA}	0.1506^{cdA}	0.1566^{cdeA}
GR2	84.26^{aB}	74.65^{aB}	196.62^{abcA}	100.28^{efAB}	0.0704^{bcA}	0.1209^{aA}	0.1627^{bcdA}	0.1276^{deA}
GR3	105.95^{aA}	84.21^{aA}	103.75^{cdA}	81.20^{fA}	0.1244^{abcA}	0.0920^{aA}	0.1474^{cdA}	0.1063^{eA}
GR4	58.46^{aA}	87.25^{aA}	133.84^{cdA}	130.92^{defA}	0.0789^{bcC}	0.0913^{aC}	0.2759^{bcB}	0.4714^{aA}
GR5	145.41^{aB}	134.33^{aB}	90.66^{cdB}	278.79^{abA}	0.1076^{bcA}	0.0998^{aA}	0.1012^{dA}	0.1509^{cdeA}
GR6	91.57^{aBC}	50.30^{aC}	166.10^{bcdB}	364.25^{aA}	0.2668^{aB}	0.0505^{aC}	0.5869^{aA}	0.3566^{abB}
GR7	81.88^{aA}	48.27^{aA}	62.45^{dA}	85.90^{fA}	0.1000^{bcA}	0.0560^{aA}	0.0946^{dA}	0.0489^{eA}
GR8	86.04^{aB}	78.88^{aB}	91.21^{cdB}	201.98^{bcdeA}	0.0651^{cB}	0.0218^{aB}	0.1138^{dAB}	0.2546^{bcdA}
GR9	77.75^{aB}	42.64^{aB}	71.96^{dB}	260.71^{abcA}	0.1603^{abcB}	0.0280^{aB}	0.0487^{dB}	0.3542^{abA}
GR10	67.57^{aB}	70.28^{aB}	294.33^{aA}	218.86^{bcdA}	0.0666^{cB}	0.0560^{aB}	0.3050^{bA}	0.1031^{eB}
GR11	116.45^{aB}	37.88^{aB}	251.22^{abA}	166.83^{cdefB}	0.2665^{aA}	0.0621^{aB}	0.2717^{bcA}	0.2913^{bcA}
GR12	111.42^{aA}	120.37^{aA}	148.18^{bcdA}	84.16^{fA}	0.0693^{cA}	0.0743^{aA}	0.1793^{bcdA}	0.0583^{eA}
Control	96.92^{aA}	48.03^{aA}	132.00^{cdA}	108.95^{efA}	0.2161^{abcA}	0.0213^{aB}	0.1613^{bcdAB}	0.0671^{eB}
Significance	S				S			
CV (%)	43.16				48.15			

Means with same lower case letter as superscript within a column are homogeneous
Means with same upper case letter as superscript within a row are homogeneous

Table 65. Uptake of Mg and Fe for each medium averaged over all growth regulators (mg per plant)

Media	Mg	Fe
M1	22.55^{bc}	4.04^{b}
M2	16.87^{c}	3.28^{b}
M3	29.81^{a}	6.62^{a}
M4	26.29^{ab}	5.83^{a}
Significance	S	S

Table 66. Uptake of Mg and Fe for each growth regulator averaged over all media (mg per plant)

Growth regulators	Mg	Fe
GR1	24.73^{a}	5.78^{ab}
GR2	24.02^{abc}	5.48^{abc}
GR3	22.49^{abc}	5.35^{abcd}
GR4	26.43^{abc}	5.69^{abc}
GR5	31.86^{a}	6.59^{a}
GR6	27.34^{ab}	5.98^{ab}
GR7	15.89^{c}	3.20^{de}
GR8	19.38^{bc}	3.49^{cde}
GR9	18.93^{bc}	3.14^{e}
GR10	27.79^{ab}	4.96^{abcd}
GR11	25.85^{abc}	5.53^{abc}
GR12	21.82^{abc}	4.14^{bcde}
Significance	S	S

Means with same letter as superscript are homogeneous

GR5 (IBA 300 ppm) recorded the highest uptake (6.59 mg plant^{-1}) among growth regulators which was significantly superior to GR7, GR8, GR9 and control (Table 66). GR9 recorded the lowest value of 3.14 mg plant^{-1}.

4.1.2.19.7 Copper

Among the media (Table 64), M1 (well rotten cow dung) recorded the highest uptake of copper (0.2161 mg plant^{-1}) which was on par with M3 (0.1613 mg plant^{-1}) and significantly superior to M2 (0.0213 mg plant^{-1}) and M4 (0.0671 mg plant^{-1}).

With respect to effect of growth regulators, GR6 (IBA 450 ppm) recorded the highest uptake of copper in M1 (0.2668 mg plant^{-1}) and M3 (0.5869 mg plant^{-1}) media. In M1 it was significantly superior to all other treatments except GR3, GR9, GR11 and control while in M3 it was significantly superior to all other treatments. In the medium M2, GR2 (IAA 300 ppm) recorded the maximum uptake of copper (0.1209 mg plant^{-1}) which was on par with all other treatments. In the medium M4, GR4 (IBA 150 ppm) had the maximum uptake of copper (0.4714 mg plant^{-1}) which was significantly superior to all other treatments except GR6 and GR9.

4.1.2.19.8 Manganese

M3 (vermicompost) recorded the highest uptake of manganese (1.5528 mg plant^{-1}) among the media (Table 67) which was significantly superior to all other media *viz.* M1 (0.5492 mg plant^{-1}), M2 (0.2308 mg plant^{-1}) and M4 (0.6439 mg plant^{-1}).

Among the growth regulators, GR10 (BA 100 ppm) recorded the highest uptake of manganese (2.8973 mg plant^{-1}) in M3 which was significantly superior to all other treatments. In the medium M1, GR6 (IBA 450 ppm) recorded the maximum

uptake of manganese (0.8743 mg plant^{-1}) which was on par with all other treatments. GR5 (IBA 300 ppm) recorded the highest uptake of manganese (0.6667 mg plant^{-1}) in M2 which was homogeneous with all other treatments. In the medium M4, GR1 (IAA 150 ppm) showed the maximum uptake of manganese (2.0803 mg plant^{-1}) which was significantly superior to all other treatments.

4.1.2.19.9 Zinc

Among the media (Table 67), M3 (vermicompost) recorded the highest uptake of zinc (0.1629 mg plant^{-1}) which was on par with all other media *viz.* M1 (0.0860 mg plant^{-1}), M2 (0.0365 mg plant^{-1}) and M4 (0.0740 mg plant^{-1}).

With respect to effect of growth regulators, GR7 (GA 100 ppm) recorded the highest uptake of zinc in M3 (0.4130 mg plant^{-1}) which differed significantly with all other treatments except GR3, GR6, GR10, GR11 and GR12. In the M1 medium, GR6 (IBA 450 ppm) recorded the highest uptake of zinc (0.2564 mg plant^{-1}) which was on par with all other treatments. In the medium M2, GR11 (BA 200 ppm) recorded the maximum uptake of zinc (0.2755 mg plant^{-1}) which differed significantly with GR3, GR6, GR8, GR9 and control. GR5 (IBA 300 ppm) recorded the highest uptake of zinc (0.6758 mg plant^{-1}) in M4 which was significantly superior to all other treatments.

Table 67. Influence of media and growth regulators on uptake of Mn and Zn (mg per plant)

Growth regulators	Uptake at 12 months (mg plant^{-1})							
	Mn				Zn			
	M1	M2	M3	M4	M1	M2	M3	M4
GR1	0.7359^{aB}	0.5263^{aB}	0.6652^{efB}	2.0803^{aA}	0.1679^{aB}	0.1483^{abB}	0.1151^{cdB}	0.4496^{bA}
GR2	0.5114^{aB}	0.5360^{aB}	1.1254^{cdeA}	1.2646^{bcA}	0.1163^{aA}	0.1780^{abA}	0.1988^{bcdA}	0.1679^{cA}
GR3	0.6247^{aB}	0.4528^{aB}	0.8378^{defAB}	1.3234^{bA}	0.1090^{aB}	0.0772^{bB}	0.3898^{aA}	0.1594^{cB}
GR4	0.4356^{aB}	0.3267^{aB}	1.4083^{bcdA}	0.8189^{bcdeAB}	0.1065^{aA}	0.1165^{abA}	0.2053^{bcdA}	0.2411^{cA}
GR5	0.6160^{aB}	0.6667^{aB}	0.7184^{efAB}	1.3076^{bA}	0.1448^{aB}	0.1148^{abB}	0.1625^{cB}	0.6758^{aA}
GR6	0.8743^{aB}	0.2188^{aC}	1.9474^{bA}	1.3665^{bAB}	0.2564^{aAB}	0.0848^{bB}	0.3608^{abA}	0.1490^{cB}
GR7	0.6028^{aA}	0.3197^{aA}	0.6583^{efA}	0.4290^{eA}	0.2005^{aB}	0.0920^{abB}	0.4130^{aA}	0.0598^{cB}
GR8	0.3057^{aA}	0.2180^{aA}	0.5737^{efA}	0.5422^{deA}	0.1178^{aA}	0.0395^{bA}	0.0934^{dA}	0.0648^{cA}
GR9	0.3766^{aA}	0.1833^{aA}	0.4244^{fA}	0.8232^{bcdeA}	0.1112^{aA}	0.0531^{bA}	0.0996^{dA}	0.0727^{cA}
GR10	0.6591^{aC}	0.2780^{aC}	2.8973^{aA}	1.1530^{bcdB}	0.1370^{aB}	0.1046^{abB}	0.3683^{abA}	0.1141^{cB}
GR11	0.5543^{aB}	0.4104^{aB}	1.6866^{bcA}	0.7545^{bcdeB}	0.1342^{aB}	0.2755^{aAB}	0.3316^{abcA}	0.1297^{cB}
GR12	0.4020^{aB}	0.3694^{aB}	1.1966^{cdeA}	0.5349^{deB}	0.1634^{aA}	0.1016^{abA}	0.2696^{abcdA}	0.1040^{cA}
Control	0.5492^{aB}	0.2308^{aB}	1.5528^{bcA}	0.6439^{cdeB}	0.0860^{aA}	0.0365^{bA}	0.1629^{cdA}	0.0740^{cA}
Significance	S				S			
CV (%)	38.31				52.22			

Means with same small letter within a column are homogenous within a period
Means with same capital letter within a row are homogenous within a period

4.1.2 *Effect of growth promoting substances*

4.1.2.1 Plant, leaf and root characters

Observations on various growth parameters are presented in Tables 68 to 78.

4.1.2.1.1 Plant height

Table 68 shows the influence of various growth promoting substances on plant height. Up to third month, no significant difference in plant height was observed among the treatments. Mean height of the plants differed significantly at sixth, ninth, twelfth and fifteenth month stages.

During the sixth month, plants under treatment B11 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm] had significantly higher mean height (14.80 cm). This was on par with the plants under treatment B1, B2, B7, B8, B9, B10, B12, B13 and B14. However, it was significantly different from the control (B15) and treatments B3, B4, B5 and B6. During this period plants under treatment B5 had the lowest mean height (9.36 cm).

During ninth month, treatment B11 had significantly higher plant height (23.18 cm) which was on par with the treatments B13 and B14. All other treatments including control were homogeneous and were significantly lower than the plant height under treatment B11, B13 and B14. During this period also plants under treatment B5 had the lowest mean height (10.52 cm).

During 12^{th} and 15^{th} months, plants under treatment B11 had highest mean height of 29.96 cm and 37.04 cm respectively and plants under control treatment had the lowest (12.34 and 13.62 cm) plant height (Fig. 7). During these two months, B11 was significantly superior to all other treatments except B14.

B11 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm] recorded maximum plant height during the entire period of study.

Table 68. Influence of growth promoting substances on plant height (cm)

Treatments	3 months[@]	6 months	9 months	12 months	15 months
B1	11.38	12.24^{abcd}	13.00 ^{b}	15.84^{de}	19.30^{cd}
B2	11.76	12.42^{abcd}	13.30 ^{b}	17.96^{cde}	23.02^{bcd}
B3	10.72	11.20^{bcd}	11.74 ^{b}	15.54^{de}	20.72^{cd}
B4	10.46	11.20^{bcd}	11.64 ^{b}	13.78^{de}	17.70^{cd}
B5	8.02	9.36^{d}	10.52 ^{b}	14.20^{de}	18.80^{cd}
B6	9.30	10.24^{cd}	10.70 ^{b}	13.22^{de}	17.66^{cd}
B7	10.44	11.40^{abcd}	12.76 ^{b}	14.38^{de}	17.24^{cd}
B8	11.88	13.10^{abc}	14.16 ^{b}	17.46^{cde}	22.64^{bcd}
B9	11.00	11.36^{abcd}	12.90 ^{b}	19.18^{cd}	26.06^{bc}
B10	12.06	13.10^{abc}	13.80 ^{b}	15.40^{de}	18.38^{cd}
B11	10.42	14.80^{a}	23.18^{a}	29.96^{a}	37.04^{a}
B12	11.50	12.26^{abcd}	14.04 ^{b}	16.58^{cde}	20.52^{cd}
B13	10.62	14.04^{ab}	21.60^{a}	22.92^{bc}	25.00^{bc}
B14	12.22	14.22^{ab}	22.94^{a}	26.14^{ab}	30.02^{ab}
B15	9.94	10.70^{bcd}	11.10^{b}	12.34^{e}	13.62^{d}
Significance	NS	S	S	S	S
CV (%)	18.1	19.7	18.9	25.5	29.0

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

(Treatment details: B1 – *Pseudomonas* sp. (2 %), B2 – *Azospirillum* sp. (10 g per plant), B3 – AMF (10 g per plant), B4 – Fresh cow dung solution (3g/ l), B5 – Cow's urine (25 times dilution with water), B6 – Vermiwash (5 times dilution), B7 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 %, B8 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %, B9 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm, B10 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + BA 100 ppm, B11 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm, B12 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm, B13 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm + BA 100 ppm, B14 – Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm, B15 – Control)

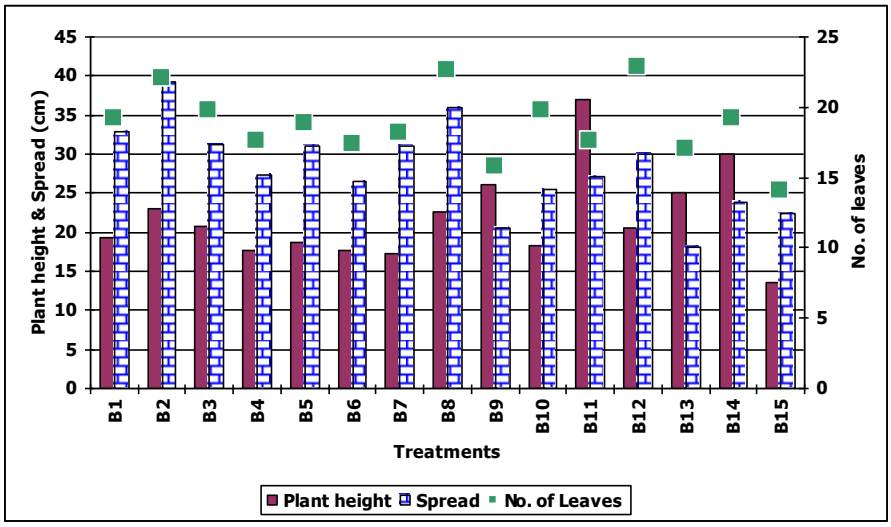


Fig. 7. Effect of growth promoting substances on growth parameters of mangosteen seedlings at 15th month

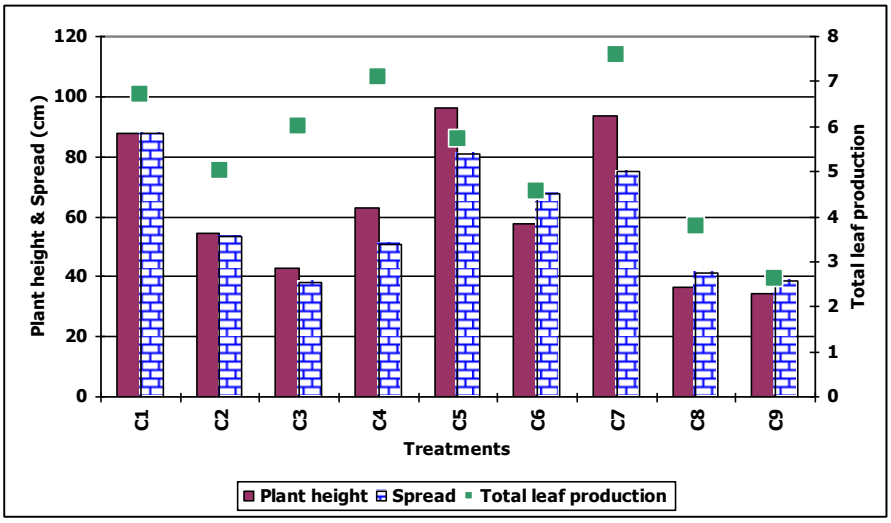


Fig. 8. Effect of growth regulators on growth parameters of two year old mangosteen grafts at 12th month

4.1.2.1.2 Plant spread

The treatments did not record significant difference with respect to plant spread up to ninth month (Table 69). During 12th month, maximum spread (35.24 cm) was recorded in treatment B2 (*Azospirillum* sp. - 10 g per plant) which was on par with B1, B3, B5, B7, B8 and B12. All other treatments including control treatment were significantly different from B2.

During 15th month also treatment B2 recorded maximum spread (39.32 cm) which was found to be statistically on par with B1, B3, B5, B7 and B8 but was significantly different from other treatments including control. Treatment B13 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm + BA 100 ppm] recorded the lowest mean spread during the entire period of study.

4.1.2.1.3 Number of leaves

Up to sixth month, no significant difference with respect to number of leaves was observed among the treatments (Table 69). During ninth month, significant difference in number of leaves per plant was observed among the treatments. The treatment B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded the maximum number of leaves per plant (16.40) which was on par with all other treatments except B1, B4, B5, B6, B9, and B15. At this stage, B9 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm] had the lowest (10.20) number of leaves per plant.

B12 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm] recorded the maximum number of leaves per plant at twelve (19.20) and fifteen (22.80) month stage and was on par with B1, B2, B3, B8, B10 and B14 at both the stages in addition to B11 at 12 month stage. The control treatment (B15) had the lowest number of 12.20 and 14.00 leaves respectively at these two stages.

Table 69. Influence of growth promoting substances on plant spread (cm) and number of leaves

Treatments	Plant spread (cm)					Number of leaves				
	3 months@	6 months	9 months	12 months	15 months	3 months@	6 months	9 months	12 months	15 months
B1	18.76	19.58	21.38	29.54^{abc}	32.80^{abc}	11.00	12.20	12.80^{bcde}	16.20^{abcd}	19.20^{abcd}
B2	19.12	19.74	22.36	35.24^{a}	39.32^{a}	11.20	12.60	13.80^{abcd}	17.40^{abc}	22.00^{abc}
B3	20.30	21.18	21.80	27.46^{abcd}	31.28^{abcd}	10.80	12.80	13.80^{abcd}	16.20^{abcd}	19.80^{abcd}
B4	17.50	20.06	18.40	24.30^{bcde}	27.42^{bcde}	9.40	10.40	11.40^{de}	14.80^{cde}	17.60^{de}
B5	18.20	18.70	19.34	27.60^{abcd}	30.96^{abcd}	9.40	10.40	11.40^{de}	14.60^{cde}	18.80^{bcd}
B6	21.28	21.66	21.66	23.78^{bcde}	26.58^{cdef}	10.60	11.60	12.00^{cde}	14.20^{cde}	17.40^{de}
B7	20.60	21.64	21.68	27.16^{abcd}	31.06^{abcd}	11.20	13.00	13.60^{abcd}	15.20^{cde}	18.20^{cd}
B8	20.00	20.80	23.50	31.96^{ab}	35.88^{ab}	11.00	13.60	16.40^{a}	19.00^{ab}	22.60^{ab}
B9	18.30	18.36	18.36	19.84^{de}	20.48^{ef}	11.20	11.20	10.20^{e}	13.60^{de}	15.80^{de}
B10	17.84	19.18	19.28	22.98^{bcde}	25.42^{cdef}	11.40	13.40	14.60^{abcd}	16.80^{abcd}	19.80^{abcd}
B11	20.90	21.00	22.62	25.22^{bcde}	27.18^{bcde}	11.00	12.80	14.80^{abc}	15.80^{abcde}	17.60^{de}
B12	18.34	19.92	21.96	26.76^{abcde}	30.08^{bcd}	10.60	12.20	16.00^{ab}	19.20^{a}	22.80^{a}
B13	17.40	17.66	17.68	17.76^{e}	18.02^{ef}	10.80	12.80	15.80^{ab}	15.40^{bcde}	17.00^{de}
B14	23.20	23.92	23.92	23.92^{bcde}	23.92^{cdef}	11.60	12.80	15.60^{ab}	17.20^{abcd}	19.20^{abcd}
B15	19.54	19.84	19.84	22.32^{cde}	22.50^{def}	11.00	12.20	11.60^{de}	12.20^{e}	14.00^{e}
Significance	NS	NS	NS	S	S	NS	NS	S	S	S
CV (%)	17.5	17.4	17.7	23.8	23.2	13.9	14.6	15.9	15.7	14.3

@ Experiment was initiated with six month old polybag plants, means with same letter as superscript are homogeneous

4.1.2.1.4 Total leaf production and senescence

Influence of growth promoting substances on total leaf production and senescence is presented in Table 70.

During the initial period to 15 months, highest total leaf production was observed in B12 (14.40) [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm] which was significantly different from B1, B4, B6, B7, B9, B13 and B15 (control). The lowest leaf production was noted in control treatment (6.00) which was on par with only B6, B7 and B9. During the same period, the minimum leaf senescence was also recorded in B12 (1.60) which was homogeneous with all other treatments except B9, B11 and B14. The maximum leaf senescence (4.00) was noted in B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm].

4.1.2.1.5 Leaf length

No significant difference was noticed with respect to leaf length up to six months (Table 71). During ninth, twelfth and fifteenth month stages, significant difference among treatments was noticed. The treatment B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded the maximum value of 10.81 cm at nine month stage which was on par with all other treatments except B9, B11, B13 and B14. At twelve month stage, B2 had the maximum leaf length (16.10 cm) and was significantly different from B4, B9, B10, B11, B13, B14 and the control treatment (B15). B8 had the highest leaf length (19.01 cm) and was on par with B1, B2, B3, B5, B6, B7 and B12 at fifteenth month. The treatment B13 recorded the minimum value of 4.72 cm at ninth month while B13 had the lowest value of 5.24 cm and 5.62 cm at twelfth and fifteenth months respectively.

Table 70. Influence of growth promoting substances on leaf production and senescence

Treatments	Total leaf production (initial to 15 months)[@]	Leaf senescence (initial to 15 months)
B1	10.4^{bc}	2.2^{bcd}
B2	12.4^{abc}	1.8^{cd}
B3	11.2^{abc}	2.2^{bcd}
B4	10.0^{bc}	1.8^{cd}
B5	11.2^{abc}	2.0^{cd}
B6	8.8^{cd}	2.0^{cd}
B7	8.8^{cd}	2.2^{bcd}
B8	13.6^{ab}	2.4^{abcd}
B9	8.4^{cd}	3.4^{abc}
B10	11.6^{abc}	2.0^{cd}
B11	11.2^{abc}	3.8^{ab}
B12	14.4^{a}	1.6^{d}
B13	10.0^{bc}	2.6^{abcd}
B14	11.6^{abc}	4.0^{a}
B15	6.0^{d}	3.0^{abcd}
Significance	S	S
CV (%)	12.4	17.0

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 71. Influence of growth promoting substances on leaf length and breadth (cm)

Treatments	Leaf length (cm)					Leaf breadth (cm)				
	3 months	6 months	9 months	12 months	15 months	3 months	6 months	9 months	12 months	15 months
B1	9.07	9.24	10.00^{a}	13.98^{abc}	16.25^{ab}	2.88	2.89	3.30^{a}	4.59^{abc}	5.07^{abc}
B2	9.49	9.86	10.11^{a}	16.10^{a}	18.35^{a}	3.16	3.23	3.39^{a}	5.53^{a}	5.90^{a}
B3	9.48	10.07	10.30^{a}	13.09^{abc}	15.52^{abc}	2.93	3.12	3.27^{a}	4.08^{bcd}	4.52^{abcd}
B4	8.64	9.38	9.77^{a}	10.82^{bcde}	12.39^{bcde}	3.08	3.23	3.37^{a}	3.89^{bcd}	4.11^{cd}
B5	8.06	8.56	9.48^{ab}	13.12^{abc}	14.70^{abcd}	2.90	2.94	3.08^{ab}	4.52^{abcd}	4.87^{abc}
B6	9.58	9.90	10.00^{a}	12.16^{abcd}	14.18^{abcd}	3.03	3.22	3.28^{a}	4.10^{bcd}	4.54^{abcd}
B7	9.39	9.74	10.66^{a}	12.63^{abcd}	15.22^{abc}	2.92	3.23	3.54^{a}	4.24^{bcd}	4.78^{abc}
B8	9.16	9.65	10.81^{a}	14.91^{ab}	19.01^{a}	3.01	3.34	3.68^{a}	4.99^{ab}	5.60^{ab}
B9	8.68	8.77	7.52^{bcd}	7.49^{ef}	7.51^{ef}	2.88	2.91	2.60^{b}	2.34^{ef}	2.34^{ef}
B10	8.41	9.02	9.38^{ab}	10.58^{cde}	11.31^{bcde}	3.00	3.11	3.19^{ab}	3.47^{cdef}	3.66^{cde}
B11	9.39	10.21	7.23^{cd}	8.40^{def}	9.66^{def}	3.07	2.94	1.90^{c}	2.23^{ef}	2.38^{ef}
B12	8.86	9.31	10.62^{a}	12.49^{abcd}	14.32^{abcd}	2.95	3.20	3.35^{a}	3.91^{bcd}	4.30^{bcd}
B13	8.17	8.48	4.72^{e}	5.24^{f}	5.62^{f}	2.79	2.70	1.18^{d}	1.56^{f}	1.62^{f}
B14	9.90	10.15	6.54^{de}	5.78^{f}	5.84^{f}	3.28	3.40	1.47^{cd}	1.29^{f}	1.31^{f}
B15	8.88	8.90	8.82^{abc}	9.97^{cde}	10.50^{cdef}	2.83	2.97	3.04^{ab}	3.22^{de}	3.33^{de}
Significance	NS	NS	S	S	S	NS	NS	S	S	S
CV (%)	15.0	15.9	17.1	26.3	28.6	12.3	12.4	15.8	25.1	25.2

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

4.1.2.1.6 Leaf breadth

Data regarding influence of different treatments on leaf breadth are presented in Table 71. No significant difference in leaf breadth was observed in all treatments up to six month stage.

At nine month stage B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded maximum leaf breadth (3.68 cm) which was on par with all other treatments except B9, B11, B13 and B14. At this stage, the lowest value of 1.18 cm was observed in B13.

B2 (*Azospirillum* sp. - 10 g per plant) showed the maximum leaf breadth of 5.53 cm and 5.90 cm respectively at twelve and fifteen month stage while B14 had the lowest value of 1.29 cm and 1.31 cm respectively at these two stages. The treatments B1, B5 and B8 were on par with B2 at twelve month stage while in addition to these treatments, B3, B6 and B7 were also statistically on par with B2 at fifteen months.

4.1.2.1.7 Leaf area

No significant difference in mean leaf area was observed among the treatments up to six month stage (Table 72). During ninth, twelfth and fifteenth month stages, significant difference among treatments was noticed.

At nine month stage, B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded maximum mean leaf area (24.95 cm^2) which was on par with all other treatments except B9 (12.24 cm^2), B11 (9.38 cm^2), B13 (3.55 cm^2), B14 (6.15 cm^2) and B15 (16.67 cm^2). B2 (*Azospirillum* sp. - 10 g per plant) had the maximum mean leaf area both at twelve (56.84 cm^2) and fifteen (68.57 cm^2) months stages while B13 and B14 had the minimum mean leaf area respectively at twelve (5.29 cm^2) and fifteen (5.50 cm^2) month stages. At twelve month stage, B2 was statistically on par with B1, B5 and B8 while in addition to these, B3 and B7 were also homogenous with B2 at fifteen month stage.

Table 72. Influence of growth promoting substances on mean leaf area and total leaf area (cm^2)

Treatments	Mean leaf area (cm^2)					Total leaf area (cm^2)				
	3 months	6 months	9 months	12 months	15 months	3 months	6 months	9 months	12 months	15 months
B1	16.36	16.69	20.79^{ab}	42.46^{abc}	55.50^{ab}	180.32	204.65	267.87^{bcd}	721.38^{abc}	1119.83^{abc}
B2	19.01	20.18	21.61^{ab}	56.84^{a}	68.57^{a}	212.06	255.63	303.50^{abcd}	986.36^{a}	1510.61^{ab}
B3	17.37	19.53	21.10^{ab}	34.75^{bcd}	45.96^{abc}	188.55	249.75	293.38^{abcd}	595.57^{abcd}	971.23^{abcd}
B4	16.62	18.95	20.55^{ab}	26.51^{cde}	32.55^{bcde}	157.41	197.56	231.48^{cdef}	396.23^{cdef}	584.50^{cde}
B5	14.95	16.05	18.79^{abc}	37.98^{abcd}	46.03^{abc}	146.08	171.28	218.36^{cdefg}	566.06^{bcde}	884.32^{bcd}
B6	18.01	19.85	20.44^{ab}	30.99^{bcde}	40.09^{bcd}	192.01	232.52	250.19^{bcde}	442.06^{cdef}	701.48^{cde}
B7	17.21	19.71	23.62^{ab}	34.94^{bcd}	48.38^{abc}	195.31	257.53	327.78^{abc}	561.52^{bcde}	945.26^{abcd}
B8	17.30	20.36	24.95^{a}	48.73^{ab}	67.88^{a}	195.17	284.09	411.40^{a}	968.83^{ab}	1566.80^{a}
B9	15.52	15.89	12.24^{cd}	11.49^{ef}	11.61^{e}	172.94	176.92	119.55^{fgh}	152.57^{ef}	182.06^{e}
B10	15.88	17.46	18.60^{abc}	23.30^{cdef}	26.60^{cde}	187.72	238.22	271.72^{bcd}	401.67^{cdef}	541.11^{cde}
B11	18.27	19.36	9.38^{de}	13.24^{ef}	16.89^{de}	206.61	247.78	140.43^{efgh}	210.26^{def}	296.55^{de}
B12	16.41	18.82	22.51^{ab}	31.79^{bcde}	40.44^{bcd}	174.39	230.80	359.86^{ab}	622.04^{abcd}	947.56^{abcd}
B13	14.24	14.23	3.55^{e}	5.29^{f}	6.11^{e}	157.19	180.01	54.82^{h}	80.50^{f}	102.53^{e}
B14	20.33	21.58	6.15^{de}	5.35^{f}	5.50^{e}	238.97	283.23	96.37^{gh}	92.71^{f}	109.66^{e}
B15	15.68	16.53	16.67^{bc}	20.30^{def}	22.21^{cde}	174.45	204.62	195.30^{defg}	256.24^{def}	318.70^{de}
Significance	NS	NS	S	S	S	NS	NS	S	S	S
CV (%)	12.3	12.7	14.5	23.9	25.3	17.1	16.8	18.6	29.9	30.9

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

4.1.2.1.8 Total leaf area

No significant difference was noticed with respect to total leaf area up to six months (Table 72). B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded the maximum total leaf area at nine (411.40 cm^2), twelve (968.83 cm^2) and fifteen (1566.80 cm^2) month stages while B13 had the minimum values of 54.82 cm^2, 80.50 cm^2 and 102.53 cm^2 respectively at these stages.

At nine month stage, B8 was on par with B2, B3, B7 and B12 while B1, B2, B3 and B12 were statistically homogenous with B8 at twelve month stages. At fifteen month stage, B8 was on par with B1, B2, B3, B7 and B12. At all these stages, B8 significantly differed with control (B15).

4.1.2.1.9 Petiole length

Data pertaining to the influence of different treatments on petiole length are presented in Table 73. The treatments showed significant difference in petiole length at three, six, twelve and fifteen month stages while the treatments were homogeneous at nine month stage.

B2 (*Azospirillum* sp. -10 g per plant) showed maximum petiole length at three (0.50 cm), six (0.51 cm) and nine (0.53 cm) month stages, while B3 (AMF - 10 g per plant) (0.68 cm) and B8 (0.70 cm) had the highest values for petiole length respectively at twelve and fifteen month stages. The control treatment (B15) recorded the lowest values of 0.33 cm and 0.34 cm respectively at three and six month stages while B13 showed the lowest petiole length both at twelve (0.44 cm) and fifteen (0.42) months. At nine month stage B14 showed the lowest value of 0.41 cm though it was on par with other treatments.

Table 73. Influence of growth promoting substances on petiole length (cm) and internodal length (cm)

Treatments	Petiole length (cm)					Internodal length (cm)				
	3 months	6 months	9 months	12 months	15 months	3 months	6 months	9 months	12 months	15 months
B1	0.45^{abc}	0.49^{ab}	0.50	0.58^{abcd}	0.58^{abcd}	1.21	1.22^{bc}	1.23^{c}	1.32^{cd}	1.42^{cd}
B2	0.50^{a}	0.51^{a}	0.53	0.64^{ab}	0.67^{ab}	1.05	1.06^{bcd}	1.09^{cd}	1.29^{cd}	1.48^{cd}
B3	0.46^{ab}	0.47^{abc}	0.52	0.68^{a}	0.69^{a}	1.14	1.17^{bcd}	1.18^{c}	1.31^{cd}	1.48^{cd}
B4	0.37^{cd}	0.41^{bcde}	0.48	0.51^{bcd}	0.52^{abcd}	1.17	1.19^{bcd}	1.20^{c}	1.21^{cd}	1.36^{cd}
B5	0.40^{bcd}	0.47^{abc}	0.48	0.59^{abc}	0.61^{abcd}	1.05	1.05^{bcd}	1.06^{cd}	1.10^{cd}	1.27^{d}
B6	0.37^{cd}	0.43^{abcde}	0.51	0.56^{abcd}	0.56^{abcd}	1.13	1.13^{bcd}	1.14^{cd}	1.14^{cd}	1.30^{cd}
B7	0.43^{abc}	0.43^{abcde}	0.45	0.53^{abcd}	0.53^{abcd}	1.09	1.10^{bcd}	1.10^{cd}	1.18^{cd}	1.32^{cd}
B8	0.43^{abc}	0.44^{abcde}	0.48	0.63^{ab}	0.70^{a}	0.84	0.84^{cd}	0.86^{cd}	0.86^{d}	1.10^{d}
B9	0.41^{abcd}	0.44^{abcde}	0.46	0.45^{cd}	0.46^{cd}	1.02	1.04^{bcd}	1.17^{c}	1.77^{bc}	2.08^{bc}
B10	0.33^{d}	0.38^{cdef}	0.43	0.50^{bcd}	0.52^{abcd}	1.09	1.09^{bcd}	1.12^{cd}	1.14^{cd}	1.26^{d}
B11	0.43^{abc}	0.41^{bcde}	0.48	0.54^{abcd}	0.59^{abcd}	1.32	1.81^{a}	2.68^{a}	3.24^{a}	3.56^{a}
B12	0.41^{abcd}	0.44^{abcde}	0.50	0.58^{abcd}	0.64^{abc}	1.25	1.28^{bc}	1.28^{c}	1.34^{cd}	1.45^{cd}
B13	0.40^{bcd}	0.35^{de}	0.46	0.44^{d}	0.42^{d}	1.05	1.29^{bc}	1.95^{b}	2.01^{b}	2.06^{bc}
B14	0.42^{abcd}	0.36^{de}	0.41	0.46^{cd}	0.48^{bcd}	1.34	1.35^{b}	2.01^{b}	2.23^{b}	2.33^{b}
B15	0.33^{d}	0.34^{e}	0.44	0.46^{cd}	0.46^{cd}	0.78	0.74^{d}	0.62^{d}	0.71^{d}	0.78^{d}
Significance	S	S	NS	S	S	NS	S	S	S	S
CV (%)	14.8	15.2	16.3	18.9	22.8	27.4	27.1	27.0	30.2	33.0

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

4.1.2.1.10 Internodal length

The treatments showed significant difference in intermodal length during the entire period of study except in the initial three months period (Table 73). B11 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm] recorded the maximum intermodal length at six (1.81 cm), nine (2.68 cm), twelve (3.24 cm) and fifteen (3.56 cm) month stages and it was significantly different from all other treatments during all these stages. In the same way, B15 (control) had the lowest intermodal length during the entire period though it was not significantly different from other treatments.

4.1.2.1.11 Number of branches

All the treatments did not produce any branches during the entire period of study.

4.1.2.1.12 Leaf thickness

During the entire period of study, treatments did not show any significant difference in leaf thickness (Table 74). The leaf thickness ranged from 0.254 mm (B3 at three month stage) to 0.312 mm (B11 and B12 both at nine month stage).

4.1.2.1.13 Leaf duration

On emergence of new leaves, these leaves were tagged to find out the leaf duration (period from emergence to senescence). These newly produced leaves did not fall and remained on the plant during the entire experimental period of fifteen months. Hence duration of leaves could not be ascertained.

4.1.2.1.14 Colour development of leaf at different stages

Young emerging leaves of mangosteen seedlings are purple, light brick red or pink in colour. Later they turn light green and to dark green on maturity. No variation was observed among treatments in leaf colour development.

Table 74. Influence of growth promoting substances on leaf thickness (mm)

Treatments	3 months[@]	6 months	9 months	12 months	15 months
B1	0.280	0.294	0.288	0.283	0.290
B2	0.273	0.288	0.296	0.287	0.297
B3	0.254	0.267	0.280	0.271	0.282
B4	0.283	0.290	0.272	0.268	0.274
B5	0.277	0.286	0.288	0.275	0.287
B6	0.278	0.282	0.294	0.278	0.286
B7	0.286	0.289	0.281	0.282	0.286
B8	0.279	0.276	0.299	0.293	0.298
B9	0.288	0.294	0.292	0.285	0.291
B10	0.272	0.280	0.300	0.297	0.301
B11	0.279	0.283	0.312	0.302	0.308
B12	0.280	0.288	0.312	0.306	0.310
B13	0.277	0.285	0.286	0.280	0.286
B14	0.289	0.298	0.293	0.281	0.288
B15	0.290	0.290	0.280	0.259	0.267
Significance	NS	NS	NS	NS	NS
CV (%)	5.8	6.6	9.0	9.2	8.8

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

4.1.2.1.15 Fresh and dry weight of plant parts

4.1.2.1.15.1 Fresh weight

The treatments showed significant difference in fresh weight of whole plant, shoot and leaf at twelve month stage only (Table 75). In the case of fresh weight of root, treatments differed significantly both at six and twelve month stages.

Fresh weight of whole plant ranged from 5.97 g (B11) to 9.64 g (B3) at six month stage and at twelve month stage the highest value (38.20 g) was noted in B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] which differed significantly with all other treatments. In the case of fresh weight of shoot, the values varied from 4.22 g (B14) to 7.26 g (B2) at six months and at twelve months the highest value of 32.41 g was recorded again by B8 which also differed significantly with all other treatments. The lowest leaf fresh weight was noticed in B11 (2.35 g) at six months and the highest value by B7 (4.59 g) at the same period. B8 with a leaf fresh weight of 23.25 g was superior to all other treatments at twelve month stage.

The treatments showed significant difference in root fresh weight at six month stage and the highest root fresh weight (3.14 g) was recorded in B5 [Cow's urine (25 times dilution with water)] which was on par with B1, B2, B3, B6 and B7. At twelve months, B8 was significantly superior to all other treatments with a root fresh weight of 5.78 g.

4.1.2.1.15.2 Dry weight

At six month stage, as in the case of fresh weight of plant parts, treatments were homogeneous with respect to dry weight also except in the case of root dry weight which differed significantly among treatments (Table 76). At twelve months, the treatments showed significant difference with respect to all the parameters.

Table 75. Influence of growth promoting substances on fresh weight of plant parts (g)

Treatments	Shoot		Root		Whole plant		Leaf	
	6 months@	12 months	6 months	12 months	6 months	12 months	6 months	12 months
B1	5.96	18.33^{cdef}	2.09^{abc}	3.61^{bc}	8.05	21.94^{bcd}	3.89	12.96^{bc}
B2	7.26	22.53^{b}	2.03^{abc}	2.90^{cde}	9.29	25.43^{b}	3.92	15.25^{b}
B3	6.94	20.68^{bc}	2.70^{ab}	3.90^{b}	9.64	24.59^{b}	4.17	13.44^{bc}
B4	5.36	19.40^{bcde}	1.75^{bc}	3.33^{bcd}	7.11	22.72^{bc}	3.49	13.00^{bc}
B5	6.38	15.23^{f}	3.14^{a}	2.83^{cdef}	9.51	18.07^{d}	3.91	10.15^{cd}
B6	5.00	15.35^{ef}	2.69^{ab}	2.60^{def}	7.68	17.95^{d}	3.08	10.37^{cd}
B7	7.39	20.43^{bc}	1.94^{abc}	2.76^{cdef}	9.33	23.19^{bc}	4.59	13.95^{bc}
B8	5.96	32.41^{a}	1.90^{bc}	5.78^{a}	7.86	38.20^{a}	4.16	23.25^{a}
B9	4.24	15.67^{ef}	1.73^{bc}	2.13^{efg}	5.98	17.80^{d}	2.69	8.37^{de}
B10	6.58	17.57^{cdef}	1.88^{bc}	2.37^{efg}	8.46	19.94^{cd}	4.38	10.70^{cd}
B11	4.98	16.34^{def}	0.98^{c}	1.55^{g}	5.97	17.89^{d}	2.35	7.27^{def}
B12	5.46	19.77^{bcd}	1.45^{bc}	1.97^{fg}	6.91	21.74^{bcd}	3.85	11.00^{bcd}
B13	5.87	10.46^{g}	0.92^{c}	1.50^{g}	6.79	11.96^{e}	3.21	4.05^{f}
B14	4.22	8.28^{g}	1.71^{bc}	1.98^{fg}	5.93	10.27^{e}	2.61	3.14^{f}
B15	6.21	9.00^{g}	1.69^{bc}	1.54^{g}	7.90	10.54^{e}	3.56	4.46^{ef}
Significance	NS	S	S	S	NS	S	NS	S
CV (%)	36.3	52.8	38.8	52.9	38.5	54.3	33.0	55.4

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 76. Influence of growth promoting substances on dry weight of plant parts (g)

Treatments	Shoot		Root		Whole plant		Leaf	
	6 months[@]	12 months	6 months	12 months	6 months	12 months	6 months	12 months
B1	2.20	7.47^{c}	0.70^{ab}	1.87^{b}	2.90	9.34^{bc}	1.28	5.34^{bc}
B2	2.24	9.49^{b}	0.70^{ab}	1.33^{bcd}	2.94	10.81^{b}	1.16	6.38^{ab}
B3	2.24	8.83^{bc}	1.00^{ab}	1.69^{bc}	3.24	10.52^{b}	1.23	5.47^{bc}
B4	2.10	8.23^{bc}	0.58^{bc}	1.44^{bcd}	2.68	9.67^{bc}	1.26	5.10^{bcd}
B5	2.22	5.55^{de}	1.09^{a}	1.42^{bcd}	3.31	6.97^{def}	1.27	3.99^{cd}
B6	1.99	5.40^{de}	0.89^{ab}	1.46^{bcd}	2.88	6.86^{def}	1.24	3.42^{de}
B7	2.54	7.95^{bc}	0.65^{ab}	1.68^{bc}	3.19	9.63^{bc}	1.43	5.00^{bcd}
B8	2.08	12.06^{a}	0.57^{bc}	2.50^{a}	2.65	14.56^{a}	1.31	7.49^{a}
B9	1.55	6.87^{cd}	0.71^{ab}	1.09^{cd}	2.26	7.96^{cde}	0.89	3.67^{cd}
B10	2.14	7.42^{c}	0.53^{bc}	1.16^{bcd}	2.67	8.58^{bcd}	1.33	4.38^{cd}
B11	1.81	7.59^{bc}	0.32^{c}	1.13^{cd}	2.12	8.71^{bcd}	0.74	3.37^{de}
B12	1.70	8.46^{bc}	0.34^{c}	1.12^{cd}	2.04	9.57^{bc}	1.18	4.54^{bcd}
B13	1.96	4.56^{ef}	0.31^{c}	0.92^{d}	2.27	5.48^{fg}	0.98	1.69^{ef}
B14	1.52	4.75^{ef}	0.71^{ab}	1.46^{bcd}	2.23	6.20^{efg}	0.98	1.37^{f}
B15	1.98	3.26^{f}	0.68^{ab}	0.92^{d}	2.66	4.18^{g}	1.08	1.63^{ef}
Significance	NS	S	S	S	NS	S	NS	S
CV (%)	35.9	55.0	35.9	55.3	34.7	53.9	38.2	55.8

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Dry weight of whole plant ranged from 2.04 g (B12) to 3.24 g (B3) at six month stage and at 12 months B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded the highest value of 14.56 g which differed significantly with all other treatments. In the case of dry weight of shoot, the values varied from 1.52 g (B14) to 2.54 g (B7) at six months and at twelve months, B8 with a value of 12.06 g was significantly superior to all other treatments. The lowest leaf dry weight was noticed in B11 (0.74 g) and the highest in B7 (1.43 g) at six month period. At twelve months stage B8 with a leaf dry weight of 7.49 g was significantly superior to all other treatments.

Significant difference in root dry weight was observed at six month stage and the highest root dry weight (1.09 g) was recorded in B5 [Cow's urine (25 times dilution with water)] which was on par with all other treatments except B4, B8, B10, B11, B12 and B13. B8 recorded the highest root dry weight at twelve month stage (2.50 g) which differed significantly with all other treatments.

4.1.2.1.16 Root parameters

4.1.2.1.16.1 Root number

Treatments showed significant difference with respect to primary, secondary, tertiary and total root number both at six and twelve months stage (Table 77).

At six month stage, the maximum number of primary roots (24.00) was recorded in B2 (*Azospirillum* sp. - 10 g per plant) which was superior to all other treatments. B7 had the lowest value (11.67) at this stage. At twelve months also, B2 had the maximum number of primary roots (27.00) which was at par with B1 (24.33) and B8 (22.67). B13 recorded the lowest value of 13.00 at this stage.

Table 77. Influence of growth promoting substances on number of roots

Treatments	Primary roots		Secondary roots		Tertiary roots		Total roots	
	6 months[@]	12 months	6 months	12 months	6 months	12 months	6 months	12 months
B1	14.00^{bc}	24.33^{ab}	35.33^{ab}	38.00^{ab}	7.00^{bcd}	7.00^{ef}	56.33^{abcd}	69.33^{ab}
B2	24.00^{a}	27.00^{a}	32.67^{abc}	42.33^{a}	8.67^{bcd}	16.67^{abcd}	65.33^{ab}	86.00^{a}
B3	13.00^{bc}	17.33^{def}	18.00^{def}	34.33^{ab}	5.33^{bcd}	8.67^{def}	36.33^{d}	60.33^{bc}
B4	16.00^{bc}	16.67^{def}	33.00^{abc}	33.33^{ab}	9.67^{bcd}	21.67^{ab}	58.67^{abc}	71.67^{ab}
B5	14.33^{bc}	15.00^{ef}	21.00^{cdef}	27.00^{bcd}	7.33^{bcd}	13.30^{cde}	42.67^{cd}	55.30^{bcd}
B6	15.00^{bc}	16.00^{def}	13.00^{f}	27.00^{bcd}	8.00^{bcd}	13.00^{cde}	36.00^{d}	47.33^{cd}
B7	11.67^{c}	16.00^{def}	18.00^{def}	21.00^{cde}	11.67^{abc}	15.00^{bcd}	41.34^{cd}	52.00^{bcd}
B8	16.67^{bc}	22.67^{abc}	30.00^{abcde}	37.33^{ab}	17.33^{a}	24.33^{a}	64.00^{ab}	84.33^{a}
B9	17.00^{bc}	20.67^{bcd}	17.33^{ef}	21.33^{cde}	6.33^{bcd}	20.33^{abc}	40.67^{cd}	62.33^{bcd}
B10	18.67^{b}	21.00^{bcd}	37.33^{a}	38.00^{ab}	12.33^{ab}	13.33^{cde}	68.33^{a}	72.33^{ab}
B11	13.33^{bc}	19.67^{bcde}	21.67^{bcdef}	30.00^{bc}	5.00^{cd}	15.33^{bcd}	40.00^{cd}	65.00^{bc}
B12	13.67^{bc}	15.00^{f}	17.00^{ef}	18.00^{de}	8.33^{bcd}	15.00^{bcd}	39.00^{cd}	48.00^{cd}
B13	11.68^{c}	13.00^{f}	18.10^{def}	21.10^{cde}	4.33^{d}	8.60^{def}	34.11^{d}	42.70^{d}
B14	15.33^{bc}	21.00^{bcd}	21.60^{bcdef}	27.33^{bcd}	7.00^{bcd}	16.00^{bcd}	43.93^{cd}	64.33^{bc}
B15	14.67^{bc}	16.00^{def}	17.10^{ef}	21.30^{cde}	9.67^{bcd}	13.10^{cde}	41.44^{cd}	50.40^{bcd}
Significance	S	S	S	S	S	S	S	S
CV (%)	18.9	14.8	27.1	23.3	43.1	40.3	21.4	18.8

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

The maximum number of secondary roots (37.33) was noted in B10 (Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + BA 100 ppm) at six month stage which differed significantly with B3, B5, B6, B7, B9, B11, B12, B13, B14 and B15 (control). The lowest number of secondary roots (13.00) was recorded in B6 at this stage. At twelve month stage, the highest number of secondary roots was observed in B2 (42.33) which was on par with B1, B3, B4, B8 and B10. The lowest number (18.00) was recorded in B12 at this stage.

At six month stage, maximum number of tertiary roots was noted in B8 (17.33) which was superior to all other treatments except B7 (11.67) and B10 (12.33). B13 recorded the lowest number of roots (4.33) at this stage. At twelve months also, B8 recorded maximum number of tertiary roots (24.33) which was on par with B2 (16.67), B4 (21.67) and B9 (20.33). The lowest value (7.00) was recorded in B1 at this stage.

The highest number of total roots (68.33) was observed in B10 at six month stage which was on par with B1 (56.33), B2 (65.33), B4 (58.67) and B8 (64.00). B13 recorded the lowest value (34.11) at this stage. At twelve months, maximum number of total roots (86.00) was observed in B2 which was superior to all other treatments except B1 (69.33), B4 (71.67), B8 (84.33) and B10 (72.33). The lowest number (42.70) was recorded in B13 at this stage (Plate 6).

4.1.2.1.16.2. Root length and spread

Significant difference was observed among the treatments with respect to root length and spread both at six and twelve months (Table 78).

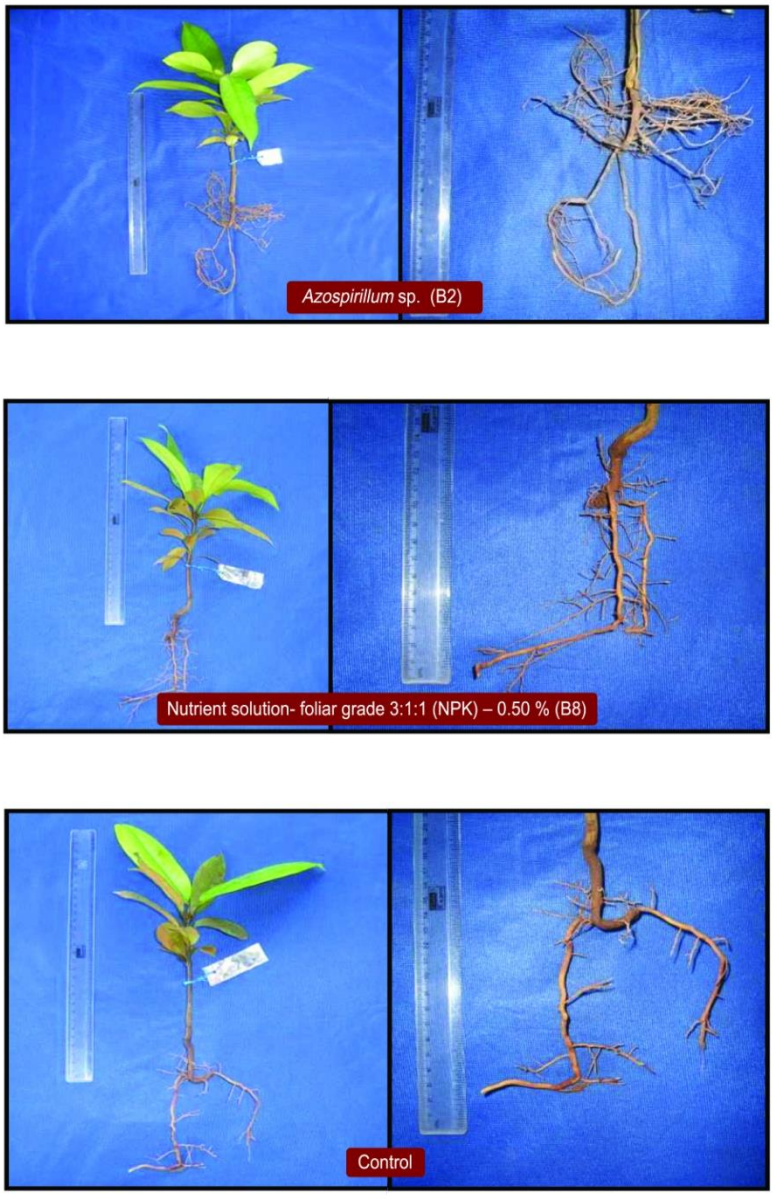


Plate 6. Influence of growth promoting substances on total number of roots

Table 78. Influence of growth promoting substances on root length (cm) and spread (cm)

Treatments	Root length		Root spread	
	6 months[@]	12 months	6 months	12 months
B1	15.70^{bcd}	24.27^{ab}	13.20^{bc}	19.07^{a}
B2	13.07^{d}	23.07^{abc}	12.03^{c}	19.50^{a}
B3	13.43^{d}	21.33^{abc}	11.53^{c}	15.53^{bcd}
B4	17.97^{abcd}	20.07^{bc}	14.77^{bc}	18.17^{ab}
B5	19.83^{abc}	27.83^{a}	21.80^{a}	23.00^{a}
B6	18.97^{abcd}	19.27^{bc}	12.23^{c}	16.53^{b}
B7	15.57^{bcd}	19.13^{bc}	13.20^{bc}	17.77^{ab}
B8	20.70^{ab}	22.37^{abc}	18.40^{ab}	18.90^{a}
B9	22.93^{a}	24.27^{ab}	12.33^{bc}	17.47^{ab}
B10	19.10^{abcd}	20.05^{bc}	14.67^{bc}	16.53^{ab}
B11	14.53^{cd}	20.33^{bc}	11.53^{c}	13.00^{bcd}
B12	18.40^{abcd}	18.63^{bc}	12.03^{c}	13.17^{bcd}
B13	13.57^{d}	23.60^{ab}	12.87^{bc}	15.93^{bc}
B14	14.03^{cd}	27.50^{a}	13.70^{bc}	16.17^{bc}
B15	13.60^{d}	16.20^{c}	10.47^{c}	10.50^{d}
Significance	S	S	S	S
CV (%)	18.8	16.8	22.5	17.1

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

The maximum root length (22.93 cm) at six month stage was observed in B9 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm] which was at par with B4, B5, B6, B8, B10 and B12. B2 recorded the minimum (13.07 cm) at this stage. At twelve month stage, B5 had maximum root length (27.83 cm) which significantly differed with B4, B6, B7, B10, B11, B12 and B15. The lowest value (16.20 cm) was observed in B15 (control) at this stage.

B5 [Cow's urine (25 times dilution with water)] recorded the maximum root spread (21.80 cm) at six month stage which was superior to all other treatments except B8 (18.40 cm). The lowest value of 10.47 cm was observed in the control (B15). At twelve months also, B5 recorded the maximum root spread (23.00 cm) which significantly differed with B3, B6, B11, B12, B13, B14 and B15. At this stage also control had the least (10. 50 cm) root spread.

4.1.2.1.17 Physiological parameters

4.1.2.1.17.1 Leaf Area Index (LAI)

Up to sixth month, no significant difference in LAI was observed among the treatments (Table 79). B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded the highest LAI values at nine (1.193), twelve (1.325) and fifteen (1.881) month stages while B14 had the lowest values of 0.280, 0.256 and 0.300 respectively at these stages.

At nine months stage B8 showed significant difference with B5, B6, B9, B11, B13, B14 and B15 while at twelve months stage, B9, B11, B13, B14 and B15 had significant difference with B8. The treatment B8 had significant difference with all the treatments except B2 and B12 at fifteen months stage.

Table 79. Influence of growth promoting substances on Leaf Area Index

Treatments	3 months[@]	6 months	9 months	12 months	15 months
B1	0.867	0.886	0.989^{abcd}	1.112^{a}	1.258^{bc}
B2	0.881	0.972	0.988^{abcd}	1.222^{a}	1.497^{ab}
B3	0.756	0.901	0.932^{abcd}	1.095^{a}	1.355^{bc}
B4	0.830	0.782	1.022^{abcd}	1.042^{ab}	1.192^{bc}
B5	0.697	0.792	0.847^{bcd}	1.035^{ab}	1.305^{bc}
B6	0.756	0.773	0.823^{cd}	1.169^{a}	1.444^{b}
B7	0.751	0.817	1.015^{abcd}	1.048^{ab}	1.319^{bc}
B8	0.752	0.950	1.193^{a}	1.325^{a}	1.881^{a}
B9	0.957	0.855	0.577^{ef}	0.612^{cd}	0.662^{de}
B10	0.860	0.975	1.095^{ab}	1.124^{a}	1.246^{bc}
B11	0.726	0.791	0.388^{fg}	0.468^{de}	0.561^{de}
B12	0.811	0.918	1.053^{abc}	1.289^{a}	1.484^{ab}
B13	0.757	0.900	0.282^{g}	0.401^{de}	0.490^{e}
B14	0.798	0.824	0.280^{g}	0.256^{e}	0.300^{e}
B15	0.852	0.860	0.786^{de}	0.782^{bc}	0.946^{cd}
Significance	NS	NS	S	S	S
CV (%)	18.9	19.0	22.0	22.8	26.8

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

4.1.2.1.17.2 Specific Leaf Weight (SLW)

Treatments differed significantly with respect to Specific Leaf Weight both at six and twelve month stages (Table 80). B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm] recorded the highest SLW both at six months (115.68 g m^{-2}) and twelve months (117.37 g m^{-2}). At six month stage, B14 was superior to all other treatments including control. At this period, B9 recorded the lowest value (48.92 g m^{-2}). At twelve month stage, B14 differed significantly with all other treatments except B11 (107.46 g m^{-2}). The lowest value (35.53 g m^{-2}) at this stage was recorded in B6.

4.1.2.1.17.3 Relative Growth Rate (RGR)

All the treatments were homogeneous with respect to RGR at twelve month stage (Table 80). The highest RGR value (0.00775 g g^{-1} day) was recorded in B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] and the lowest in control (0.00245 g g^{-1} day).

4.1.2.1.17.4 Shoot – root ratio

Shoot – root ratio showed significant difference both at six and twelve month stages (Table 80). At six month stage, highest shoot- root ratio (6.71) was recorded in B13 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm + BA 100 ppm] which was superior to all other treatments. The lowest shoot – root ratio was observed in B6 (1.83) at this stage. At twelve months, the maximum ratio (10.54) was observed in B12 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm] which was homogeneous with B7 (9.58) and B11 (10.04). The lowest value was noted in B14 (3.62).

Table 80. Influence of growth promoting substances on physiological parameters

Treatments	SLW (g m^{-2})		Shoot – root ratio		Dry matter production (g $plant^{-1}$)		RGR (g g^{-1} day)
	6 months@	12 months	6 months	12 months	6 months	12 months	12 months
B1	62.00 ^{b}	44.51^{d}	2.85^{def}	5.35^{c}	2.90	9.34^{bc}	0.00426
B2	67.77 ^{b}	49.09 ^{d}	3.29^{def}	7.12^{bc}	2.94	10.81^{b}	0.00425
B3	72.66 ^{b}	50.35 ^{d}	2.44^{def}	5.29^{c}	3.24	10.52^{b}	0.00410
B4	74.07 ^{b}	41.91 ^{d}	3.06^{def}	6.22^{bc}	2.68	9.67^{bc}	0.00371
B5	62.12 ^{b}	45.52 ^{d}	1.98^{ef}	5.08^{c}	3.31	6.97^{def}	0.00480
B6	70.44 ^{b}	35.53 ^{d}	1.83^{f}	5.93^{bc}	2.88	6.86^{def}	0.00359
B7	74.65 ^{b}	39.92 ^{d}	3.88^{bcd}	9.58^{ab}	3.19	9.63^{bc}	0.00290
B8	51.54 ^{b}	41.65 ^{d}	3.39^{def}	5.60^{bc}	2.65	14.56^{a}	0.00775
B9	48.92 ^{b}	70.38^{c}	2.52^{def}	7.31^{bc}	2.26	7.96^{cde}	0.00432
B10	64.02 ^{b}	51.56 ^{d}	3.55^{cde}	6.54^{bc}	2.67	8.58^{bcd}	0.00569
B11	67.46 ^{b}	107.46^{ab}	5.29^{b}	10.04^{ab}	2.12	8.71^{bcd}	0.00550
B12	58.25 ^{b}	45.70 ^{d}	3.88^{bcd}	10.54^{a}	2.04	9.57^{bc}	0.00375
B13	58.27 ^{b}	94.90^{b}	6.71^{a}	4.42^{c}	2.27	5.48^{fg}	0.00297
B14	115.68^{a}	117.37^{a}	5.02^{bc}	3.62^{c}	4.54	6.20^{efg}	0.00338
B15	75.32^{b}	48.24 ^{d}	3.96^{bcd}	4.830^{c}	2.67	4.18^{g}	0.00245
Significance	S	S	S	S	NS	S	NS
CV (%)	19.8	16.7	23.8	30.9	13.1	22.0	77.8

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

4.1.2.1.17.5 Dry matter production

Significant difference was observed among the treatments in dry matter production at twelve month stage only (Table 80). The values ranged from 2.04 g per plant (B12) to 4.54g per plant (B14) at six month stage and at twelve months, B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded the highest dry matter production (14.56 g per plant) which was significantly superior to all other treatments. B15 (control) recorded the lowest value (4.18 g per plant) at this stage.

4.1.2.1.17.6 Chlorophyll content

Treatments showed significant difference in chlorophyll a, b and total chlorophyll content in all the three stages studied (Table 81).

The highest chlorophyll 'a' content (0.543 mg g^{-1}) was noted in B1 [*Pseudomonas* sp. (2 %)], at four month stage which was superior to all other treatments. At this stage, the minimum value was recorded by B7 (0.128 mg g^{-1}). At eight month stage, the highest value (0.457 mg g^{-1}) was recorded by B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm] which was superior to rest of the treatments. B12 recorded the lowest value (0.101 mg g^{-1}) at this period. At twelve months, the maximum value was noted in B6 (1.127 mg g^{-1}) which was at par with B1 (1.124 mg g^{-1}) and B8 (1.123 mg g^{-1}). Control (B15) had the lowest chlorophyll 'a' content (0.489 mg g^{-1}) at this stage.

Chlorophyll 'b' content was highest in B1 (0.330 mg g^{-1}) at four month stage and it was superior to rest of the treatments. The lowest chlorophyll 'b' content at this stage was noted in B13 (0.071 mg g^{-1}). At eight month stage, the highest chlorophyll 'b' content was recorded by B14 (0.238 mg g^{-1}) which was superior to all other treatments. The lowest value of 0.016 mg g^{-1} was observed in B12. B1 showed the maximum chlorophyll 'b' content (0.688 mg g^{-1}) at twelve month stage and differed

significantly with rest of the treatments. The lowest value of 0.262 mg g $^{-1}$ was recorded in the control (B15) at this stage.

The total chlorophyll content was highest in B1 (0.873 mg g $^{-1}$) at four month stage and it differed significantly with all other treatments. The lowest value was noted in B13 (0.204 mg g $^{-1}$) at this period. B14 recorded the maximum total chlorophyll content (0.695 mg g $^{-1}$) at eight months and was superior to rest of the treatments. The lowest value at this period was noted in B12 (0.117 mg g $^{-1}$). At twelve months, the highest value of 1.813 mg g $^{-1}$ was observed in B1 and it differed significantly with all other treatments. Control (B15) had the lowest total chlorophyll content (0.751 mg g $^{-1}$) at this stage.

4.1.2.1.18. Nutrient content

Data pertaining to nutrient content of plant is presented in Tables 82 and 83. Treatments differed significantly with respect to N, P, K, Ca, Mg, Fe, Cu, Mn and Zn contents both at six and twelve month stages.

4.1.2.18.1 Nitrogen content

At six month period (Table 82), the highest nitrogen content (1.8457 %) was recorded in B10 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + BA 100 ppm] which differed significantly with all other treatments except B8, B14 and B15. Nitrogen content was lowest in B13 (0.7226 %).

At twelve months, B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm] recorded the highest nitrogen content (1.8473 %) which was superior to all other treatments except B15 (1.8342 %). At this stage, lowest nitrogen content was noted in B11 (0.9768 %).

Table 81. Influence of growth promoting substances on chlorophyll content of leaves (mg g^{-1})

Treatments	Chlorophyll a			Chlorophyll b			Total chlorophyll		
	4 months@	8 months	12 months	4 months	8 months	12 months	4 months	8 months	12 months
B1	0.543^{a}	0.334^{e}	1.124^{a}	0.330^{a}	0.147^{e}	0.688^{a}	0.873^{a}	0.481^{e}	1.813^{a}
B2	0.305^{b}	0.198^{hi}	0.878^{d}	0.175^{c}	0.090^{g}	0.429^{f}	0.481^{c}	0.288^{j}	1.307^{g}
B3	0.313^{b}	0.205^{h}	0.841^{f}	0.192^{b}	0.086^{g}	0.417^{f}	0.505^{b}	0.291^{j}	1.258^{h}
B4	0.179^{h}	0.214^{g}	0.759^{h}	0.145^{ef}	0.195^{c}	0.393^{g}	0.324^{h}	0.409^{f}	1.152^{ij}
B5	0.260^{c}	0.199^{hi}	0.783^{g}	0.196^{b}	0.141^{e}	0.389^{g}	0.456^{d}	0.340^{h}	1.172^{i}
B6	0.212^{f}	0.187^{j}	1.127^{a}	0.170^{cd}	0.052^{i}	0.660^{b}	0.382^{e}	0.238^{l}	1.787^{b}
B7	0.128^{i}	0.367^{d}	0.529^{j}	0.106^{h}	0.144^{e}	0.298^{i}	0.233^{j}	0.511^{d}	0.827^{l}
B8	0.229^{d}	0.118^{k}	1.123^{a}	0.152^{e}	0.028^{j}	0.626^{c}	0.380^{e}	0.146^{m}	1.749^{c}
B9	0.174^{h}	0.439^{b}	0.497^{k}	0.128^{g}	0.212^{b}	0.264^{j}	0.302^{i}	0.651^{b}	0.762^{m}
B10	0.182^{h}	0.187^{j}	1.080^{b}	0.157^{de}	0.067^{h}	0.492^{e}	0.339^{fgh}	0.254^{k}	1.571^{d}
B11	0.222^{de}	0.299^{f}	1.021^{c}	0.131^{fg}	0.091^{g}	0.524^{d}	0.353^{f}	0.390^{g}	1.544^{e}
B12	0.216^{ef}	0.101^{l}	0.751^{h}	0.127^{g}	0.016^{k}	0.391^{g}	0.343^{fg}	0.117^{n}	1.143^{j}
B13	0.133^{i}	0.401^{c}	0.603^{i}	0.071^{i}	0.170^{d}	0.328^{h}	0.204^{k}	0.571^{c}	0.931^{k}
B14	0.196^{g}	0.457^{a}	0.865^{e}	0.132^{fg}	0.238^{a}	0.479^{e}	0.327^{gh}	0.695^{a}	1.345^{f}
B15	0.226^{d}	0.195^{i}	0.489^{k}	0.123^{g}	0.113^{f}	0.262^{j}	0.349^{f}	0.308^{i}	0.751^{m}
Significance	S	S	S	S	S	S	S	S	S
CV (%)	2.3	1.8	0.6	5.9	5.6	2.1	2.4	2.2	1.0

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscript are homogeneous

Table 82. Influence of growth promoting substances on N, P, K, Ca and Mg content of leaves (%)

Treatments	N		P		K		Ca		Mg	
	6 months[@]	12 months	6 months	12 months	6 months	12 months	6 months	12 months	6 months	12 months
B1	1.2482^{f}	1.5253^{b}	0.1640^{bc}	0.1234^{f}	1.6467^{b}	1.5500^{b}	1.5123^{c}	1.5865^{fg}	0.2195^{ef}	0.1668^{ef}
B2	1.2912^{ef}	1.4852^{bc}	0.1804^{bc}	0.1337^{ef}	1.4056^{f}	1.4167^{cd}	1.4120^{def}	1.5600^{fg}	0.3080^{cd}	0.1528^{f}
B3	0.8248^{g}	1.2858^{f}	0.2547^{a}	0.1874^{bc}	1.2906^{g}	1.0467^{g}	1.4537^{cde}	1.7847^{cd}	0.3368^{bc}	0.2593^{bc}
B4	1.5589^{bcd}	1.5123^{b}	0.2020^{abc}	0.1375^{ef}	1.5533^{c}	1.4433^{c}	1.3847^{efg}	1.6428^{ef}	0.3355^{bc}	0.1845^{ef}
B5	1.5096^{bcde}	1.3144^{ef}	0.2001^{abc}	0.1760^{bcd}	1.4284^{ef}	1.0983^{g}	1.0498^{h}	1.8025^{cd}	0.2812^{cde}	0.2010^{de}
B6	1.3668^{def}	1.3614^{e}	0.1809^{bc}	0.2973^{a}	1.5811^{bc}	1.3700^{de}	1.5129^{c}	1.7415^{de}	0.3078^{cd}	0.2660^{bc}
B7	1.4411^{cdef}	1.2603^{f}	0.2211^{ab}	0.1323^{f}	1.5439^{c}	1.5000^{b}	1.4063^{def}	2.3868^{b}	0.3822^{b}	0.2817^{b}
B8	1.6691^{abc}	1.1808^{g}	0.2048^{abc}	0.1617^{bcdef}	1.5267^{cd}	1.3950^{cd}	1.4628^{cd}	2.7548^{a}	0.2272^{ef}	0.2732^{bc}
B9	1.5135^{bcde}	1.4379^{cd}	0.1727^{bc}	0.1477^{def}	1.4428^{def}	1.3200^{e}	1.1022^{h}	1.5250^{fg}	0.4747^{a}	0.2030^{de}
B10	1.8457^{a}	1.3217^{ef}	0.2541^{a}	0.1528^{cdef}	1.8683^{a}	1.3767^{de}	1.3592^{fg}	1.4883^{gh}	0.3298^{bc}	0.2332^{cd}
B11	1.5336^{bcd}	0.9768^{h}	0.2164^{ab}	0.1370^{ef}	1.5033^{cde}	1.5150^{b}	1.8794^{b}	1.8837^{d}	0.2727^{cde}	0.2352^{cd}
B12	1.5230^{bcd}	1.3797^{de}	0.1824^{bc}	0.1719^{bcde}	1.5500^{c}	1.5567^{b}	1.3283^{g}	1.3687^{h}	0.2582^{de}	0.1783^{ef}
B13	0.7226^{g}	1.5025^{bc}	0.1371^{c}	0.1936^{b}	1.5606^{c}	1.2367^{f}	1.9612^{a}	1.5398^{fg}	0.3909^{b}	0.3841^{a}
B14	1.6664^{abc}	1.8473^{a}	0.2107^{ab}	0.1480^{def}	1.4550^{def}	1.3767^{de}	1.4003^{def}	1.3781^{h}	0.1757^{f}	0.3590^{a}
B15	1.7116^{ab}	1.8342^{a}	0.1702^{bc}	0.1571^{bcdef}	1.5539^{c}	1.7033^{a}	1.8418^{b}	0.8725^{i}	0.2288^{ef}	0.2695^{bc}
Significance	S	S	S	S	S	S	S	S	S	S
CV (%)	8.5	2.7	18.1	12.1	3.2	2.3	2.7	4.2	11.8	9.4

@ Experiment was initiated with six month old polybag plants

Means with same letter as superscript are homogeneous

4.1.2.18.2 Phosphorous content

B3 [AMF (10 g per plant)] recorded highest phosphorous content (0.2547 %) at six month stage (Table 82) which was significantly different from all other treatments except B4, B5, B7, B8, B10, B11 and B14. The lowest phosphorous content at this period was noted in B13 (0.1371 %).

At twelve months, the highest phosphorous content (0.2973 %) was recorded in B6 [vermiwash (5 times dilution)] which showed significant difference with all other treatments including control. The lowest phosphorous content at this stage was noted in B7 (0.1323 %).

4.1.2.18. 3 Potassium content

The highest potassium content (1.8683 %) at six month stage (Table 82) was noted in B10 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + BA 100 ppm] which was superior to rest of the treatments. B3 recorded the lowest content (1.2906 %) at this stage. At twelve months, the maximum potassium content was recorded in B15 (1.7033 %) which was significantly different from all other treatments. The lowest content of 1.0467 % was observed in B3.

4.1.2.18.4 Calcium content

The highest calcium content (1.9612 %) at six month stage (Table 82) was recorded in B13 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm + BA 100 ppm] and it was superior to rest of the treatments. B5 had the lowest calcium content (1.0498 %) at this stage.

The highest calcium content (2.7548 %) at twelve month stage was recorded in B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] which differed significantly with all other treatments. The lowest value (0.8725 %) was noted in control (B15).

4.1.2.18.5 Magnesium content

The highest magnesium content (0.4747 %) was recorded by B9 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm] at six month stage (Table 82) which was superior to all other treatments. B14 showed the lowest value (0.1757 %) at this stage.

At twelve month stage, the maximum magnesium content (0.3841 %) was observed in B13 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm + BA 100 ppm] which was significantly different from all other treatments except B14 (0.3590 %). The lowest value (0.1528 %) was noted in B2 at this stage.

4.1.2.18.6 Iron content

At six month stage (Table 83), highest iron content (1063.22 mg kg^{-1}) was noted in B3 [AMF (10 g per plant)] which was superior to all other treatments except B6 (1017.33 mg kg^{-1}). The lowest iron content at this stage was recorded by B14 (470.67 mg kg^{-1}).

At twelve months also B3 recorded the maximum iron content (980.67 mg kg^{-1}) which differed significantly with all other treatments. B8 showed the lowest iron content (353.00 mg kg^{-1}) at this stage.

4.1.2.18.7 Copper content

The highest copper content of 93.39 mg kg^{-1} was recorded by B6 [vermiwash (5 times dilution)] which was homogeneous with only B15 (83.69 mg kg^{-1}) at six month stage (Table 83). B1 recorded the lowest value (8.43 mg kg^{-1}) at this stage.

At twelve month stage, the maximum copper content (11.97 mg kg^{-1}) was observed in B3 [AMF (10 g per plant)] which was superior to rest of the treatments. Both B14 and B15 recorded the lowest value (2.93 mg kg^{-1}) at this stage.

4.1.2.18.8 Manganese content

At six months (Table 83), the highest manganese content (152.80 mg kg $^{-1}$) was noted in B9 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm] which was at par with only B15 (139.78 mg kg $^{-1}$). The lowest value of 23.46 mg kg $^{-1}$ was recorded by B10 at this stage.

The maximum manganese content (151.47 mg kg $^{-1}$) at twelve months was observed in B11 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm] which was homogeneous with only B6 (143.87 mg kg $^{-1}$). B10 recorded the lowest value (75.37 mg kg $^{-1}$) at this stage also.

4.1.2.18.9 Zn content

The highest zinc content was recorded by B11 (68.44 mg kg $^{-1}$) at six months (Table 83), which was superior to all other treatments except B3 (65.84 mg kg $^{-1}$). B1 showed the lowest value (11.87 mg kg $^{-1}$) at this period.

At twelve month stage, the maximum zinc content (36.22 mg kg $^{-1}$) was noted in B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm] which was at par with only B5 (35.60 mg kg $^{-1}$). The lowest value at this period was recorded in B10 (9.03 mg kg $^{-1}$).

Table 83. Influence of growth promoting substances on Fe, Cu, Mn and Zn content of leaves (mg kg^{-1})

Treatments	Fe		Cu		Mn		Zn	
	6 months[@]	12 months	6 months	12 months	6 months	12 months	6 months	12 months
B1	745.33^{c}	757.33^{de}	8.43^{f}	8.87^{bc}	95.97^{b}	117.27^{d}	11.87^{e}	17.47^{efg}
B2	800.56^{bc}	812.33^{bc}	25.71^{e}	7.30^{bcdef}	65.48^{def}	116.67^{d}	21.28^{d}	22.17^{cde}
B3	1063.22^{a}	980.67^{a}	53.66^{c}	11.97^{a}	68.17^{de}	133.60^{b}	65.84^{a}	23.53^{cd}
B4	802.00^{bc}	508.00^{h}	40.50^{d}	9.57^{b}	64.23^{def}	103.30^{d}	14.83^{de}	25.57^{bc}
B5	761.33^{bc}	658.00^{f}	28.17^{e}	6.33^{defg}	94.83^{bc}	103.97^{d}	14.53^{de}	35.60^{a}
B6	1017.33^{a}	675.00^{f}	93.39^{a}	8.47^{bcd}	50.22^{ef}	143.87^{ab}	13.48^{de}	29.03^{b}
B7	804.44^{bc}	649.67^{f}	53.32^{c}	6.20^{defg}	76.58^{cd}	104.17^{d}	12.93^{de}	14.53^{g}
B8	843.00^{b}	353.00^{j}	52.33^{c}	6.87^{cdefg}	77.10^{cd}	133.10^{b}	18.23^{de}	15.80^{fg}
B9	747.89^{c}	646.00^{f}	77.69^{b}	4.90^{fgh}	152.80^{a}	100.73^{d}	39.47^{c}	21.10^{cdef}
B10	612.33^{d}	601.67^{g}	23.14^{e}	5.50^{efg}	23.46^{g}	75.37^{d}	15.01^{de}	9.03^{h}
B11	626.33^{d}	724.33^{e}	48.73^{cd}	5.50^{efg}	70.90^{d}	151.47^{a}	68.44^{a}	19.17^{defg}
B12	640.00^{d}	772.67^{cd}	51.37^{cd}	4.77^{gh}	68.43^{de}	81.97^{d}	15.40^{de}	19.53^{defg}
B13	840.11^{b}	842.33^{b}	81.19^{b}	7.60^{bcde}	76.66^{cd}	76.84^{d}	56.60^{b}	22.90^{cde}
B14	470.67^{e}	745.67^{de}	48.10^{cd}	2.93^{h}	47.73^{g}	100.72^{c}	19.23^{de}	36.22^{a}
B15	751.33^{c}	462.67^{i}	83.69^{ab}	2.93^{h}	139.78^{a}	106.90^{c}	20.10^{de}	18.50^{defg}
Significance	S	S	S	S	S	S	S	S
CV (%)	5.8	3.6	12.0	19.4	13.0	7.9	16.6	13.1

@ Experiment was initiated with six month old polybag plants

Means with same letter as superscripts are homogeneous

4.1.2.19 Nutrient uptake

Data pertaining to uptake of nutrients by the plants after one year of study is presented in Table 84.

Treatments did not record any significant difference with respect to uptake of P, K, Ca, Mg and Zn after one year of study. Significant difference was observed with respect to uptake of N, Fe, Cu and Mn only at the end of this period.

4.1.2.19.1 Nitrogen

Treatments showed significant difference with respect to uptake of nitrogen by the plant and the highest uptake (131.39 mg plant^{-1}) was noted in B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] which was significantly superior to B13, B14 and B15. Control recorded the lowest value of 44.91 mg plant^{-1} at the end of one year of study.

4.1.2.19.2 Phosphorous

The highest value for phosphorous uptake was noted in B8 (14.37 mg plant^{-1}) and B11 recorded the lowest value (5.84 mg plant^{-1}) though not statistically significant.

4.1.2.19.3 Potassium

All the treatments were at par and the maximum potassium uptake (107.81 mg plant^{-1}) was noted in B2 [(*Azospirillum* sp. - 10 g per plant)] and the minimum in B13 (45.52 mg plant^{-1}).

4.1.2.19.4 Calcium

The highest calcium uptake was noted in B8 (114.59 mg plant^{-1}) and the lowest (35.45 mg plant^{-1}) in B15 (control) and all the treatments were homogeneous.

4.1.2.19.5 Magnesium

B8 recorded the highest magnesium uptake (20.24 mg plant^{-1}) and the lowest was noted in B15 (7.83mg plant^{-1}) with all the treatments statistically at par.

4.1.2.19.6 Iron

Treatments differed significantly with respect to uptake of iron after one year of study. B2 recorded the highest value (4.78 mg plant^{-1}) which showed significant difference with all treatments except B1, B3 and B12. The lowest uptake (1.40 mg plant^{-1}) was noted in B15 (control).

4.1.2.19.7 Copper

Significant difference was observed between the treatments with respect to uptake of copper. The highest uptake was recorded in B3 (0.1075 mg plant^{-1}) which differed significantly with all other treatments. The lowest uptake was noted in B9 (0.0209 mg plant^{-1}).

4.1.2.19.8 Manganese

Treatments showed significant difference in uptake of manganese and B8 recorded the maximum value (0.7095 mg plant^{-1}) which was homogeneous to B1, B2 and B3 only and varied significantly from other treatments. The lowest value was observed in B13 (0.1527 mg plant^{-1}).

4.1.2.19.9 Zinc

All the treatments were homogeneous in the uptake of zinc and the values ranged from 0.0379 mg plant^{-1} (control) to 0.1288 mg plant^{-1} (B2).

Table 84. Influence of growth promoting substances on nutrient uptake (mg per plant)

Treatments	Uptake at 12 months (mg plant^{-1})$^{@}$								
	N	P	K	Ca	Mg	Fe	Cu	Mn	Zn
B1	122.78^{a}	9.36	95.42	90.25	11.84	3.62^{abcd}	0.0695^{bc}	0.5608^{abc}	0.0865
B2	128.15^{a}	12.43	107.81	107.25	14.53	4.78^{a}	0.0610^{c}	0.6770^{ab}	0.1288
B3	105.17^{ab}	13.97	66.29	94.63	15.69	4.36^{abc}	0.1075^{a}	0.5615^{abc}	0.1098
B4	109.82^{ab}	8.45	65.01	84.13	9.63	1.70^{cd}	0.0349^{c}	0.3049^{bc}	0.0820
B5	67.39^{ab}	9.36	54.46	82.01	10.44	2.02^{cd}	0.0345^{c}	0.3317^{bc}	0.1157
B6	67.77^{ab}	10.63	49.64	65.65	8.86	1.59^{cd}	0.0285^{c}	0.3298^{bc}	0.0710
B7	86.08^{ab}	8.09	65.67	93.00	12.67	2.21^{bcd}	0.0254^{c}	0.3260^{bc}	0.0555
B8	131.39^{a}	14.37	97.45	114.59	20.24	2.16^{bcd}	0.0459^{c}	0.7095^{a}	0.0970
B9	83.71^{ab}	9.11	61.21	71.23	11.52	2.40^{bcd}	0.0209^{c}	0.3331^{bc}	0.0629
B10	88.12^{ab}	9.46	70.80	79.10	11.82	2.35^{bcd}	0.0225^{c}	0.2965^{bc}	0.0939
B11	69.88^{ab}	5.84	57.23	78.60	14.15	2.21^{bcd}	0.0254^{c}	0.3298^{bc}	0.0643
B12	105.01^{ab}	11.04	80.10	92.23	11.13	3.50^{abcd}	0.0247^{c}	0.3111^{bc}	0.0838
B13	48.19^{b}	7.31	45.52	62.39	10.24	1.61^{cd}	0.0537^{c}	0.1527^{c}	0.0470
B14	54.07^{b}	10.26	60.95	99.72	15.06	2.34^{bcd}	0.0232^{c}	0.2651^{bc}	0.0745
B15	44.91^{b}	5.97	46.26	35.45	7.83	1.40^{d}	0.0488^{c}	0.2160^{c}	0.0379
Significance	S	NS	NS	NS	NS	S	S	S	NS
CV (%)	50.0	50.0	53.3	61.7	55.3	51.3	51.8	56.3	48.4

@ Experiment was initiated with six month old polybag plants
Means with same letter as superscripts are homogeneous

4.2 Experiment II: Accelerating plant growth in the main field

4.2.1 *Two year old grafted plants*

4.2.1.1 Plant characters

Observations on various growth parameters are presented in Tables 85 to 90.

4.2.1.1.1 Plant height

The data pertaining to influence of growth regulators on plant height is given in Table 85. Treatments differed significantly in all the stages with respect to plant height.

At three months, the maximum plant height (57.87 cm) was recorded by C7 (GA 200 ppm + BA 100 ppm) which was at par with C1, C4, C5 and C6 and differed significantly with control (27.97 cm). At similar trend was noted at six month period also and the maximum plant height was noted in C7 (68.33 cm) which was homogeneous with C1, C4, C5 and C6 and showed significant difference with control (30.13 cm). At nine month period, C7 with the maximum plant height (78.40 cm) was statistically at par with C1, C4, C5 and C6. Control treatment recorded the lowest plant height (31.67 cm) at this stage. At twelve month stage, the maximum plant height (96.33 cm) was observed in C5 (GA 100 ppm + BA 100 ppm) which was homogeneous with C1, C4, and C7. Control treatment recorded the lowest plant height (34.33 cm) at this stage also (Fig. 8).

4.2.1.1.2 Plant girth

Significant difference in plant girth was observed among the treatments throughout the experimental period (Table 85). At three month stage, C1 (GA 100 ppm) recorded the maximum plant girth (5.13 cm) which was on par with C7 (5.00 cm), C5 (3.93 cm) and C6 (3.80 cm). C9 (control) recorded the lowest value (2.50 cm) at this stage.

Table 85. Influence of growth regulators on plant height (cm) and girth (cm) in two year old grafts

Treatments	Plant height (cm)				Plant girth (cm)			
	3 months[@]	6 months	9 months	12 months	3 months[@]	6 months	9 months	12 months
C1	43.10^{ab}	52.83^{ab}	68.83^{a}	87.67^{ab}	5.13^{a}	5.90^{a}	6.27^{ab}	6.90^{ab}
C2	31.33^{b}	32.67^{b}	40.70^{b}	54.33^{bc}	2.93^{b}	3.33^{bc}	3.73^{bc}	4.63^{bc}
C3	30.30^{b}	33.70^{b}	36.87^{b}	42.97^{c}	2.87^{b}	3.07^{bc}	3.27^{c}	3.60^{bc}
C4	42.97^{ab}	47.83^{ab}	52.57^{ab}	62.97^{abc}	3.27^{b}	3.43^{bc}	4.07^{bc}	5.23^{abc}
C5	57.10^{a}	65.33^{a}	78.23^{a}	96.33^{a}	3.93^{ab}	4.67^{ab}	5.50^{abc}	6.70^{ab}
C6	45.17^{ab}	47.27^{ab}	53.40^{ab}	57.53^{bc}	3.80^{ab}	3.90^{bc}	4.50^{bc}	5.40^{abc}
C7	57.87^{a}	68.33^{a}	78.40^{a}	93.70^{a}	5.00^{a}	5.93^{a}	7.67^{a}	8.27^{a}
C8	24.87^{b}	29.70^{b}	31.87^{b}	36.43^{c}	3.13^{b}	3.37^{bc}	3.90^{bc}	4.40^{bc}
C9	27.97^{b}	30.13^{b}	31.67^{b}	34.33^{c}	2.50^{b}	2.63^{c}	2.73^{c}	3.00^{c}
Significance	S	S	S	S	S	S	S	S
CV (%)	27.2	26.7	26.6	30.2	24.1	24.5	32.2	32.4

@ The experiment was initiated with two year old grafted plants

Means with same letter as superscript are homogeneous

[Treatment details: C1 – GA 100 ppm, C2 – GA 200 ppm, C3 – BA 100 ppm, C4 – BA 200 ppm, C5 – GA 100 ppm + BA 100 ppm, C6 – GA 100 ppm + BA 200 ppm, C7 – GA 200 ppm + BA 100 ppm, C8 – GA 200 ppm + BA 200 ppm, C9 – control (no growth regulator)]

At six, nine and twelve month stages, C7 (GA 200 ppm + BA 100 ppm) recorded the maximum plant girth of 5.93 cm, 7.67 cm and 8.27 cm respectively while C9 (control) had the lowest plant girth of 2.63 cm, 2.73 cm and 3.00 cm respectively at these stages.

At six and nine month stages C7 was homogenous with C1 and C5 and the rest of the treatments including control showed significant difference with C7. At twelve months, in addition to C1 and C5, C4 and C6 were also statistically on par with C7.

4.2.1.1.3 Number of primary branches

Significant difference in number of primary branches was observed among the treatments throughout the experimental period (Table 86). C5 (GA 100 ppm + BA 100 ppm) recorded the highest number of primary branches at three (4.67), six (5.33) and nine (8.00) month stages while at twelve months, C7 (GA 200 ppm + BA 100 ppm) showed maximum number (14.00) of primary branches.

At three month stage, treatments C1 and C6 were on par with C5, while at six and nine month stages, in addition these two treatments, C7 was also homogeneous with C5. C7 showed significant difference with C3, C8 and C9 and all other treatments were on par with C7 at twelve month stage. At all these stages, C9 (control) showed significant difference with both C5 and C7.

Treatments also differed significantly with respect to total production of primary branches for a period of one year. The highest production of primary branches was noticed in C7 (12.67) which was superior to C2, C3, C8 and C9. Total production of primary branches was lowest in C9 (1.00).

Table 86. Influence of growth regulators on number of primary and secondary branches in two year old grafts

Treatments	Primary branches						Secondary branches					
	Initial[@]	3 months	6 months	9 months	12 months	Total production	Initial[@]	3 months	6 months	9 months	12 months	Total production
C1	3.33 (2.06)	3.33 (2.06)^{ab}	4.33 (2.3)^{ab}	6.67 (2.75)^{ab}	10.33 (3.34)^{ab}	7.00 (2.82)^{ab}	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.33 (1.14)	2.67 (1.90)^{ab}	2.67 (1.90)^{abc}
C2	1.67 (1.58)	1.67 (1.58)^{bcd}	1.67 (1.58)^{bcd}	2.67 (1.91)^{bcd}	4.33 (2.3)^{abc}	2.67 (1.87)^{b}	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.33 (1.14)	1.00 (1.33)^{ab}	1.00 (1.33)^{bc}
C3	0.00 (1)	0.00 (1)^{d}	0.00 (1)^{d}	0.33 (1.14)^{d}	1.67 (1.62)^{c}	1.67 (1.63)^{b}	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)^{b}	0.00 (1.00)^{c}
C4	1.33 (1.41)	1.33 (1.41)^{bcd}	1.67 (1.48)^{bcd}	3.33 (1.94)^{bcd}	5.33 (2.29)^{abc}	4.00 (2.10)^{ab}	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.67 (1.24)	1.33 (1.41)^{ab}	1.33 (1.41)^{bc}
C5	4.67 (2.38)	4.67 (2.38)^{a}	5.33 (2.52)^{a}	8 .00 (2.97)^{a}	11.33 (3.43)^{ab}	6.67 (2.61)^{ab}	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	1.67 (1.62)	5.00 (2.45)^{a}	5.00 (2.44)^{a}
C6	1.67 (1.58)	2.33 (1.82)^{abc}	2.33 (1.82)^{abcd}	4.33 (2.3)^{abc}	5.33 (2.51)^{abc}	3.67 (2.10)^{ab}	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)^{b}	0.00 (1.00)^{c}
C7	1.33 (1.49)	1.67 (1.62)^{bcd}	2.67 (1.86)^{abc}	7.00 (2.72)^{ab}	14.00 (3.62)^{a}	12.67 (3.45)^{a}	1.67 (1.48)	1.67 (1.48)	1.67 (1.48)	5.00 (2.16)	6.00 (2.37)^{a}	4.33 (2.13)^{ab}
C8	1.33 (1.49)	1.33 (1.49)^{bcd}	1.67 (1.62)^{bcd}	2.33 (1.79)^{bcd}	3.00 (1.99)^{bc}	1.67 (1.63)^{b}	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.33 (1.14)^{b}	0.33 (1.14)^{c}
C9	0.67 (1.24)	0.67 (1.24)^{cd}	1.00 (1.38)^{cd}	1.67 (1.61)^{cd}	1.67 (1.61)^{c}	1.00 (1.41)^{b}	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.33 (1.14)^{b}	0.33 (1.14)^{c}
Significance	NS	S	S	S	S	S	NS	NS	NS	NS	S	S
CV (%)	28.9	24.0	24.9	24.4	30.4	25.8	26.6	26.6	26.6	39.6	38.8	33.7

@ The experiment was initiated with two year old grafted plants

Means with same letter as superscript are homogeneous

Values in the parenthesis are mean of square root transformed values

4.2.1.1.4 Number of secondary branches

Presence of secondary branches was seen only in treatment C7 (GA 200 ppm + BA 100 ppm) up to six month stage. Plants under other treatments did not have secondary branches up to this stage (Table 86). All the treatments were non significant at ninth month and showed significant difference only at twelve month stage.

At twelve months, C7 recorded the maximum number of secondary branches (6.00), which was on par with C5 (5.00), C1 (2.67), C4 (1.33) and C2 (1.00). All other treatments including control (0.33) showed significant difference with C7.

Treatments also showed significant difference with respect to total production of secondary branches for a period of one year. The highest production of secondary branches was observed in C5 (5.00) which differed significantly with all other treatments except C1 (2.67) and C7 (4.33). Treatments C3 and C6 did not produce any secondary branches during the period of study.

4.2.1.1.5 Total number of branches

There was significant difference among the treatments with respect to total number of branches throughout the experimental period (Table 87). Treatment C5 (GA 100 ppm + BA 100 ppm) recorded the maximum number of branches at three (4.67) and six (5.33) month stages, while C7 had the highest values of 12.00 and 20.00 respectively at nine and twelve month stages. At all these stages, control (C9) was significantly lower to both C5 and C7.

The total production of branches also showed significant difference between the treatments for a period of one year. Highest production of total branches was recorded in C7 (17.00) which was homogeneous with C1, C4 and C5. The lowest production was noted in control (1.33).

Table 87. Influence of growth regulators on total number of branches in two year old grafts

Treatments	Initial[@]	3 months	6 months	9 months	12 months	Total production
C1	3.33 (2.06)	3.33 (2.06)^{ab}	4.33 (2.30)^{ab}	7.00 (2.82)^{ab}	13.00 (3.73)^{ab}	9.67 (3.26)^{abc}
C2	1.67 (1.58)	1.67 (1.58)^{abc}	1.67 (1.58)^{bcd}	3.00 (1.99)^{abc}	5.33 (2.48)^{abc}	3.67 (2.05)^{bc}
C3	0.00 (1.00)	0.00 (1.00)^{c}	0.00 (1.00)^{d}	0.33 (1.14)^{c}	1.67 (1.62)^{c}	1.67 (1.63)^{c}
C4	1.33 (1.41)	1.33 (1.41)^{bc}	1.67 (1.48)^{bcd}	4.00 (2.05)^{abc}	6.67 (2.46)^{abc}	5.33 (2.30)^{abc}
C5	4.67 (2.38)	4.67 (2.38)^{a}	5.33 (2.52)^{a}	9.67 (3.23)^{a}	16.33 (4.10)^{a}	11.67 3.46)^{ab}
C6	1.67 (1.58)	2.33 (1.82)^{abc}	2.33 (1.82)^{abcd}	4.33 (2.30)^{abc}	5.33 (2.51)^{abc}	3.67 (2.10)^{bc}
C7	3.00 (1.85)	3.33 (1.99)^{ab}	4.33 (2.23)^{abc}	12.00 (3.33)^{a}	20.00 (4.19)^{a}	17.00 (3.91)^{a}
C8	1.33 (1.49)	1.33 (1.49)^{bc}	1.67 (1.62)^{bcd}	2.33 (1.79)^{bc}	3.33 (2.08)^{bc}	2.00 (1.72)^{c}
C9	0.67 (1.24)	0.67 (1.24)^{bc}	1.00 (1.38)^{cd}	1.67 (1.61)^{bc}	2.00 (1.69)^{c}	1.33 (1.52)^{c}
Significance	NS	S	S	S	S	S
CV (%)	32.5	26.6	25.2	31.7	35.4	36.9

@ The experiment was initiated with two year old grafted plants
Means with same letter as superscript are homogeneous
Values in the parenthesis are mean of square root transformed values

4.2.1.1.6 Plant spread

The treatments showed significant difference with respect to plant spread throughout the study period (Table 88).

C5 (GA 100 ppm + BA 100 ppm) recorded the maximum plant spread of 68.73 cm both at three and six month stages, while C1 (GA 100 ppm) showed the highest values of 78.00 cm and 87.67 cm respectively at nine and twelve month stages. At all these stages, C9 (control) recorded the lowest values for plant spread.

At three and six month stages, C5 differed significantly from C3, C8 and C9 and was homogeneous with rest of the treatments. At nine and twelve month stages, C1 recorded significant difference with C2, C3, C4, C8 and C9 and was statistically on par with all other treatments.

4.2.1.1.8 Leaf area

The treatments showed significant difference for leaf area only at three and twelve month stages (Table 89). At six and nine month stages, the treatments did not vary significantly.

At three month stage, C1 (GA 100 ppm) recorded the highest mean leaf area (115.51 cm^2), and was significantly superior to all other treatments except C2, C6 and C7. At twelve month stage, C6 (GA 100 ppm + BA 200 ppm) which recorded the highest mean leaf area (138.87 cm^2) was homogeneous with only C1 and differed significantly with all other treatments including control.

Table 88. Influence of growth regulators on plant spread (cm) in two year old grafts

Treatments	3 months[@]	6 months	9 months	12 months
C1	67.30[a]	67.73[a]	78.00[a]	87.67[a]
C2	44.27[ab]	48.00[ab]	49.00[bc]	53.30[bcd]
C3	34.53[b]	35.00[b]	36.40[c]	37.90[d]
C4	45.37[ab]	46.27[ab]	46.93[bc]	50.53[bcd]
C5	68.73[a]	68.73[a]	70.23[ab]	80.73[a]
C6	57.83[ab]	57.83[ab]	59.70[abc]	67.53[abc]
C7	55.00[ab]	59.40[ab]	61.33[abc]	75.00[ab]
C8	36.67[b]	38.00[b]	38.00[c]	41.10[cd]
C9	33.63[b]	34.37[b]	34.37[c]	38.53[d]
Significance	S	S	S	S
CV (%)	26.9	26.0	26.5	24.6

@ The experiment was initiated with two year old grafted plants
Means with same letter as superscript are homogeneous

Table 89. Influence of growth regulators on leaf area (cm^2) and leaf production in two year old grafts

Treatments	Leaf area (cm^2)				Leaf production				
	3 months[@]	6 months	9 months	12 months	Up to 3 months[@]	3 to 6 months	6 to 9 months	9 to 12 months	Total leaf production
C1	115.51^{a}	105.23	97.17	107.99^{ab}	0.27 (1.11)	1.47 (1.55)^{a}	2.24 (1.8)^{a}	2.73 (1.93)^{ab}	6.71 (2.77)^{abc}
C2	93.18^{abc}	93.18	90.92	87.23^{b}	0.67 (1.24)	0.00 (1.00)^{c}	2.00 (1.73)^{a}	2.34 (1.82)^{abc}	5.01 (2.44)^{bcd}
C3	71.57^{cd}	73.19	75.55	72.29^{b}	2.00 (1.73)	1.33 (1.49)^{ab}	0.67 (1.24)^{bc}	2.00 (1.73)^{bc}	6.00 (2.65)^{abc}
C4	84.69^{bcd}	77.60	80.88	88.85^{b}	1.33 (1.49)	1.87 (1.69)^{a}	1.33 (1.52)^{abc}	2.56 (1.87)^{ab}	7.09 (2.84)^{ab}
C5	78.80^{bcd}	69.93	86.41	103.40^{b}	0.27 (1.11)	1.68 (1.60)^{a}	0.61 (1.25)^{bc}	3.15 (2.02)^{a}	5.71 (2.57)^{abcd}
C6	96.59^{ab}	97.08	83.79	138.87^{a}	1.33 (1.49)	0.22 (1.1)^{bc}	1.01 (1.39)^{abc}	1.97 (1.72)^{bc}	4.54 (2.34)^{cd}
C7	97.88^{ab}	94.01	93.75	87.02^{b}	1.33 (1.49)	1.99 (1.72)^{a}	1.80 (1.67)^{ab}	2.47 (1.86)^{ab}	7.59 (2.93)^{a}
C8	71.88^{cd}	75.23	82.76	91.32^{b}	0.00 (1.00)	1.00 (1.41)^{ab}	1.22 (1.48)^{abc}	1.53 (1.59)^{cd}	3.76 (2.18)^{de}
C9	69.50^{d}	70.84	70.78	72.95^{b}	0.00 (1.00)	1.17 (1.45)^{ab}	0.50 (1.21)^{c}	0.93 (1.39)^{d}	2.60 (1.88)^{e}
Significance	S	NS	NS	S	NS	S	S	S	S
CV (%)	14.0	19.9	12.0	19.3	21.9	14.9	15.8	7.9	9.0

@ The experiment was initiated with two year old grafted plants
Means with same letter as superscript are homogeneous
Values in the parenthesis are mean of square root transformed values

4.2.1.1.9 Leaf production

The data pertaining to influence of growth regulators on leaf production is presented in Table 89. Significant variation in mean leaf production was noted during all the periods except initial to three months period. During the initial to three months period, C3 (BA 100 ppm) recorded the highest mean leaf production (2.00) followed by C4, C6 and C7 all having a mean leaf production of 1.33 leaves. C8 and C9 (control) did not produce any leaves during this period.

From three to six months period, C7 (GA 200 ppm + BA 100 ppm) recorded the maximum mean leaf production (1.99) which was on par with all other treatments except C2 without any leaf production during the period. From six to nine months stage, C1 (GA 100 ppm) recorded the highest mean leaf production (2.24) which differed significantly with C3 (0.67), C5 (0.61) and the control treatment (C9) having the lowest mean leaf production of 0.50 leaf. C5 (GA 100 ppm + BA 100 ppm) recorded the maximum mean leaf production (3.15) during nine to twelve months period which was on par with C1, C2, C4 and C7. The lowest mean leaf production (0.93) was observed in the control treatment which was homogeneous with C8 (1.53).

Treatments also showed significant difference with respect to mean total leaf production for one year. The maximum leaf production was recorded in C7 (7.59) which was at par with C1 (6.71), C3 (6.00), C4 (7.09) and C5 (5.71). The lowest leaf production (2.60) was recorded by C9 (control) which was homogeneous only with C8 (3.76).

4.2.1.1.10 Extension of shoot in the main stem

The treatments showed significant difference only during the period from third to sixth month and also in the total extension for one year period (Table 90).

In the initial three months period, the maximum extension of shoot in the main stem (6.20 cm) was noted in C6 (GA 100 ppm + BA 200 ppm) and the lowest in control (1.70 cm). During the period from third to sixth month, C7 (GA 200 ppm + BA 100 ppm) recorded the highest value (10.47 cm) which was significantly superior to control (2.17 cm) and all other treatments except C1 and C5.

The maximum values for extension of shoot in the main stem were observed in C1 during the sixth to nine month (16.00 cm) and nine to twelve month (18.83 cm) periods, but the difference was not statistically significant from other treatments. During both these periods, the control treatment recorded the minimum shoot extension of 1.53 cm and 2.67 cm respectively.

The total extension of shoot for one year was highest in C1 (46.37 cm) which was at par with C2 (27.30 cm), C4 (24.00 cm), C5 (43.17 cm) and C7 (41.93 cm) and differed significantly with other treatments including control. Control (C9) recorded the lowest extension of shoot (8.07 cm) for one year period.

4.2.1.1.11 Extension of shoot in branches

The treatments showed significant difference in mean extension of shoot in branches during the entire period of investigation except in the initial period up to three months (Table 90). During the initial period up to three months, the highest mean extension of shoot (4.07 cm) was observed in C6 (GA 100 ppm + BA 200 ppm) and the lowest in C2, C3 and C4 without any growth of the branches. At this period, plants of C3 were also devoid of any branches.

Table 90. Influence of growth regulators on extension of shoot in the main stem (cm) and branches (cm) in two year old grafts

Treatments	Extension of shoot in main stem (cm)					Extension of shoot in branches (cm)				
	Up to 3 months@	3 to 6 months	6 to 9 months	9 to 12 months	Total extension of shoot	Up to 3 months@	3 to 6 months	6 to 9 months	9 to 12 months	Total extension of shoot
C1	1.80	9.73^{ab}	16.00	18.83	46.37^{a}	0.61	4.68^{a}	6.59^{a}	10.54^{ab}	22.42^{ab}
C2	4.30	1.33^{d}	8.03	13.63	27.30^{abc}	0.00	0.87^{b}	5.06^{ab}	7.10^{abc}	13.02^{cde}
C3	3.90	3.40^{cd}	3.17	6.10	16.57^{bc}	0.00	0.00^{b}	1.03^{b}	3.88^{cd}	4.92^{ef}
C4	4.00	4.87^{bcd}	4.73	10.40	24.00^{abc}	0.00	0.87^{b}	2.56^{ab}	5.78^{bcd}	9.21^{def}
C5	3.93	8.23^{abc}	12.90	18.10	43.17^{ab}	0.22	4.00^{a}	4.06^{ab}	10.49^{ab}	18.77^{abc}
C6	6.20	2.10^{d}	6.13	4.13	18.57^{bc}	4.07	0.73^{b}	3.78^{ab}	5.90^{bcd}	14.47^{bcd}
C7	6.10	10.47^{a}	10.07	15.30	41.93^{ab}	2.39	5.92^{a}	6.62^{a}	11.62^{a}	26.55^{a}
C8	2.30	4.83^{bcd}	2.17	4.57	13.87^{c}	0.11	1.43^{b}	2.37^{b}	4.71^{cd}	8.63^{def}
C9	1.70	2.17^{d}	1.53	2.67	8.07^{c}	0.05	0.58^{b}	1.23^{b}	1.44^{d}	3.31^{f}
Significance	NS	S	NS	NS	S	NS	S	S	S	S
CV (%)	96.6	52.3	90.0	81.4	53.6	82.8	68.0	57.2	41.3	37.0

@ The experiment was initiated with two year old grafted plants
Means with same letter as superscript are homogeneous

Plants of C7 (GA 200 ppm + BA 100 ppm) recorded the maximum mean extension of shoot in branches (5.92 cm) at three to six month stage which was significantly superior to all other treatments except C1 (4.68 cm) and C5 (4.00 cm). At this stage also plants of C3 which were devoid of any branches had the lowest mean extension of shoot (0.00).

From six to nine months stage, the maximum mean extension of shoot was observed in C7 (6.62 cm) which significantly differed with C3 (1.03 cm), C8 (2.37 cm) and C9 (1.23 cm). During the nine to twelve month period also C7 recorded the highest mean extension of shoot (11.62 cm) which was on par with all other treatments except C3, C4, C8 and C9. At this period, the control treatment (C9) recorded the lowest value of 1.44 cm.

The mean total extension of shoot in the branches was highest in C7 (26.55 cm) which was superior to all other treatments except C1 (22.42 cm) and C5 (18.77 cm). The lowest value (3.31 cm) for this parameter was recorded in C9 (control).

4.2.1.1.12 Bud break and time of flushing

As a result of application of growth regulators at monthly interval, treated two year old grafts produced leaves throughout the year without any definite period of bud break. Further a distinct period of flushing was also not observed in these grafts as leaf production was continuous throughout the year.

4.2.2 *Five year old orchard trees*

4.2.2.1 Plant characters

Observations on various growth parameters are presented in Tables 91 to 97.

4.2.2.1.1 Plant height

The treatments did not show any significant effect on plant height during the period of study (Table 91). Height of the plants ranged from 2.36 m (D4) to 3.27 m

(D11) during third month and the same trend was observed by twelfth month also with D4 recording the lowest height (3.16 m) and D11 the highest (3.83 m).

4.2.2.1.2 Plant girth

The treatments did not show any significant effect on plant girth during the period of study (Table 91). Girth of the plants ranged from 18.80 cm (D1) to 25.23 cm (D11) during third month and after one year of study, it ranged from 23.73 cm (D1 and D5) to 29.17 cm (D11).

4.2.2.1.3 Number of primary branches

The treatments did not show any significant effect on number of primary branches during the period of study (Table 92).

The number of primary branches ranged from 24.00 (D4) to 34.67 (D9) during the initial period. At twelve month stage, D4 recorded the lowest (28.67) and D11 the highest (40.67) number of primary branches. Total production of primary branches during a period of one year also showed no significant difference between the treatments and the values varied from 4.00 (D9) to 9.67 (D2).

4.2.2.1.4 Number of secondary branches

The treatments did not show any significant effect on number of secondary branches and total production of secondary branches during the period of investigation (Table 92). Number of secondary branches ranged from 107.00 (D8) to 150.00 (D10) in the initial stage and after one year, it ranged from 169.47 (D3) to 297.81 (D10). The total production of secondary branches varied from 56.14 (D3) to 147.81 (D10) during a period of one year.

Table 91. Influence of growth regulators on plant height (m) and girth (cm) in five year old trees

Treatments	Plant height (m)				Plant girth (cm)			
	3 months[@]	6 months	9 months	12 months	3 months[@]	6 months	9 months	12 months
D1	2.77	2.81	3.17	3.52	18.80	20.43	23.27	23.73
D2	3.05	3.08	3.47	3.74	21.10	23.07	26.23	27.37
D3	2.76	2.83	3.00	3.44	19.50	21.10	24.13	24.63
D4	2.36	2.53	2.89	3.16	18.87	19.90	22.80	25.47
D5	2.63	2.65	2.78	3.17	18.83	21.60	22.77	23.73
D6	3.12	3.15	3.38	3.68	19.23	20.50	23.23	24.30
D7	2.65	2.77	3.00	3.25	19.07	20.03	23.63	24.53
D8	2.98	3.02	3.27	3.51	20.73	22.13	24.83	25.33
D9	2.93	3.07	3.16	3.39	21.23	21.70	23.30	24.13
D10	3.23	3.32	3.37	3.47	23.57	25.10	27.17	27.77
D11	3.27	3.37	3.55	3.83	25.23	26.50	28.10	29.17
Significance	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	11.1	10.5	9.2	6.8	15.1	14.8	13.0	13.9

@ The experiment was initiated with five year old orchard trees

[Treatment details: D1 – GA 100 ppm, D2 – GA 200 ppm, D3 – BA 100 ppm, D4 – BA 200 ppm, D5 – GA 100 ppm + BA 100 ppm, D6 – GA 100 ppm + BA 200 ppm, D7 – GA 200 ppm + BA 100 ppm, D8 – GA 200 ppm + BA 200 ppm, D9 – Paclobutrazol 1.5 g a.i./tree, D10 – Paclobutrazol 2.0 g a.i./tree, D11 – Control (no growth regulator)]

Table 92. Influence of growth regulators on number of primary and secondary branches in five year old trees

Treatments	Number of primary branches						Number of secondary branches					
	Initial[@]	3 months	6 months	9 months	12 months	Total production	Initial[@]	3 months	6 months	9 months	12 months	Total production
D1	28.67	29.33	31.00	33.00	35.33	6.67	121.33	123.83	123.83	174.70	202.11	80.78
D2	28.33	28.33	30.67	34.00	38.00	9.67	126.33	126.33	140.46	168.76	196.83	70.50
D3	26.00	26.00	29.33	31.67	35.33	9.33	113.33	123.83	129.25	153.47	169.47	56.14
D4	24.00	24.67	25.33	26.67	28.67	4.67	107.67	122.67	128.08	166.25	188.46	80.79
D5	25.67	27.00	28.33	30.33	33.00	7.33	109.67	137.75	139.83	185.88	222.44	112.77
D6	31.00	32.33	34.00	36.67	39.33	8.33	101.33	117.88	117.92	169.17	218.48	117.15
D7	27.67	27.67	29.33	31.33	33.67	6.00	120.67	128.29	138.10	172.82	219.78	99.12
D8	28.33	28.33	31.33	33.67	37.00	8.67	107.00	111.33	117.63	156.97	188.99	81.99
D9	34.67	34.67	36.00	36.67	38.67	4.00	110.00	118.00	126.00	192.67	226.58	116.58
D10	30.67	31.33	33.33	36.00	39.33	8.67	150.00	166.10	185.35	254.73	297.81	147.81
D11	32.00	32.00	35.67	37.67	40.67	8.67	142.67	142.67	150.67	238.40	282.15	139.48
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	10.5	11.5	10.8	10.5	11.8	44.4	20.0	20.4	22.3	20.2	20.7	38.5

@ The experiment was initiated with five year old orchard trees

4.2.2.1.5 Number of total branches

The treatments showed significant difference in total number of branches only during ninth and twelfth months (Table 93). During ninth month, D10 (Paclobutrazol 2.0 g a.i./tree) recorded the maximum number of total branches (290.73) and was superior to all other treatments except D9 (229.33) and D11 (276.07). Number of total branches was minimum in D8 (190.64) at this period.

The number of total branches was highest in D10 (337.15) in twelfth month also and was statistically superior to D1, D2, D3, D4 and D8 at this stage. D3 recorded the minimum number of total branches (204.81) and differed significantly with D10 and D11 at this stage. The total production of all the branches during the one year period was also not significantly different between the treatments and the values ranged from 65.47 (D3) to 156.48 (D10).

4.2.2.1.6 Plant spread

The treatments did not show any significant effect on plant spread during the period of study (Table 94). Plant spread ranged from 2.47 m (D4) to 3.09 m (D11) during third month and after one year, it varied from 3.03 m (D3) to 3.92 m (D11).

4.2.2.1.8 Leaf area

With respect to leaf area, the treatments did not show any significant difference during the period of study (Table 94). Mean leaf area ranged from 107.31 cm^2 (D10) to 152.15 cm^2 (D1) at third month and after one year, it ranged from 107.44 cm^2 (D4) to 172.75 cm^2 (D7).

Table 93. Influence of growth regulators on number of total branches in five year old trees

Treatments	Initial[@]	3 months	6 months	9 months	12 months	Total production
D1	150.00	153.17	154.83	207.70^{bc}	237.44^{bc}	87.44
D2	154.67	154.67	171.13	202.76^{c}	234.83^{c}	80.16
D3	139.33	149.83	158.58	185.14^{c}	204.81^{c}	65.47
D4	131.67	147.33	153.41	192.91^{c}	217.13^{c}	85.46
D5	135.33	164.75	168.17	216.21^{bc}	255.44^{abc}	120.11
D6	132.33	150.22	151.92	205.83^{bc}	257.81^{abc}	125.48
D7	148.33	155.96	167.43	204.15^{c}	253.45^{abc}	105.12
D8	135.33	139.67	148.97	190.64^{c}	225.99^{c}	90.65
D9	144.67	152.67	162.00	229.33^{abc}	265.25^{abc}	120.58
D10	180.67	197.43	218.68	290.73^{a}	337.15^{a}	156.48
D11	174.67	174.67	186.33	276.07^{ab}	322.82^{ab}	148.15
Significance	NS	NS	NS	S	S	NS
CV (%)	16.5	17.1	18.6	17.2	17.8	35.2

@ The experiment was initiated with five year old orchard trees
Means with same letter as superscript are homogeneous

Table 94. Influence of growth regulators on plant spread (m) and mean leaf area (cm^2) in five year old trees

Treatments	Plant spread (m)				Mean leaf area (cm^2)			
	3 months[@]	6 months	9 months	12 months	3 months[@]	6 months	9 months	12 months
D1	2.70	2.77	3.08	3.19	152.15	164.07	156.40	133.51
D2	2.87	3.02	3.40	3.54	126.72	162.27	153.46	140.62
D3	2.71	2.79	2.98	3.03	129.42	139.82	149.22	135.86
D4	2.47	2.75	3.00	3.08	110.02	137.97	148.88	107.44
D5	3.07	3.12	3.51	3.55	141.45	158.69	172.87	146.40
D6	2.72	2.79	3.02	3.20	138.69	147.24	163.21	112.04
D7	2.65	2.95	3.22	3.25	114.51	183.65	166.82	172.75
D8	2.82	2.92	3.21	3.50	116.92	156.02	168.39	147.06
D9	2.63	2.86	3.33	3.59	142.89	156.07	161.35	164.12
D10	3.08	3.24	3.33	3.52	107.31	151.56	154.30	122.52
D11	3.09	3.20	3.82	3.92	134.73	139.93	147.52	166.13
Significance	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	12.0	12.2	13.8	12.2	23.0	19.5	15.1	19.2

@ The experiment was initiated with five year old orchard trees

4.2.2.1.8 Leaf production in primary branches

Mean leaf production in primary branches (Table 95) ranged from 0.00 (D2 and D11) to 3.42 (D5) in the initial three months period and in the final three months period of study, it ranged from 2.58 (D3) to 9.50 (D5). The highest values for mean leaf production were recorded in D5 during the initial (3.42) and final three months period (9.50) while D2 and D11 had the highest values during three to six months period (2.08) and six to nine months period (9.36) respectively. The mean total leaf production for one year period ranged from 8.96 (D3) to 21.06 (D5) during the period. The treatments did not record any significant difference with respect to mean leaf production and mean total leaf production in primary branches during the period of study.

4.2.2.1.9 Leaf production in secondary branches

During the period of study, treatments did not show any significant difference with respect to mean leaf production and mean total leaf production in secondary branches (Table 95). Mean leaf production in secondary branches ranged from 0.00 (D2) to 1.56 (D6) in the initial three months period and in the final three months period of study, it ranged from 1.27 (D1) to 3.12 (D9). The highest values for mean leaf production were recorded in D6 (1.56), D10 (0.93), D1 (4.55) and D9 (3.12) respectively for initial to three months, three to six months, six to nine months and nine to twelve months periods. The mean total leaf production for the one year period was highest in D9 (7.79) and lowest in D2 (4.02).

4.2.2.1.10 Extension of shoot in primary branches

No significant difference was observed among the treatments with respect to mean and total extension of shoot in primary branches during the period of investigation (Table 96). Mean extension of shoot in primary branches ranged from 0.00 (D2 and D11) to 8.63 cm (D5) in the initial three months period and in the final three months period of study, it ranged from 2.96 cm (D10) to 11.55 cm (D7).

Table 95. Influence of growth regulators on leaf production in primary and secondary branches in five year old trees

Treatments	Leaf production in primary branches					Leaf production in secondary branches				
	Up to 3 months@	3 to 6 months	6 to 9 months	9 to 12 months	Total leaf production	Up to 3 months	3 to 6 months	6 to 9 months	9 to 12 months	Total leaf production
D1	0.42 (1.17)	0.00 (1.00)	8.29 (3.04)	3.75 (2.17)	12.46 (3.66)	0.47 (1.20)	0.00 (1.00)	4.55 (2.32)	1.27 (1.51)	6.29 (2.68)
D2	0.00 (1.00)	2.08 (1.61)	4.00 (2.17)	3.58 (2.13)	9.67 (3.26)	0.00 (1.00)	0.68 (1.26)	1.57 (1.58)	1.78 (1.66)	4.02 (2.24)
D3	1.79 (1.61)	1.00 (1.33)	3.58 (2.13)	2.58 (1.87)	8.96 (3.14)	1.21 (1.45)	0.50 (1.19)	2.25 (1.77)	1.38 (1.54)	5.35 (2.51)
D4	1.42 (1.48)	1.25 (1.49)	5.46 (2.46)	3.25 (2.06)	11.38 (3.49)	0.93 (1.35)	0.82 (1.32)	3.19 (2.03)	2.22 (1.77)	7.16 (2.84)
D5	3.42 (1.98)	0.25 (1.11)	7.89 (2.95)	9.50 (2.91)	21.06 (4.57)	1.12 (1.43)	0.06 (1.03)	4.35 (2.22)	1.28 (1.47)	6.81 (2.72)
D6	1.75 (1.62)	0.00 (1.00)	6.42 (2.71)	5.29 (2.41)	13.46 (3.78)	1.56 (1.55)	0.00 (1.00)	3.44 (2.06)	1.54 (1.55)	6.53 (2.66)
D7	1.00 (1.33)	1.42 (1.51)	5.75 (2.52)	6.96 (2.75)	15.13 (3.93)	0.89 (1.31)	0.41 (1.18)	2.91 (1.95)	2.44 (1.74)	6.66 (2.64)
D8	0.58 (1.23)	0.83 (1.29)	6.08 (2.5)	4.13 (2.25)	11.63 (3.51)	0.50 (1.21)	0.49 (1.19)	2.44 (1.82)	1.57 (1.58)	5.00 (2.43)
D9	0.83 (1.29)	0.75 (1.27)	6.33 (2.68)	3.33 (1.96)	11.25 (3.45)	0.28 (1.12)	0.78 (1.27)	3.62 (2.15)	3.12 (1.92)	7.79 (2.9)
D10	0.58 (1.22)	2.00 (1.59)	7.17 (2.84)	3.92 (2.03)	13.67 (3.82)	0.61 (1.23)	0.93 (1.36)	3.40 (2.1)	1.78 (1.62)	6.72 (2.75)
D11	0.00 (1.00)	0.83 (1.29)	9.36 (3.14)	4.17 (2.27)	14.36 (3.89)	0.49 (1.19)	0.63 (1.23)	2.72 (1.91)	1.33 (1.52)	5.18 (2.47)
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	33.5	39.1	22.4	35.4	18.8	26.0	25.6	20.0	21.7	21.2

@ The experiment was initiated with five year old orchard trees, values in the parenthesis are mean of square root transformed values

Table 96. Influence of growth regulators on extension of shoot in primary and secondary branches (cm) in five year old trees

Treatments	Extension of shoot in primary branches (cm)					Extension of shoot in secondary branches (cm)				
	Up to 3 months[@]	3 to 6 months	6 to 9 months	9 to 12 months	Total	Up to 3 months[@]	3 to 6 months	6 to 9 months	9 to 12 months	Total
D1	1.66	0.00	12.24	6.67	20.57	1.46	0.00	6.82	2.34	10.62
D2	0.00	2.06	9.16	9.50	20.71	0.00	1.33	4.26	3.82	9.42
D3	5.52	3.06	5.64	7.86	22.09	3.03	1.39	3.24	3.23	10.89
D4	3.62	2.17	7.59	5.67	19.05	2.56	0.96	3.54	3.76	10.82
D5	8.63	0.31	11.02	8.50	28.46	3.84	0.06	5.04	2.29	11.22
D6	5.38	0.00	11.23	10.58	27.19	4.71	0.00	6.15	3.67	14.53
D7	3.08	1.65	7.72	11.55	24.00	2.60	0.50	4.59	4.69	12.38
D8	1.46	1.21	8.47	7.88	19.02	1.16	0.69	4.65	2.53	9.02
D9	3.17	1.02	8.07	7.77	20.03	0.93	1.79	5.17	6.77	14.66
D10	1.29	0.69	4.39	2.96	9.32	0.94	0.27	1.86	1.61	4.68
D11	0.00	0.62	15.32	6.66	22.60	0.73	0.74	6.68	3.43	11.58
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	144.3	205.7	55.6	65.6	47.4	129.7	223.6	56.5	93.1	66.2

@ The experiment was initiated with five year old orchard trees

The highest values for mean extension of shoot in primary branches were recorded in D5 (8.63 cm), D3 (3.06 cm), D11 (15.32 cm) and D7 (11.55 cm) respectively for initial to three months, three to six months, six to nine months and nine to twelve months periods. The mean total extension of shoot in primary branches for the one year period was highest in D5 (28.46 cm) and lowest in D10 (9.32 cm).

4.2.2.1.11 Extension of shoot in secondary branches

Treatments did not show any significant difference with respect to mean and total extension of shoot in secondary branches during the period of study (Table 96). Mean extension of shoot in secondary branches ranged from 0.00 (D2) to 4.71 cm (D6) in the initial three months period and in the final three months period of study, it ranged from 1.61 cm (D10) to 6.77 cm (D9). The highest values for mean extension of shoot in secondary branches were recorded in D6 (4.71 cm), D9 (1.79 cm), D1 (6.82 cm) and D9 (6.77 cm) respectively for initial to three months, three to six months, six to nine months and nine to twelve months periods. The mean total extension of shoot for one year period was highest in D9 (14.66 cm) and lowest in D10 (4.68 cm).

4.2.2.1.12 Bud break and time of flushing

As a result of application of growth regulators at monthly interval, treated trees produced leaves throughout the year without any definite period of bud break and flushing. Even then, two distinct periods of flushing were noted in orchard trees (Table 97). The first flushing started by the middle of April (D5, D8 and D10) and lasted till last week of June (D11). All the trees showed flushing during this period and the major flushing was in the month of June. Following this, a second flushing was also observed from middle of October to middle of November. Out of the total trees treated, 54.55 per cent trees flushed during second season also leading to an additional growth of the trees in a year. This second flushing was observed in the treatments D1, D3, D4, D5, D6, D7 and D8 (Plate 7). In rest of the trees (D2, D9, D10 and control) only single flushing was observed limited to April – June period.

Table 97. Influence of growth regulators on time of flushing in five year old trees

Treatments	First flushing		Second flushing	
	Period of flushing	Number of trees flushed	Period of flushing	Number of trees flushed
D1	June 3rd week	All (3)	October 3rd week	2
D2	June 1st week	All (3)	Nil	0
D3	May 1st week	All (3)	October 2nd week	2
D4	June 1st week	All (3)	October last week	2
D5	April 2nd week	All (3)	October last week	3
D6	June 2nd week	All (3)	October last week	3
D7	June 2nd week	All (3)	October 3rd week	3
D8	April 2nd week	All (3)	November 2nd week	3
D9	June 2nd week	All (3)	Nil	0
D10	April 2nd week	All (3)	Nil	0
D11	June last week	All (3)	Nil	0
	Total	33		18

Plate 7. Influence of growth regulators on flushing

4.2.2.2 Floral and fruit characters

4.2.2.2.1. Flowering

During the first year of study, out of the 33 trees studied, only 11 trees flowered. During the second year, except two trees in the control treatment all other trees flowered.

4.2.2.2.1.1 Time of flowering

During the first year of study (Table 98), flowering started in the last week of December (D2- GA 200 ppm) and the last flowering was observed during first week of March in D6 (GA 100 ppm + BA 200 ppm). In the second year, flowering was first observed in D3 (BA 100 ppm), D5 (GA 100 ppm + BA 100 ppm) and D10 (Paclobutrazol 2.0 g a.i./tree) during last week of January and last flowering was noted in D7 (GA 200 ppm + BA 100 ppm), D8 (GA 200 ppm + BA 200 ppm) and D11 (control) during last week of February.

4.2.2.2.1.2 Duration of flowering

Duration of flowering ranged from one day (single flower in D1 and D6) to 51 days (D10) during the first year. In the second year, flowering duration ranged from 18.33 (D1) to 55.67 (D10) days and all the treatment were at par with respect to this character (Table 99).

4.2.2.2.1.3 Number of flowers per unit area of tree canopy

The number of flowers per unit area (0.50 m x 0.50 m) of tree canopy ranged from 0.00 (D3, D4, D11) to 1.20 (D2) in the first year. Treatments differed significantly in the second year and maximum number of 8.53 flowers per 0.50 m x 0.50 m area of tree canopy was noticed in D10 which was superior to all other treatments (Table 99). Normally flowers are produced singly in mangosteen and in D10 as result of paclobutrazol application, flowers were produced mostly in clusters of 2 to 3 per cluster. Control treatment (D11) showed the lowest value of 0.60.

Table 98. Influence of growth regulators on time of flowering in five year old trees

Treatments	Fifth year		Sixth year	
	Time of flowering	Number of trees flowered	Time of flowering	Number of trees flowered
D1	January 1st week	1	February 1st week	All (3)
D2	December last week	1	February 2nd week	All (3)
D3	Nil	0	January last week	All (3)
D4	Nil	0	February 2nd week	All (3)
D5	January 1st week	1	January last week	All (3)
D6	March 1st week	1	February 1st week	All (3)
D7	February 1st week	2	February last week	All (3)
D8	January last week	2	February last week	All (3)
D9	February 1st week	1	February 2nd week	All (3)
D10	January 2nd week	2	January last week	All (3)
D11	Nil	0	February last week	1
	Total	11		31

Table 99. Influence of growth regulators on floral and fruit characters

Treatments	Duration of flowering (days)	Number of flowers per 0.50 m x 0.50 m canopy area	Flower and fruit drop (%)	Days from flowering to harvest	Average fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Fruit circumference (cm)
D1	18.33 (4.36)	2.03 (1.69)^{bc}	32.60^{bc}	84.87^{c}	65.82	3.93	4.96	16.12
D2	28.33 (5.40)	3.13 (2.02)^{b}	38.52^{abc}	88.80^{abc}	81.56	4.27	5.32	17.57
D3	46.33 (6.46)	1.53 (1.56)^{bc}	37.61^{abc}	93.17^{ab}	65.99	3.76	4.85	15.96
D4	21.00 (4.63)	1.60 (1.59)^{bc}	53.61^{ab}	93.33^{ab}	82.30	4.21	5.23	16.72
D5	38.33 (6.26)	3.23 (2.06)^{b}	41.20^{abc}	92.80^{ab}	84.56	4.41	5.36	17.99
D6	25.67 (5.15)	3.23 (2.06)^{b}	49.43^{ab}	96.40^{a}	73.85	4.14	5.11	16.81
D7	27.67 (5.27)	2.90 (1.97)^{bc}	57.99^{ab}	87.80^{bc}	78.25	3.98	5.19	17.19
D8	27.00 (5.27)	1.60 (1.61)^{bc}	60.21^{ab}	89.47^{abc}	62.93	3.95	4.77	15.72
D9	23.33 (4.91)	2.83 (1.94)^{bc}	48.70^{ab}	86.60^{bc}	89.96	4.26	5.65	18.30
D10	55.67 (7.27)	8.53 (3.00)^{a}	65.65^{a}	90.27^{abc}	73.27	3.99	5.21	16.96
D11	30.00 (5.55)	0.60 (1.26)^{c}	14.29^{c}	86.80^{bc}	58.09	3.92	4.55	14.97
Significance	NS	S	S	S	NS	NS	NS	NS
CV (%)	24.1	20.7	35.3	4.5	15.4	9.3	8.0	7.1

Means with same letter as superscript are homogeneous
Values in the parenthesis are mean of square root transformed values

4.2.2.2.1.4 Flower and fruit drop

Flower and fruit drop was noticed mostly within fifteen days of anthesis and thereafter fruit drop was almost nil. In the first year, the maximum number of flower/ fruit drop was noticed in D5 (21.43 per cent) and the minimum in D1, D3, D4, D8 and D9 (0.00 per cent). Treatments showed significant difference with respect to this character (Table 99) and maximum drop was noticed in D10 (65.65 per cent) which differed significantly with only D1 (32.60 per cent) and control (14.29 per cent). The control treatment recorded the lowest flower/ fruit drop as flowering was scanty in this treatment giving the lowest yield of all the treatments compared.

4.2.2.2.1.5. Days from flowering to harvest

Time required from flowering to harvesting varied from 85.80 (D2) to 103.00 (D5) days during the first year. Treatments showed significant difference in this period during second year (Table 99). The minimum period of 84.87 days from flowering to harvesting was recorded in D1 and the treatment D6 required a period of 96.40 days from flowering to harvesting.

4.2.2.2.2 Fruit characters

4.2.2.2.2.1 Fruit weight

During the first year of study, average fruit weight ranged from 73.66 g (D7) to 139.02 g (D1), but the number of fruits per tree and yield per tree were very less and yielding trees were very few.

No significant difference was observed between the treatments with respect to average fruit weight in second year also (Table 99). The maximum average fruit weight of 89. 96 g was recorded in D9 (Paclobutrazol 1.5 g a.i./tree), followed closely by D5 (84.56 g), D4 (82.30 g) and D2 (81.56 g). The lowest average fruit weight (58.09 g) was recorded in control (Fig 9).

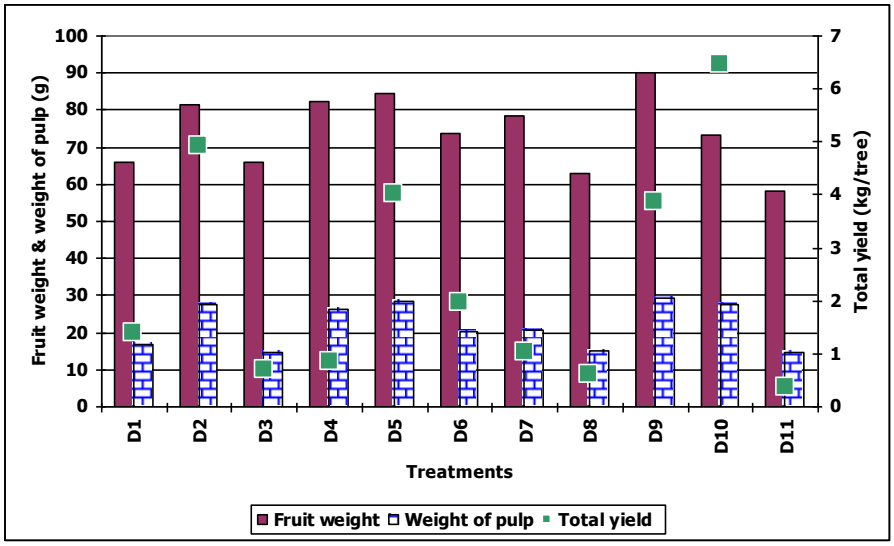


Fig. 9. Effect of growth regulators on fruit characters and yield of five year old mangosteen trees

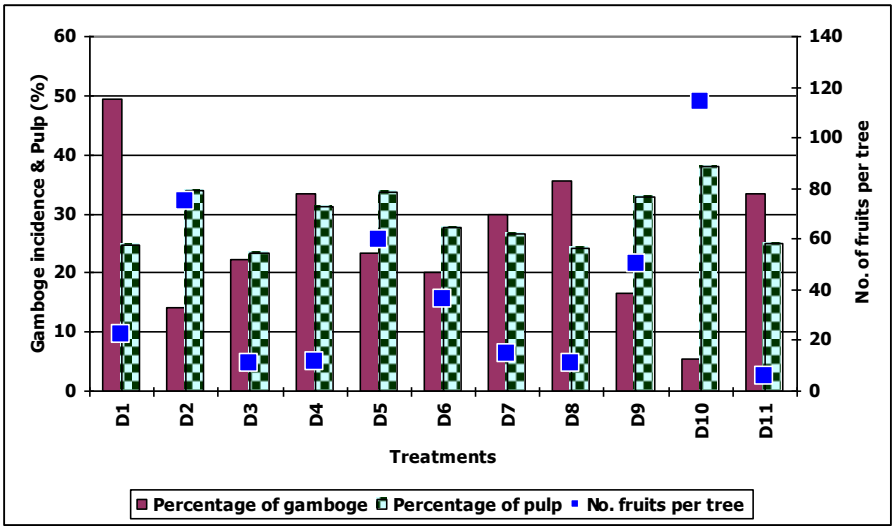


Fig. 10. Effect of growth regulators on incidence of gamboge, percentage of pulp and number of fruits per tree of five year old mangosteen trees

Based on fruit size, the trees were classified into three groups as detailed below (IPGRI, 2003).

a) Large: > 140 g per fruit
b) Medium: 90 – 140 g per fruit
c) Small: < 90 g per fruit

During first year of study, D1, D5, D6 and D10 produced fruits of medium size and all other trees produced fruits of smaller size. In the second year, even though all trees produced fruits of smaller size, fruit number and average yield per tree were much higher compared to first year (Table 100).

Table 100. Classification of treated trees based on fruit size

Fruit size and trees under each group					
Large		Medium		Small	
First year	Second year	First year	Second year	First year	Second year
--	--	D1, D5, D6, D10	---	D2, D7, D8, D9	All treated trees

4.2.2.2.2.2 Fruit length, breadth and circumference

The treatments did not differ significantly with respect to fruit length, breadth and circumference (Table 99). The mean fruit length varied from 3.76 cm (D3) to 4.41 cm (D5), breadth from 4.55 cm (D11) to 5.65 cm (D9) and circumference from 14.97 cm (D11) to 18.30 cm (D9) among the treatments.

4.2.2.2.2.3 Pulp weight and percentage of edible portion

The treatments showed significant difference in pulp weight and percentage of edible portion (Table 101 and Fig. 10). The highest pulp weight of 29.41 g was recorded in D9 (Paclobutrazol 1.5 g a.i./tree) which was superior to D1, D3, D6, D8 and D11 (control). The control treatment had the lowest pulp weight of 14.44 g.

Percentage of edible portion was highest (37.96 per cent) in D10 (Paclobutrazol 2.0 g a.i./tree) which was homogeneous only with D2, D5 and D9 and differed significantly with all other treatments including control (24.87 per cent).

4.2.2.2.2.4 Number of segments

The treatments differed significantly with respect to number of big segments only and all the treatments were on par with regard to number of medium, small and total segments (Table 101). The number of big segments was maximum (1.57) in D10 and was superior to D1, D7 and D11. The control treatment (D11) had the lowest number of big segments (0.82). The mean number of medium, small and total segments in the fruit ranged from 1.97 (D6), 1.11 (D3) and 5.48 (D10) respectively to 3.81 (D1), 2.68 (D11) and 6.08 (D9) in this period.

In all the treatments, the total number of segments ranged from a minimum of four to a maximum of seven without any variation between the treatments.

4.2.2.2.2.5 Thickness of rind

All the treatments were on par (Table 101) and rind thickness varied from 0.60 cm (D10) to 0.83 cm (D3 and D4).

4.2.2.2.2.6 Cavity size of fruits

Significant difference was observed between treatments with respect to cavity size (Table 101) and the maximum cavity size (4.18 cm) was recorded in D9 (Paclobutrazol 1.5 g a.i./tree) which differed significantly with all other treatments except D2, D4, D5 and D10. Control treatment (D11) had the lowest cavity size of 3.21 cm.

Table 101. Influence of growth regulators on fruit characters

Treatments	Weight of pulp (g)	Percentage of edible portion	Number of segments per fruit				Thickness of rind (cm)	Cavity size (cm)
			Big	Medium	Small	Total		
D1	16.97^{c}	24.75^{e}	0.85^{c}	3.81	1.38	6.04	0.77	3.42^{de}
D2	27.67^{ab}	33.99^{ab}	1.47^{ab}	2.05	2.12	5.64	0.72	4.06^{abc}
D3	14.85^{c}	22.38^{e}	1.08^{abc}	3.71	1.11	5.90	0.83	3.26^{e}
D4	26.15^{ab}	31.26^{bcd}	1.13^{abc}	2.40	1.97	5.50	0.83	3.88^{abcd}
D5	28.44^{ab}	33.66^{abc}	1.49^{ab}	2.31	1.70	5.50	0.73	4.15^{ab}
D6	20.30^{bc}	27.59^{cde}	1.23^{abc}	1.97	2.47	5.68	0.68	3.69^{bcde}
D7	20.73^{abc}	26.55^{de}	1.02^{bc}	2.95	1.73	5.71	0.81	3.65^{cde}
D8	15.01^{c}	24.14^{e}	1.07^{abc}	3.11	1.67	5.84	0.70	3.26^{e}
D9	29.41^{a}	32.73^{abc}	1.24^{abc}	2.34	2.50	6.08	0.76	4.18^{a}
D10	27.70^{ab}	37.96^{a}	1.57^{a}	2.06	1.86	5.48	0.60	4.02^{abc}
D11	14.44^{c}	24.87^{e}	0.82^{c}	2.12	2.68	5.62	0.72	3.21^{e}
Significance	S	S	S	NS	NS	NS	NS	S
CV (%)	20.8	11.4	23.5	36.2	37.8	7.7	11.3	7.0

Means with same letter as superscript are homogeneous

4.2.2.2.2.7 Colour of ripe fruits

No variation was observed between the treatments with respect to colour of mature and ripe fruits in both the years of study. A uniform pattern of colour development was noted in the fruits from maturity to full ripeness. A fully mature fruit is identified by a light yellow pink colour with the occurrence of pink or red lines or patches appearing on the outer surface of rind. Then the fruit completely changes to pink, red, dark red, red purple and finally to purple, dark purple or black. After this stage, fruit shell hardens with difficulty in opening of fruits rendering it unfit for consumption.

4.2.2.2.2.8 Number of seeds per fruit

Seeds were classified into bold (above 1.50 g weight) and small (below 1.50 g weight) based on seed weight. No significant difference was noticed between the treatments with respect to bold, small and total number of seeds per fruit (Table 102). Total seeds per fruit varied from 0.21 (D8) to 1.46 (D10). Only D1 (0.19 bold seed per fruit), D4 and D5 (both 0.03 bold seed each per fruit) produced bold sized seeds. All other treatments produced seeds of small size which ranged from 0.29 (D3) to 1.46 (D10) seeds per fruit.

Treatments differed significantly in percentage of seedless fruits (Table 102) and the highest number of seedless fruits (79.93 per cent) was recorded in D8 which was on par with all other treatments except D5 (27.78 per cent), D9 (33.03 per cent) and D10 which had the lowest value of 5.56 per cent.

4.2.2.2.2.9 Seed weight

Treatments showed significant difference with respect to mean seed weight (Table 102). The maximum mean seed weight of 0.80 g was recorded by D10 which was at par with all other treatments except D1, D4 and D8. D8 had the minimum seed weight of 0.34 g.

Table 102. Influence of growth regulators on fruit characters

Treatments	Number of seeds per fruit			Percentage of seedless fruits	Weight of seed (g)	TSS (° Brix)	Incidence of gamboge (%)
	Bold (>1.5 g)	Small (<1.5 g)	Total				
D1	0.19 (1.08)	0.32 (0.46)	0.51 (0.57)	58.10^{ab}	0.36^{b}	17.23	49.52
D2	0.00 (1.00)	0.77 (0.88)	0.77 (0.88)	35.22^{abc}	0.72^{ab}	18.02	14.16
D3	0.00 (1.00)	0.29 (0.54)	0.29 (0.54)	73.05^{ab}	0.41^{ab}	17.87	22.22
D4	0.03 (1.02)	0.57 (0.61)	0.60 (0.63)	40.00^{abc}	0.36^{b}	16.52	33.33
D5	0.03 (1.02)	0.98 (0.99)	1.01 (1.00)	27.78^{bc}	0.71^{ab}	17.98	23.33
D6	0.00 (1.00)	0.61 (0.78)	0.61 (0.78)	43.73^{abc}	0.74^{ab}	18.93	20.00
D7	0.00 (1.00)	0.45 (0.67)	0.45 (0.67)	56.29^{ab}	0.44^{ab}	17.81	30.00
D8	0.00 (1.00)	0.81 (0.69)	0.21 (0.37)	79.93^{a}	0.34^{b}	17.16	35.55
D9	0.00 (1.00)	0.77 (0.87)	0.77 (0.87)	33.03^{bc}	0.76^{a}	17.62	16.66
D10	0.00 (1.00)	1.46 (1.19)	1.46 (1.19)	5.56^{c}	0.80^{a}	18.87	5.56
D11	0.00 (1.00)	0.52 (0.72)	0.52 (0.72)	58.64^{ab}	0.43^{ab}	17.02	33.33
Significance	NS	NS	NS	S	S	NS	NS
CV (%)	4.5	39.6	36.3	49.8	36.8	5.2	37.4

Means with same letter as superscript are homogeneous
Values in the parenthesis are mean of square root transformed values

4.2.2.2.2.10 Total Soluble Solids (TSS)

No significant variation was observed in the TSS content among the treatments (Table 102). The value ranged from 16.52 (D4) to 18.93 ° Brix (D6) in the second year.

4.2.2.2.2.11 Incidence of gamboge disorder of fruits

It is a major physiological disorder found in mangosteen. It is evidenced by the oozing of latex onto fruit surface and also inside the fruit spoiling the fruit. Treatments showed variation in incidence of this disorder (Table 102). Gamboge was highest in D1 (49.52 per cent) and lowest (5.56 per cent) in D10.

4.2.2.3 Yield and yield attributes

4.2.2.3.1 Time of harvesting and duration of harvesting

The first harvesting was recorded in the treatment D1 (GA 100 ppm) during the first week of April and harvesting started in the treatment D6 (GA 100 ppm + BA 200 ppm) as late as first week of June during the first year of study. Harvesting was also first completed in D1 (first week of April) and extended up to third week of June in D7 (GA 200 ppm + BA 100 ppm).

During second year, first harvesting was recorded in D3 (BA 100 ppm) during first week of March and harvesting started as late as last week of May in the treatment D8 (GA 200 ppm + BA 200 ppm). Harvesting was first completed in D4 (BA 200 ppm) during third week of May and the last harvesting was recorded in D8 and D11 (control) during last week of June.

The period required to complete harvesting varied from one day (single fruit in D1 and D6) to 47.00 days (D10) in the first year (Table 103). All the treatments were at par with respect to this period in the second year (Table 104). Duration of harvesting was minimum (16.67 days) in D4 and the maximum time was recorded in D10 (58.67 days).

Table 103. Influence of growth regulators on yield and yield attributes (first year)

Treatments	Duration of harvesting (days)	Total yield per tree (kg)	Number of fruits per tree	Number of harvests
D1	1.00	0.046	1.00	1.00
D2	19.00	0.634	23.00	8.00
D3	0.00	0.000	0.00	0.00
D4	0.00	0.000	0.00	0.00
D5	18.00	0.408	11.00	4.00
D6	1.00	0.031	1.00	1.00
D7	32.50	0.173	3.50	2.50
D8	26.50	0.173	3.00	2.50
D9	30.00	0.049	2.00	2.00
D10	47.00	1.977	29.50	7.50
D11	0.00	0.000	0.00	0.00

Table 104. Influence of growth regulators on yield and yield attributes (second year)

Treatments	Duration of harvesting (days)	Total yield per tree (kg)	Number of fruits per tree	Number of harvests
D1	17.33 (4.19)	1.39^{bc}	22.00 (4.41)^{cd}	5.67^{cd}
D2	29.33 (5.50)	4.93^{ab}	74.67 (8.57)^{ab}	8.33^{abc}
D3	52.00 (6.84)	0.69^{c}	11.00 (3.35)^{cd}	5.33^{cd}
D4	16.67 (3.83)	0.83^{c}	11.67 (3.11)^{cd}	3.67^{d}
D5	36.67 (6.13)	4.02^{abc}	59.67 (7.19)^{abc}	10.00^{ab}
D6	26.67 (5.26)	1.97^{bc}	36.00 (5.91)^{bcd}	6.67^{abcd}
D7	26.00 (5.09)	1.04^{c}	14.33 (3.90)^{cd}	6.33^{bcd}
D8	28.33 (5.41)	0.60^{c}	10.67 (3.41)^{cd}	4.33^{cd}
D9	20.00 (4.56)	3.87^{abc}	50.00 (6.72)^{abcd}	6.00^{cd}
D10	58.67 (7.32)	6.45^{a}	114.33 (10.3)^{a}	10.33^{a}
D11	32.00 (5.74)	0.35^{c}	6.00 (2.64)^{d}	4.00^{d}
Significance	NS	S	S	S
CV (%)	28.9	25.8	40.9	32.8

Means with same letter as superscript are homogeneous
Values in the parenthesis are mean of square root transformed values

4.2.2.3.2 Yield

Yield per tree showed wide variation in both the years of study. During the first year of investigation, out of the 33 trees studied, only 11 trees flowered and during the second year, except two trees in the control treatment, all other trees flowered.

Yield per tree ranged from 0.031 kg (D6) to 1.977 kg (D10) in the first year (Table 103). In the second year (Table 104), treatments showed significant difference with respect to yield and D10 (Paclobutrazol 2.0 g a.i./tree) recorded the highest yield of 6.45 kg followed by D2 (4.93 kg), D5 (4.02 kg) and D9 (3.87 kg) which were at par with D10. But D10 was significantly superior to D1, D3, D4, D6, D7, D8 and D11 (control). The lowest yield of 0.35 kg was recorded in the control.

4.2.2.3.3 Number of fruits per tree

During the first year of study (Table 103), number of fruits per tree varied from one (D1 and D6) to 29.50 (D10). In the second year (Table 104), treatments showed significant difference in fruit number per tree and the maximum number of 114.33 fruits per tree was recorded in D10 (Paclobutrazol 2.0 g a.i./tree) which was statistically superior to D1, D3, D4, D6, D7, D8 and D11 (control). The lowest number of fruits per tree was recorded in the control treatment (6.00).

4.2.2.3.4 Number of harvests

The number of harvests varied from 1.00 (D1 and D6) to 8.00 (D2) in the first year (Table 103). In the second year (Table 104), treatments showed significant difference and maximum number of harvests was recorded in D10 (10.33) which was on par with D2 (8.33) and D5 (10.00) and D6 (6.67). The lowest number (4.00) of harvests was noted in D11.

4.3 Experiment III: Use of nurse stocks and rootstocks to enhance growth

4.3.1 Nurse stock

Additional nurse stocks namely seedlings of *Garcinia mangostana, G. indica, G. hombroniana, G. gummi-gutta, G. xanthochymus and G. cowa* were approach grafted/ inarched to two year old seedlings to provide a double root system. Seedling growth was measured bimonthly for 18 months.

Grafting studies were taken up during the months of July – August on two year old mangosteen seedlings using different *Garcinia* sp. as nurse stocks. Various nurse stocks had a considerable effect on growth characters of mangosteen seedlings (Table 105). With regard to increase in plant height, mangosteen seedlings having its own seedlings as nurse stock recorded the highest value (6.26 cm) after eighteen months period followed by seedlings with *G. xanthochymus* (4.79 cm) as nurse stock. Mean increase in height of seedlings was comparatively less with other nurse stocks such as *G. hombroniana* (4.04 cm), *G. indica* (3.73 cm), *G. gummi-gutta* (3.41 cm) and *G. cowa* (2.08 cm) compared to 4.31 cm of control (mangosteen seedling without any nurse stock).

In the case of mean leaf production also nurse stocks induced a similar effect on seedling growth. The highest mean leaf production was noticed in seedlings with *Garcinia mangostana* as nurse stock (7.64) followed by *G. xanthochymus* nurse stock (6.70). The lowest mean leaf production (2.50) was noticed in seedlings with *G. cowa* as nurse stock. A similar trend was observed in the case of mean leaf area and total mean leaf area in which seedlings with *Garcinia mangostana* nurse stocks showing highest values of 52.40 cm^2 and 840.84 cm^2 respectively for these parameters and the lowest values of 16.47 cm^2 and 139.20 cm^2 respectively by *G. cowa* nurse stocks.

Table 105. Influence of nurse stock on growth of mangosteen

Nurse stock species	Growth after 18 months of grafting								
	Mean increase in height (cm)	Mean leaf production	Mean leaf area (cm^2)	Total number of leaves	Mean total leaf area (cm^2)	Mean spread (cm)		Mean internodal length (cm)	Mean number of branches
						EW	NS		
Garcinia mangostana	6.26	7.64	52.40	16.04	840.84	31.05	25.69	1.69	2.09
G. indica	3.73	5.50	22.79	8.89	202.55	19.85	17.63	1.30	1.00
G. hombroniana	4.04	4.73	34.08	11.47	391.09	27.56	22.36	1.09	0.89
G. gummi-gutta	3.41	5.64	37.09	11.83	439.11	26.86	22.33	1.25	1.80
G. xanthochymus	4.79	6.70	37.05	12.94	479.73	25.36	19.68	1.08	1.11
G. cowa	2.08	2.50	16.47	8.45	139.20	19.26	15.94	0.70	0.88
Control (mangosteen seedlings)	4.31	4.57	22.54	13.57	305.98	21.97	17.31	1.33	0.14

Mean canopy spread in EW and NS directions were also highest (31.05 cm and 25.69 cm respectively) in seedlings with *Garcinia mangostana* as nurse stocks and lowest (19.26 cm and 15.94 cm respectively) in seedlings with *G. cowa* as nurse stock. Mean intermodal length also followed a similar pattern with highest value of 1.69 cm recorded in the former and the lowest value of 0.70 cm in the latter. Mean number of branches varied from 0.14 (control) to 2.09 in seedlings with *Garcinia mangostana* as nurse stocks.

The growth of the mangosteen seedlings grafted with different nurse stocks was compared with that of seedlings (control) of same age. Control treatment showed higher values for all the parameters studied compared to seedlings with *G. cowa* as nurse stock except in the case of mean number of branches which was less compared to the latter. In the case of all other characters, control showed intermediary values compared to seedlings with nurse stocks. But seedlings with *Garcinia mangostana* nurse stock was superior to all other treatments for all the parameters under study indicating that seedling growth can be promoted considerably by nurse stock grafting with *Garcinia mangostana* seedlings (Plate 8).

4.3.2 Use of related species as rootstock

In addition to seedlings of *Garcinia mangostana,* related species *viz. G. indica, G. hombroniana, G. gummi-gutta, G. xanthochymus and G. cowa* were used as rootstocks. Softwood grafting was followed and growth of grafts was recorded bimonthly. The results of the experiment are presented in Tables 106 and 107.

Grafting studies were taken up during the months of July – August on two year old rootstocks using precured and non-precured scions taken from orthotropic and plagiotropic shoots. No difference in scion growth and success percentage of grafts was observed between scion types used.

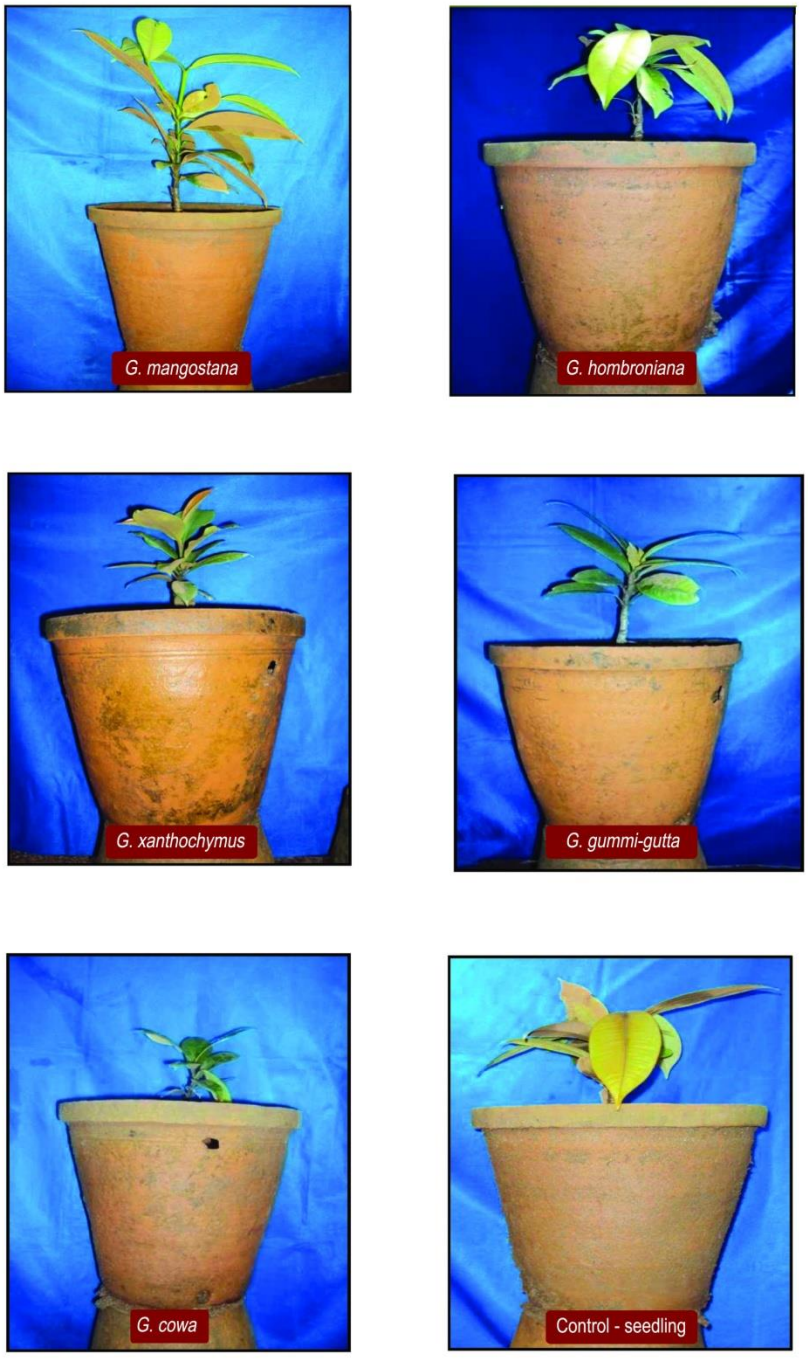


Plate 8. Performance of nurse stock grafts

Table 106. Success of grafting mangosteen using mangosteen and its related species as rootstock

Species	Time taken for sprouting of scions (days)	Percentage sprouting	Percentage of final grafting success	Remarks
Garcinia mangostana	24.40	93.33	83.33	Of the total grafts, 16.67 per cent grafts dried within a period of seven months of grafting.
G. indica	34.00	53.33	2.22	Of the total grafts, 97.78 per cent grafts dried within a period of 77 days of grafting.
G. hombroniana	57.50	86.84	55.26	Of the total grafts, 44.74 per cent of grafts dried within a period of one year of grafting.
G. gummi-gutta	31.40	71.88	0.00	All the sprouted grafts dried within a period of 50 days.
G. xanthochymus	36.10	84.21	21.05	Of the total grafts, 78.95 per cent grafts dried within a period of one year after grafting.
G. cowa	26.50	14.29	0.00	All the sprouted grafts dried within a period of 31days after grafting.

Table 107. Influence of rootstocks on growth of mangosteen grafts

Species	Growth after 18 months of grafting					
	Mean leaf production	Mean leaf area (cm^2)	Total number of leaves	Mean total leaf area (cm^2)	Mean total scion or shoot growth (cm)	Remarks
Garcinia mangostana	5.20	34.41	5.20	178.93	2.36	
G. indica	4.00	3.10	4.00	12.40	0.50	
G. hombroniana	3.60	11.43	3.60	41.15	0.76	
G. gummi-gutta	0.00	0.00	0.00	0.00	0.00	No survival of grafts at 18 month stage
G. xanthochymus	4.25	7.79	4.25	33.12	0.89	
G. cowa	0.00	0.00	0.00	0.00	0.00	No survival of grafts at 18 month stage
Control (mangosteen seedlings)	6.00	22.21	14.35	318.71	4.72	Even though leaf production for 18 months was only 6.00, the total number of leaves in the seedlings was 14.35 after 18 months.

The average time taken for sprouting of grafts ranged from 24.40 days to 57.50 days among the different rootstocks used for the study (Table 106). Scions grafted on *Garcinia mangostana* took least time for sprouting (24.40 days) followed by *G. cowa* (26.50 days), *G. gummi-gutta* (31.40 days), *G. indica* (34.00 days) and *G. xanthochymus* (36.10 days). The maximum time for sprouting of grafts was observed in *G. hombroniana* (57.50 days) rootstocks.

Wide variation was also observed in the percentage of sprouting of grafts. Out of the total grafting carried out, 93.33 per cent scions sprouted when *Garcinia mangostana* was used as the rootstock. This was followed by *G. hombroniana* (86.84 %), *G. xanthochymus* (84.21 %), *G. gummi-gutta* (71.88 %) and *G. indica* (53.33 %). Percentage of sprouting was minimum (14.29 %) when *G. cowa* was used as rootstock.

The highest success percentage of grafting (83.33 %) was recorded in *Garcinia mangostana* rootstock followed by *G. hombroniana* (55.26 %), *G. xanthochymus* (21.05 %) and *G. indica* (2.22 %). In the case of *G. gummi-gutta* and *G. cowa*, no grafts survived after a period of two year of study. In both these cases, all the sprouted grafts showed no further growth after sprouting and dried completely within a period of 50 days (*G. gummi*-gutta) and 31 days (*G. cowa*) of grafting. In the case of *G. indica* also, sprouted grafts showed stunted growth and 97.78 per cent grafts dried within a period of 77 days of grafting.

Various rootstocks had a significant effect on growth characters of the grafts (Table 107). With regard to mean leaf production, leaf area, total leaf area and scion growth for a period of 18 months after grafting, the maximum values (5.20, 34.41 cm^2, 178.93 cm^2 and 2.36 cm respectively) were recorded in grafts with *Garcinia mangostana* as rootstock. Even though grafts with G. *xanthochymus* as rootstock were having a mean leaf production of 4.25 leaves for the period, the leaf size was

very small (7.79 cm^2) with a mean total leaf area of 33.12 cm^2. The scion growth was only 0.89 cm indicating a stunted growth with short internodes. Grafts with *G. hombroniana* as rootstock produced a mean number of 3.60 leaves but with a slightly higher mean leaf area (11.43 cm^2) and mean total leaf area (41.15 cm^2). Similar to *G. xanthochymus* rootstocks, growth was poor with a scion growth of 0.76 cm for the period. A similar growth habit was also observed in grafts with *G. indica* as rootstock. These grafts recorded the lowest values for mean leaf area (3.10 cm^2), mean total leaf area (12.40 cm^2) and scion growth (0.50 cm). As none of the grafts survived till the end of the study where *G. gummi-gutta* and *G. cowa* were used as rootstocks these parameters could not be measured in such cases.

The growth of the grafts was compared with that of seedlings of same age. Seedlings had higher values for all the parameters studied compared to grafts except for mean leaf area. Mean shoot growth was double (4.72 cm) in the case of seedlings compared to grafts with *G. mangostana* (2.36 cm) as rootstock during the period of study. Mean leaf production for the period was also higher for seedlings (6.00) compared to grafts (5.20). Mean leaf area alone was slightly lower (22.21 cm^2) for the seedlings compared to grafts (34.41 cm^2).

These results indicate that among the rootstocks studied for enhancing growth of mangosteen plants, mangosteen is compatible only with its own rootstock (*G. mangostana*) only and all other rootstocks showed varying degrees of incompatibility in terms of success of grafting and growth rate. Further, on comparison of growth of grafts with seedlings, seedlings had a slightly better growth rate with respect to shoot growth, leaf production and total leaf area during the period of study (Plate 9).

4.3.3 Rooting of softwood cuttings

Studies were taken up on rooting of cuttings. Softwood cuttings from juvenile trees were raised in the normal potting mixture. Eighty six per cent of cuttings rooted within a period of two months even without the use of any growth regulators. The rooted cuttings also had profuse root growth with large number of lateral roots compared to seedlings (Plate 10).

4.4 Experiment IV

4.4.1 Induction of variability through induced mutation and polyploidy

Experiments were undertaken to induce variability in mangosteen through induced mutation and polyploidy and the results of the study are presented hereunder.

4.4.1.1 Gamma irradiation

Gamma irradiation was done using Cobalt-60 as the source. Based on percentage germination of seeds, LD_{50} value was found. Ten to 100 Gy at an interval of 10 Gy was tried on seeds and it was found that LD_{50} value was around 30 Gy. Doses for the experiment were thus fixed from 5 Gy to 50 Gy at an interval of 5 Gy.

4.4.1.1.1 Seed

Seeds were collected from freshly harvested fruits and used for irradiation. Irradiated seeds were sown on the same day in polythene bags containing potting mixture and growth parameters of the seedlings were studied for one year.

Treatments showed wide variation in days required for germination (Table 108). Seeds irradiated with 5 Gy took minimum time (19.20 days) for germination and as the dosage increased, time required for germination was also increased progressively to 21.00 days (10 Gy), 22.20 days (15 Gy), 76.40 days (20 Gy), 82.40 days (25 Gy) and the maximum time required for germination was noted in 30 Gy dosage (133.60 days). Treatments beyond 30 Gy dosage showed no germination.

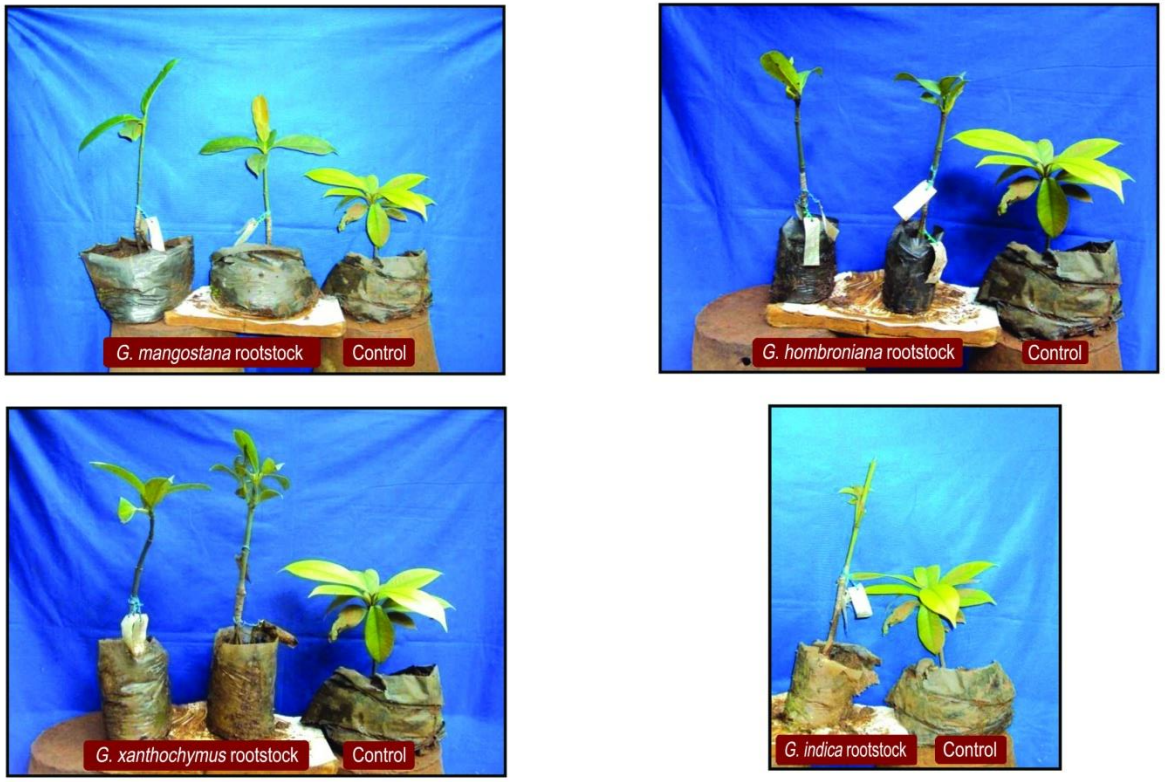
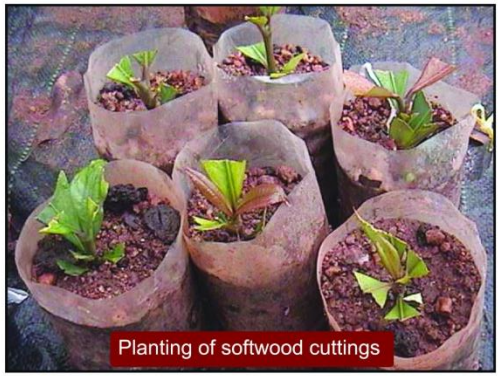


Plate 9. Softwood grafts on various rootstocks



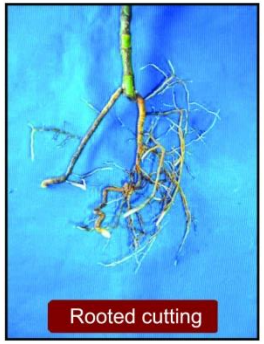


Plate 10.Rooting of cuttings for large-scale production of planting materials

Germination observed in control treatment (21.60 days) was also comparable to that of initial three doses of irradiation.

Seed germination also showed a similar pattern among the treatments. Cent per cent germination was observed in the initial three doses (5, 10 and 15 Gy) and in the control. As the dose of irradiation increased, a decline in germination was noted up to 50 per cent in 30 Gy treatment. Doses beyond 30 Gy completely prevented germination.

Morphologically seedlings of all the treatments were similar with respect to leaf length, breadth, mean leaf area and root length during the entire period of study. But seedlings irradiated with 30 Gy dose had the lowest values for plant height (5.87 cm), leaf production (3.33), total number of leaves (3.33), total leaf area (26.23 cm^2), intermodal length (0.13 cm) and root length (5.75 cm) (Plate 11). To confirm whether such a variation was originated due to changes at genomic level, molecular studies were taken up utilizing the seedlings irradiated with 30 Gy and 25 Gy gamma irradiation, the results of which are presented under the title assessment of genetic variability through RAPD analysis.

Table 108. Growth of mangosteen seedlings raised from gamma irradiated seeds

Dose of irradiation	Days for germination	Germination percentage	Growth of seedlings after 12 months								Root length in two months (cm)
			Mean height (cm)	Internodal length (cm)	Leaf production	Total number of leaves	Size of leaf		Mean leaf area (cm^2)	Mean total leaf area (cm^2)	
							Length (cm)	Breadth (cm)			
5 Gy	19.20	100.00	7.54	0.30	4.00	4.00	5.18	2.57	8.25	33.83	6.00
10 Gy	21.00	100.00	8.68	0.66	5.20	5.20	6.46	2.82	11.25	59.53	6.10
15 Gy	22.20	100.00	8.62	0.63	5.20	5.20	5.86	2.49	9.22	51.74	6.20
20 Gy	76.40	90.00	8.92	0.58	5.20	5.20	6.55	2.96	12.00	64.54	5.85
25 Gy	82.40	90.00	8.66	0.64	5.60	5.60	5.55	2.40	8.50	51.03	6.20
30 Gy	133.60	50.00	5.87	0.13	3.33	3.33	5.40	2.47	8.38	26.23	5.75
35 Gy	--	0.00	--	--	--	--	--	--	--	--	--
40 Gy	--	0.00	--	--	--	--	--	--	--	--	--
45 Gy	--	0.00	--	--	--	--	--	--	--	--	--
50 Gy	--	0.00	--	--	--	--	--	--	--	--	--
Control	21.60	100.00	8.86	0.78	5.20	5.20	6.18	2.70	10.71	55.70	5.90

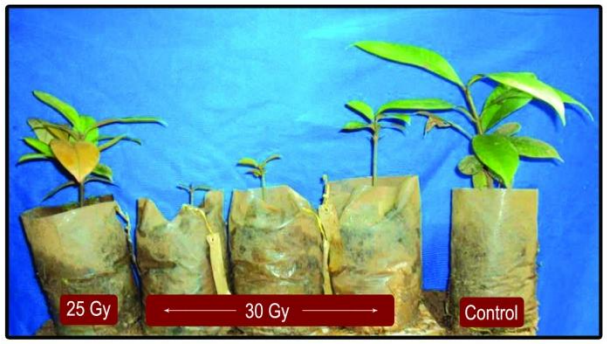


Plate 11. Mangosteen seedlings raised from gamma irradiated seeds

Plate 12. Growth of mangosteen grafts with irradiated scions

4.4.1.1.2 Scion

Grafting studies were taken up during the months of July – August on two year old rootstocks using gamma irradiated, precured and non-precured scions taken from orthotropic and plagiotropic shoots. Doses for the experiment were fixed from 5 Gy to 50 Gy at an interval of 5 Gy. Growth parameters of the grafts were studied for one year.

The average time taken for sprouting of grafts ranged from 16 to 36 days among the different doses of irradiation used for the study (Table 109). Grafts with untreated scions took least time for sprouting (16 days) and maximum time for sprouting was taken by scions irradiated with 40 Gy irradiation (36 days).

Wide variation was also observed in the percentage of sprouting of grafts and the values ranged from 40 to 100 per cent. Scions treated with 30 Gy, 40 Gy, 45 Gy and 50 Gy recorded the minimum sprouting of 40 per cent. The maximum sprouting of 100 per cent was noted in 15 Gy. The treatments 10 Gy, 20 Gy and control recorded 90 per cent sprouting.

The success percentage of grafting ranged from 0 to 80 per cent. The highest percentage of grafting success (80 %) was recorded in control (untreated scions) followed by 10 Gy (20 %) and 5 Gy and 15 Gy (both 10 %). In all other treatments, no grafts survived after a period of one year of study. In this case, even though the initial success ranged from 40 to 90 per cent, all the grafts dried within a period of 58 (30 Gy) to 180 days (20 Gy).

Table 109. Growth of mangosteen grafts with irradiated scions

Dose of irradiation	Time taken for sprouting of scions (days)	Percentage sprouting	Percentage of final grafting success	Remarks
5 Gy	17.50	80.00	10.00	Of the total grafts, 90.00 per cent grafts dried within a period of 72 days of grafting.
10 Gy	17.89	90.00	20.00	Of the total grafts, 80.00 per cent grafts dried within a period of 126 days of grafting.
15 Gy	29.00	100.00	10.00	Of the total grafts, 90.00 per cent of grafts dried within a period 121days of grafting.
20 Gy	16.89	90.00	0.00	All the grafts dried within a period of 180 days of grafting.
25 Gy	28.00	60.00	0.00	All the grafts dried within a period of 124 days of grafting.
30 Gy	21.00	40.00	0.00	All the grafts dried within a period of 58 days of grafting.
35 Gy	23.80	50.00	0.00	All the grafts dried within a period of 70 days of grafting.
40 Gy	36.00	40.00	0.00	All the grafts dried within a period of 144 days of grafting.
45 Gy	33.50	40.00	0.00	All the grafts dried within a period of 134 days of grafting.
50 Gy	27.00	40.00	0.00	All the grafts dried within a period of 64 days of grafting.
Control	16.00	90.00	80.00	Of the total grafts, 20.00 per cent of grafts dried within a period 69 days of grafting.

The irradiation treatment of scions had a significant effect on the growth of the grafts. All the grafts showed a stunted growth after sprouting and those survived after one year presented an underdeveloped appearance (Plate 12). The leaf production for one year was limited to production of just two undersized leaves without any further extension growth of the shoot. As these grafts were of stunted growth without any leaf production and shoot growth for one year period, they could not be utilized for any further variability studies. The control treatment (grafts with untreated scions) showed a normal growth with a mean production of 3.75 leaves and mean extension growth of 2.12 cm for one year period.

4.4.1.2 Colchicine application

Colchicine was used to induce variability through treating seeds as well as application in growing apex of seedlings. Doses for the experiment were fixed at 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 per cent.

4.4.1.2.1 Seed

Seeds were collected from freshly harvested fruits and used for colchicine treatment. Seeds were soaked overnight in specified concentrations of colchicine solution and sown in polythene bags containing the potting mixture and growth parameters of the seedlings were studied for one year.

Treatments showed some amount of variation in days required for germination (Table 110) and the values ranged from 19.40 days (colchicine 0.1 per cent) to 50.80 days (colchicine 3.0 per cent). Seeds treated with up to 2.0 per cent colchicine and the control showed earlier germination of less than 22 days, while those above 2.0 per cent dosage showed a slightly late germination over 30.0 days.

Germination percentage also showed a similar pattern between the treatments. Hundred per cent germination was observed in control and all the doses of colchicine except two higher doses (3.0 and 3.5 per cent) and 1.5 per cent colchicine. The higher two doses of colchicine showed 80.0 per cent germination and 90.0 per cent germination was observed in 1.5 per cent dose.

Seedlings of all the treatments were identical with respect to different morphological characters studied such as plant height, internodal length, leaf production, size of leaf, mean and total leaf area and root length during the entire period of study. Since distinct morphological variants could not be identified on treating the seeds with various doses of colchicine, the resultant seedlings were not subjected to further molecular studies.

4.4.1.2.2 Bud application

Growing shoot apices of one year old seedlings were treated with specified concentrations of colchicine solution and growth parameters of the seedlings were studied for one year.

Seedlings of all the treatments were identical with respect to different morphological characters studied with overall mean values of 18.99 cm (plant height), 1.55 cm (internodal length), 6.04 (leaf production), 17.33 (total number of leaves), 19.64 cm (length of leaves), 5.85 cm (breadth of leaves), 73.42 cm^2 (mean leaf area), 1294.42 cm^2 (total leaf area) and 6.19 cm (extension of shoot) during the entire period of study (Table 111).

Table 110. Growth of mangosteen seedlings raised from seeds treated with colchicine

Dose of colchicine (%)	Days for germination	Germination percentage	Growth of seedlings after 12 months								Root length in two months (cm)
			Mean height (cm)	Internodal length (cm)	Leaf production	Total number of leaves	Size of leaf		Mean leaf area (cm^2)	Mean total leaf area (cm^2)	
							Length (cm)	Breadth (cm)			
0.1	19.40	100.00	6.60	0.34	4.80	4.80	6.19	2.69	10.76	49.16	5.92
0.5	20.00	100.00	7.36	0.43	5.20	5.20	5.44	2.00	6.84	36.53	5.50
1.0	21.60	100.00	9.58	0.37	6.00	6.00	6.14	2.32	9.36	60.63	5.72
1.5	19.60	90.00	10.94	0.59	6.00	6.00	6.39	2.82	12.37	81.72	5.58
2.0	19.80	100.00	9.06	0.44	6.00	6.00	6.37	2.46	10.66	71.84	5.62
2.5	30.40	100.00	7.96	0.32	5.60	5.60	6.22	2.71	11.49	74.26	5.48
3.0	50.80	80.00	8.08	0.25	5.20	5.20	6.33	2.77	11.67	58.96	5.26
3.5	39.00	80.00	8.84	0.36	5.20	5.20	6.00	2.55	9.69	49.90	5.22
Control	21.40	100.00	9.72	0.36	6.00	6.00	7.54	2.80	13.01	83.82	5.50

Table 111. Growth of mangosteen seedlings treated with colchicine in the shoot apex

Dose of colchicine (%)	Growth of seedlings after 12 months of colchicine application								Extension of shoot for one year period (cm)
	Mean height (cm)	Internodal length (cm)	Leaf production for one year	Total number of leaves	Size of leaf		Mean leaf area (cm^2)	Mean total leaf area (cm^2)	
					Length (cm)	Breadth (cm)			
0.1	18.76	1.29	6.00	17.00	20.64	7.02	91.15	1553.72	5.64
0.5	18.52	1.38	5.60	16.60	18.94	5.56	65.68	1084.25	6.04
1.0	18.80	1.52	5.60	16.20	18.58	5.30	61.65	1002.27	5.84
1.5	18.70	1.54	6.40	17.00	21.56	6.14	83.28	1407.96	5.50
2.0	20.02	1.81	5.60	16.60	18.68	5.16	59.91	1002.80	6.48
2.5	18.60	1.54	6.00	18.40	18.82	5.34	64.46	1183.01	5.92
3.0	19.54	1.90	6.80	18.40	20.72	6.85	91.70	1813.11	6.76
3.5	19.18	1.65	6.40	18.00	19.80	6.03	78.40	1485.77	7.38
Control	18.84	1.38	6.00	17.80	19.06	5.31	64.56	1116.95	6.18
Growth of morphological variants									
3.0 (R2)	25.80	2.12	12.00	24.00	28.20	8.94	156.31	3751.37	11.60
3.5 (R3)	24.50	2.16	10.00	22.00	21.60	8.72	149.22	3282.77	12.80

However two distinct morphological variants were observed among the plants which were treated with two higher dose of colchicine (Plate 13). One replication (R2) treated with 3.0 per cent colchicine solution produced a vigorous plant with higher values for all the characters studied. Similarly another replication (R3) treated with 3.5 per cent colchicine solution also recorded identical values of the first variant. For these two variants, higher values were observed compared to rest of the seedlings for all the parameters recorded such as plant height (25.80 cm & 24.50 cm), internodal length (2.12 cm & 2.16 cm), leaf production (12.00 & 10.00), total number of leaves (24.00 & 22.00), length of leaves (28.20 cm & 21.60 cm), breadth of leaves (8.94 cm & 8.72 cm), mean leaf area (156.31 cm^2 & 149.22 cm^2), total leaf area (3751.37 cm^2 & 3282.77 cm^2) and extension of shoot (11.60 cm & 12.80 cm) during the entire period of study.

To confirm that such a variation was originated due to changes at genomic level, molecular studies were taken up utilizing these two morphological variants, the results of which are presented under the title assessment of genetic variability through RAPD analysis.

4.4.2 Assessment of induced variability through RAPD analysis

4.4.2.1 Characterization of mangosteen samples using RAPD markers

Out of the eleven primers used, only OPA-18 and OPC-03 yielded scorable bands. The genomic DNA isolated from the following treatments was subjected to RAPD analysis using the two selected primers in order to assess the variability induced if any.

S1: Colchicine 3.0 %

S2: Colchicine 3.5 %

S3: γ irradiation 30 Gy

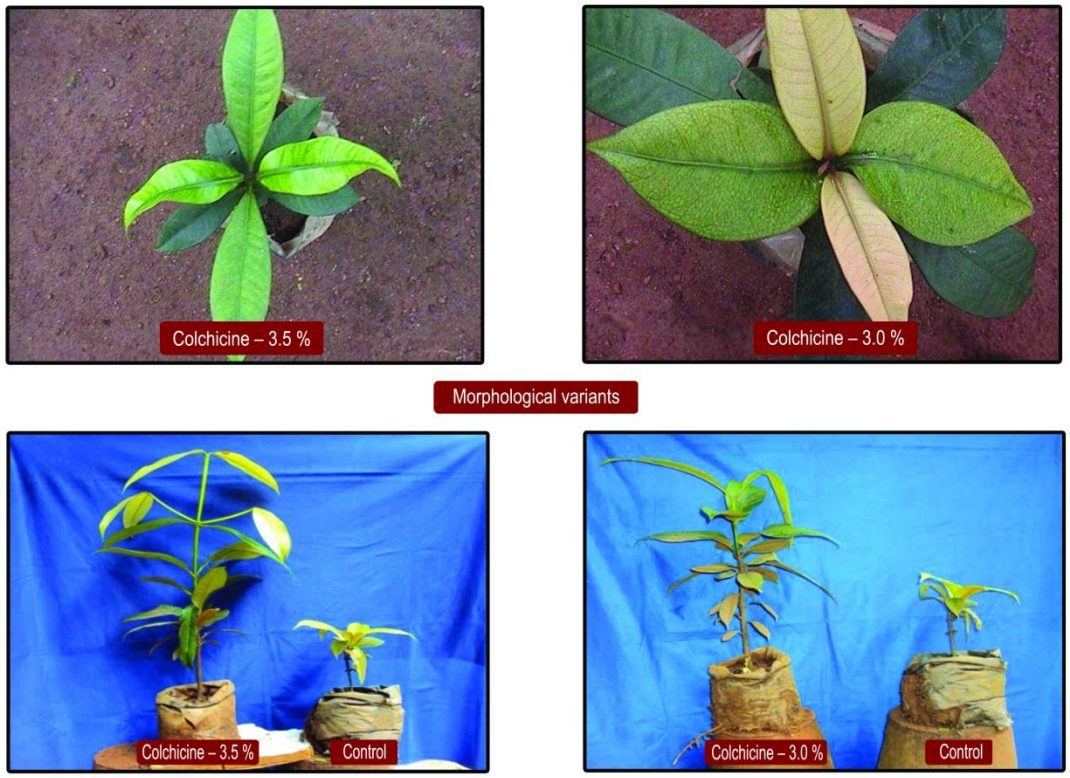


Plate 13. Mangosteen seedlings treated with colchicine in the shoot apex

S4: γ irradiation 25 Gy

S5: Control

The two random primers yielded 29 scorable amplicons (Table 112, Plate 14 and 15) of which 14 (48.28 per cent) were polymorphic and 15 bands were monomorphic (51.72 per cent). Dendrogram was drawn based on NTSYS Version 2.01 software to study the interrelationships (Fig. 11). The pooled data for Jaccard's Similarity Coefficient values ranged from 0.33 to 0.75 (Table 113).

Similarity coefficients (Table 113) were used to construct UPGMA dendrogram. The dendrogram constructed based on RAPD bands yielded two groups viz. Group I and Group II which split at a coefficient of 0.46. Group I consisted of two clusters. Maximum similarity of 75.00 per cent was observed between S2 (colchicine 3.5 %) with S4 (γ irradiation 25 Gy) in the cluster II of Group I as well as S3 (γ irradiation 30 Gy) of cluster I with S4 of cluster II. S3 (γ irradiation 30 Gy) alone formed cluster I with 73.00 per cent similarity with cluster II entities. S1 (colchicine 3.0 %) and S5 (control) formed a single cluster in Group II. Least similarity value (33.33 per cent) was obtained between S2 (colchicine 3.5 %) and S5 (control). S5 being the control treatment, clustering of the other treatments indicated that seed irradiation with 25 Gy and 30 Gy gamma rays and bud application of colchicine 3.5 per cent were effective in inducing variation in genomic DNA of mangosteen.

Table 112. RAPD primers used, total number of bands and percentage of polymorphism

Sl. No.	Primer code	Primer sequence	Total bands	Number of polymorphic bands	Number of monomorphic bands	Percentage of polymorphism
1	OPC-03	GGGGGTCTTT	16	11	5	68.75
2	OPA- 18	AGGTGACCGT	13	3	10	23.08
		Total	29	14	15	48.28

Table 113. Genetic similarity index among mangosteen variants for selected random primers

Sl. No.	Variants	S1	S2	S3	S4	S5
1	S1	1.0000				
2	S2	0.5000	1.0000			
3	S3	0.5000	0.7143	1.0000		
4	S4	0.3750	0.7500	0.7500	1.0000	
5	S5	0.5000	0.3333	0.5000	0.5556	1.0000

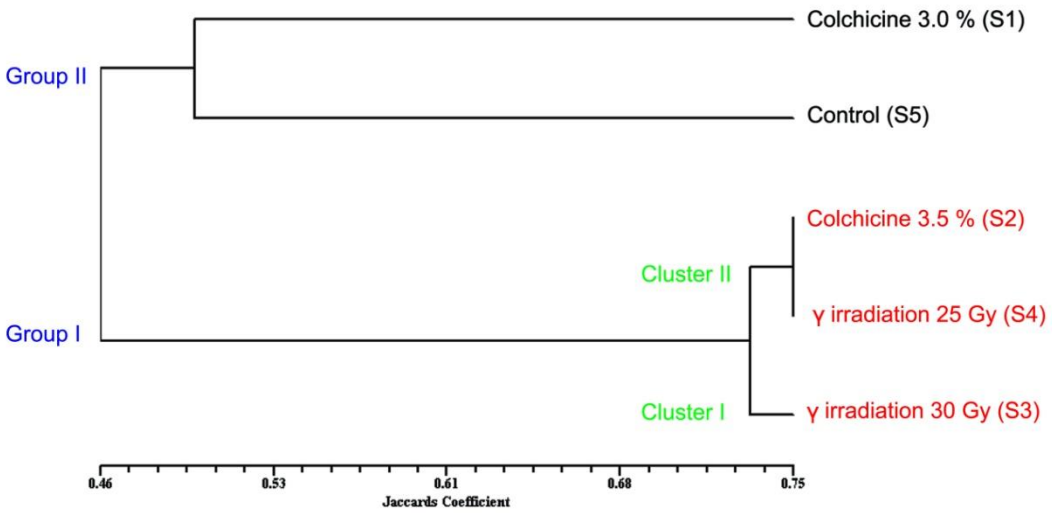


Fig. 11. Dendrogram of mangosteen variants from pooled RAPD data using UPGMA clustering

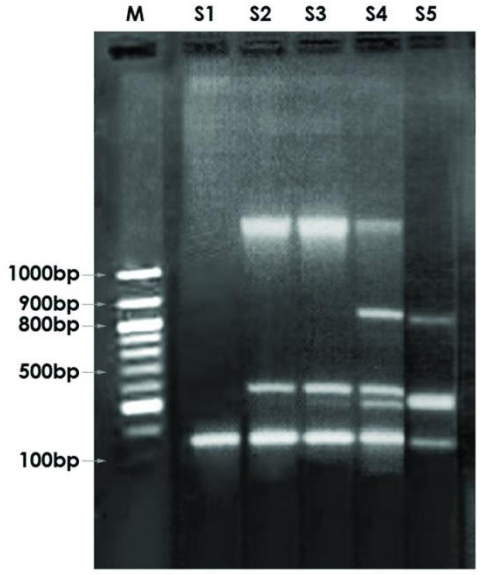


Plate 14. RAPD profile for mangosteen variants with primer OPC-03

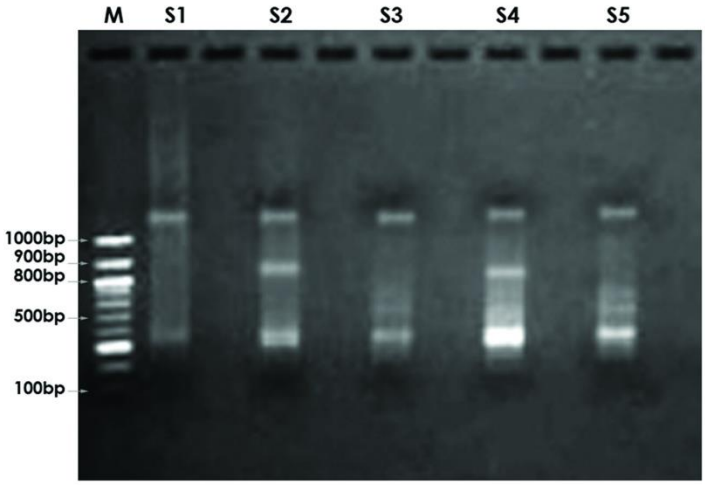


Plate 15. RAPD profile for mangosteen variants with primer OPA-18

Lane M: Marker DNA, S1: Colchicine 3.0 %, S2: Colchicine 3.5 %,
S3: γ irradiation 30 Gy, S4: γ irradiation 25 Gy, S5: Control

Discussion

5. DISCUSSION

Mangosteen is presumed to have originated in South – East Asia probably in the Indonesian region and has been in cultivation for a considerable time in various parts of humid tropics. The major producing countries are located in South- East Asia, namely Thailand, Malaysia, Philippines and Indonesia. Thailand is the world's largest producer with an approximate production of 240,000 metric tons annually with exports recorded at 15,000 MT in 2006. About 85 per cent of the total production of the four countries is in Thailand (Diczbalis, 2009).

Among the cultivated tropical tree fruits, mangosteen is perhaps one of the most localised in relation to its centre of origin, habitat and area of cultivation. The tree has remained centred around its original habitat possibly due to recalcitrant seeds that quickly lose their viability. The long gestation period is also a major constraint to the spread of its cultivation.

Mangosteen is now gaining demand in the international markets mainly due to its much acclaimed health benefits such as antioxidant properties and prophylactic action on many of the degenerative diseases. Demand always exceeds production and hence the crop fetches a premium price even in local markets. Consequently a renewed interest has been generated in its cultivation especially in Kerala and neighbouring states of Karnataka and Tamil Nadu. It yields profusely and also fits well as a component crop in the homesteads of Kerala. The foremost step to popularise its cultivation is by developing techniques to overcome its slow growth and consequent long pre-bearing period.

The present investigation was envisaged to develop techniques for accelerating seedling growth, reducing gestation period and inducing variability through mutation and polyploidy in mangosteen. The results of the study are discussed in the ensuing pages.

5.1 Enhancing seedling growth in nursery

Mangosteen seedlings are extremely slow growing in the nursery and the slow growth is attributed to poor root development especially the development of lateral roots. The crop is reported to be devoid of root hairs, the vital link responsible for absorption of nutrients and water. This slow growth necessitates the seedlings to be kept in the nursery for about two to three years so that they are sufficiently developed for transplanting to the main field. The present study indicated that different treatments have a significant influence in accelerating growth of mangosteen seedlings in the nursery. The results of various treatments employed in this study to enhance the growth of seedlings are discussed here under.

5.1.1 *Effect of media and growth regulators*

Experiments were conducted to find out the effect of growing media and application of growth regulators to enhance growth and to reduce juvenile phase of mangosteen seedlings. Seedlings were raised in polythene bags with four different media containing either well rotten cow dung, poultry manure, vermicompost or enriched coir pith compost as organic manure. Four growth regulators (IAA, IBA, GA and BA) at three concentrations (150, 300 and 450 ppm for IAA and IBA; 100, 200 and 300 ppm for GA and BA) were used for the study. The results showed that different media as well as growth regulators had a significant influence on plant characters, physiological parameters, nutrient content and uptake nutrients in of mangosteen seedlings.

5.1.1.1 *Plant, leaf and root characters*

The interaction was significant between media and growth regulators with respect to all the characters studied except for petiole length and number of branches. In the case of these parameters also significant variation was observed among the media as well as among the growth regulators.

Plant height is an important character that describes overall growth of the plant and it was found that different media had a significant influence on plant height. Among the four media compared, vermicompost was found to be the most significantly superior growing medium recording the highest plant height (46.70 cm) by the end of fifteen months. Influence of coir pith compost and cow dung on plant height was uniform but effect of poultry manure was relatively less compared to other media. Similar results were reported in the case of strawberry (Arancon *et al.,* 2004) and hot pepper (Arancon *et al.,* 2005) where vermicompost applications increased growth and yield significantly.

In the case of vermicompost medium, medium alone without any application of growth regulators recorded the highest plant height and was homogeneous with rest of the treatments using various growth regulators. This indicates that use of vermicompost medium itself without any additional use of growth regulators is sufficient enough to enhance the growth of mangosteen seedlings significantly in the nursery stage. Vermicompost contains major and minor nutrients in plant available forms, enzymes, vitamins and plant growth hormones (KAU, 2007) and this may be the possible reason for the superior performance of the seedlings in vermicompost medium.

In the case of normal potting mixture containing well rotten cow dung, all the treatments were uniform and hence additional use of growth regulators is not necessary to improve height of the seedlings. However, when media containing

poultry manure and coir pith compost were used, use of several growth regulators was found to significantly enhance the plant height compared to use of media alone. Use of IAA (all concentrations) and IBA (150 and 450 ppm) could enhance seedling growth in poultry manure medium. All concentrations of BA, two lower concentrations (150 and 300 ppm) of IAA, two higher concentrations (300 and 450 ppm) of IBA and GA 100 and 300 ppm were found to be effective in enhancing seedling growth significantly in coir pith compost medium. Similar results are reported by Yusuf (2002) in a study on selection index and activation of seedling growth in mangosteen and the study revealed that the best treatment for enhancing seedling growth was GA 150 mg/l followed by IAA 250 mg/l.

A similar trend was noticed in the case of internodal length, a parameter related to height of the plant. Among the media, vermicompost medium recorded maximum internodal length (3.46 cm) which was significantly superior to all other media. Effect of all other media on internodal length was identical. In the case of vermicompost medium, effect of medium alone was homogeneous with other superior treatments using growth regulators. This indicates that use of vermicompost medium itself without any additional use of growth regulators is sufficient to enhance the growth of mangosteen seedlings in terms of internodal length and ultimately height of the plant.

However, in the case of other three media, use of several growth regulators was found to significantly enhance the internodal length compared to use of media alone. At the end of fifteen month period, in the case of medium containing well rotten cow dung, use of IBA 450 ppm was found to enhance internodal length significantly compared to use of media alone. IBA 150 ppm and all concentrations of GA (100, 200 and 300 ppm) were more effective than medium alone in improving internodal length in poultry manure medium. Two higher concentrations of IAA (300 and 450 ppm), IBA 300 ppm, GA 100 and 300 ppm were the treatments inducing a

similar effect in medium containing coir pith compost. Similar results are reported by Yusuf (2002). Gibberellins characteristically increase growth by cell elongation and increasing the internodal growth (Hull and Lewis, 1959; Davies and Holmes, 1962; Ak *et al.,* 1995) indifferent plant species. They are known to increase plant height also (Marth and Mitchell, 1961; Marth *et al.,* 1965; Shreve and Campbell, 1967; Taylor, 1972).

Mangosteen seedlings started branching after six months of imposing treatments. Though the interaction between media and growth regulator was significant in initial period, it was not evident towards the end of the experiment. Among the media, highest number of branches was noticed in media containing well rotten cow dung (1.45), but it was homogeneous with media containing poultry manure and vermicompost. However production of branches was significantly lower in coir pith compost containing media. Different growth regulators also significantly influenced branch production with BA being the most superior treatment. BA 200 ppm recorded the highest number of branches per plant (2.45), while its higher concentration (300 ppm) was the next superior treatment with a production of 2.00 branches per plant. These results are in agreement with the report of Franchlet (1981) that cytokinins are responsible for multiple shoot production.

Plant spread was also influenced by both media and growth regulators. Among the four media, vermicompost was the most significantly superior growing medium recording the highest plant spread (52.90 cm) by the end of fifteen months. Comparison of effect of other media on plant spread showed that media containing well rotten cow dung and coir pith compost were having a uniform influence on plant spread, but effect of poultry manure medium was significantly lower compared to other media. In the case of vermicompost medium, effect of medium alone was homogeneous with other superior treatments using growth regulators such as IAA 450 ppm and BA 200 ppm. This indicates that use of vermicompost medium itself

without any additional use of growth regulators is sufficient to enhance the growth of mangosteen seedlings in terms of plant spread.

In normal potting mixture containing well rotten cow dung, all the treatments were uniform and hence additional use of growth regulators was not necessary to improve spread of the seedlings. However, when media containing poultry manure and coir pith compost were used, use of several growth regulators was found to significantly enhance the plant spread compared to use of media alone. Use of all doses of IAA and IBA 150 ppm in poultry manure medium and use of IAA 300 ppm in coir pith compost medium was found to be effective in enhancing plant spread significantly in both directions compared to use of media alone.

Even though, media and growth regulator interaction was found significant only towards the end of the experiment with respect to number of leaves, significant difference was observed among different media and among growth regulators in leaf number during the entire period of study. During the initial period up to six months, both vermicompost and coir pith compost media were equally effective in number of leaves and thereafter influence of vermicompost medium was significantly superior to all other media having the highest number of leaves (27.00) towards the end of the experiment. Comparison of other media revealed that all the three media were uniform in leaf number.

Some of the growth regulators evoked a greater response than media alone in number of leaves. In vermicompost medium, effect of all growth regulators except BA 200 ppm (33.0 leaves) was uniform with media alone (27.00 leaves) in leaf number indicating that use of BA 200 ppm can significantly improve leaf number in vermicompost medium. In medium containing well rotten cow dung, BA 200 ppm (31.00 leaves), BA 300 ppm (24.60 leaves) or IBA 150 ppm (23.80 leaves) showed superiority over medium alone (16.60 leaves) emphasizing their importance for

improving number of leaves in the plant. BA 300 ppm (30.40 leaves) and IAA 150 ppm (26.40 leaves) showed their superiority over media alone (18.00 leaves) in leaf number in medium containing poultry manure. Similar to their effect in medium containing well rotten cow dung, two higher concentrations of BA (200 and 300 ppm) and IAA 300 ppm (25.60 leaves) recorded more number of leaves (28.60 and 25.80 leaves respectively) than medium alone (17.60 leaves) when coir pith compost was used in the medium underlining their importance in improving leaf number in this medium.

In general BA treatments were found to be more effective in influencing number of leaves in a plant irrespective of the medium used. BA is reported to delay senescence (Lassoie and Hinckley, 1991) and this might be the probable reason for retention of more leaves in the plant by the treatments containing BA.

Among the four media compared, vermicompost media was superior in terms of total leaf production (20.00 leaves) for a period of fifteen months compared to other media. Leaf production in vermicompost without any growth regulator was on par with other treatments involving growth regulators inferring that medium alone is sufficient for the production of maximum number of leaves.

It was evident that treatments involving BA were able to induce a greater response compared to other growth regulators in production of leaves in mangosteen seedlings. It is reported that leaf growth and development are under the control of endogenous ratios of GA to cytokinins (Letham *et al.*, 1982; Renfroe and Brown, 1983). Further, Franchlet (1981) showed that cytokinins are responsible for multiple shoot production. This might be the possible reason for the higher leaf production per plant as there are more number of shoots per plant, there will be more number of leaves as well. The study also revealed that all the media were uniform in total leaf senescence for a period of fifteen months.

The different media and growth regulators showed a significant influence on mean as well as total leaf area during the entire period of study and their interaction was evident towards the end of the experiment. Among the four different media compared, vermicompost medium was found to be the most significantly superior growing medium recording the highest total leaf area (3275.98 cm^2) by the end of fifteen months. Influence of other two media on total leaf area was uniform but effect of poultry manure medium was relatively less compared to other media. In the case of vermicompost medium, effect of medium alone was homogeneous with rest of the treatments using various growth regulators. This indicates that use of vermicompost medium itself without any additional use of growth regulators is sufficient to enhance the total leaf area of mangosteen seedlings significantly. This is in line with the report of Arancon *et al.* (2004, 2005) that vermicompost applications significantly increased leaf area in strawberry and hot peppers.

In the case of normal potting mixture containing well rotten cow dung, all the growth regulators were uniform and hence additional use of growth regulators is not required to improve total leaf area of the seedlings. However, when media containing poultry manure and coir pith compost were used, use of several growth regulators was found to significantly enhance the total leaf area compared to use of media alone. In poultry manure medium, use of IAA 150 and 300 ppm and IBA 150 ppm was found to be effective in enhancing total leaf area significantly compared to use of media alone. In medium containing coir pith compost, in addition to IAA 150 and 300 ppm, all concentrations of IBA and BA were found to be effective in significantly improving total leaf area compared to use of media alone towards the end of the experiment.

The slow growth of the mangosteen seedlings is attributed to its poor root system which is very scanty with few laterals. Root hairs are also apparently absent at all stages of growth. Hence a faster growth of seedlings could be achieved by

inducing production of more roots in seedlings thereby leading to greater absorption of water and nutrients by the plant. In this context, the present study assumes significance and it was found that both media and growth regulators significantly influence different root parameters like number of roots, its spread and length.

Among the media, highest number of primary (25.67), secondary (50.00), tertiary (22.33) and total roots (98.00) was observed in vermicompost medium towards the end of the experiment. In addition to plant nutrients, vermicompost also contains plant hormones (KAU, 2007) and the presence of these hormones might have encouraged production of more roots by the plant. Arancon *et al.* (2004; 2005) also reported that application of vermicompost had significantly increased the growth of strawberry, marigold and pepper roots. The overall growth of mangosteen seedlings was superior in vermicompost medium compared to other media and the presence of more roots which help in enhanced absorption of nutrients may be one of the reasons for this better growth.

Comparison of influence of other media on root production revealed that media containing coir pith compost and poultry manure had a relatively higher production of roots compared to conventional medium containing well rotten cow dung. Normally seedlings are raised in this conventional medium and the present study revealed that use of medium containing vermicompost, coir pith compost and poultry manure can significantly improve root production in mangosteen seedlings.

In all the media, growth regulators had a significant influence on root production compared to use of media alone. In vermicompost medium, IAA 150 ppm, BA 100 and 200 ppm were significantly superior to medium alone in root production, while in medium containing coir pith compost, all growth regulators except GA 300 showed a significantly superior effect on root production. In the case of conventional potting mixture, IAA 300 ppm, all concentrations of IBA, GA 100

ppm, BA 200 and 300 ppm were found to be the significantly superior growth regulators that can improve root production compared to medium alone. In medium containing poultry manure, except two higher concentrations of GA and BA 200 ppm, all other growth regulators significantly improved root production compared to use of medium alone.

A similar trend was observed in the case of root length and spread with vermicompost medium being the most superior among all the media recording a length of 29.30 cm and spread of 25.50 cm. Effect of other media was on par. Various growth regulators did not show any superiority in vermicompost medium indicating that, in this medium, use of growth regulators is not required to improve root length. But in other media, use of several growth regulators had a profound influence on root length. In general IBA was more effective than other growth regulators in improving root length. This is in conformity with the report of Thimann (1977) that application of auxins generally stimulates root growth. Root spread was also significantly influenced by different growth regulators and their effect varied widely between media indicating that media - growth regulator interaction was more pronounced in the case of spread than length.

The different media and growth regulators significantly influenced fresh weight of various plant parts. Among the media, vermicompost medium was found be the most significantly superior medium recording highest fresh weight of whole plant, shoot, leaves and roots. This is in line with the report of Arancon *et al.* (2004) that vermicompost application in strawberry increased plant shoot biomass by 37 per cent. Vermicompost contains many growth promoting enzymes and plant hormones in addition to macro and micro nutrients and this may be the possible reason for better growth of the plants in vermicompost medium ultimately resulting in higher biomass for various plant parts. Next to vermicompost, coir pith and cow dung media

performed better with respect to fresh weight of plant parts. Seedlings grown in poultry manure put forth a poor performance with respect to fresh weight.

In vermicompost medium, all concentrations of BA (100, 200 and 300 ppm) were found to be superior in terms of fresh weight among which BA 100 ppm recorded the highest value for all plant parts. Since cytokinin is known to mobilize protein and activate a number of enzymes participating in a wide range of metabolic reactions associated with protein synthesis (Kulaeva, 1979), it might have resulted in higher biomass production in treatments involving BA.

In the case of normal potting mixture with well rotten cow dung, effect of media alone was homogeneous with treatments involving various growth regulators inferring that, in this medium use of growth regulators is not required to improve production of plant biomass. In media containing poultry manure, IBA 300 ppm and in coir pith compost medium, IAA 150 ppm were superior to media alone in enhancing fresh weight of plant parts indicating that these two growth regulators are to be used to accelerate growth of the seedlings through higher biomass production. A very similar trend was observed with respect to dry matter production as well.

5.1.1.2 Physiological parameters

Physiological parameters point towards the efficiency of the plant in terms of growth and yield. The present study took into consideration the physiological parameters like, Leaf Area Index (LAI), Specific Leaf Weight (SLW), Relative Growth Rate (RGR), shoot – root ratio and chlorophyll content of the plants.

LAI is a factor that influence crop growth rate and the rate of dry matter production by a crop will increase as the LAI increases. It quantifies the amount of foliage in a plant canopy. In the present study, media – growth regulator interaction was found to be significant towards the later stages of the experiment and the

influence of all the media on LAI was identical. Considering the effect of all the growth regulators in all the four media, IBA 450 ppm was the most significantly superior growth regulator recording a LAI of 3.410 in vermicompost medium towards the end of the experiment. Higher LAI is effective in increasing the photosynthetic efficiency thereby enhancing growth of the seedlings (Yoshida, 1972).

Specific Leaf Weight was shown to be positively correlated to Transpiration Efficiency in many crop species (Brown and Byrd, 1996). Thicker leaves are usually having higher photosynthetic capacity and plants with a higher SLW are able to synthesis more photo assimilates than those with lower SLW values. In the present experiment, SLW was uniform in all the four media. With respect to growth regulators, no particular trend was noticed in SLW in all the four media.

Relative Growth Rate (RGR) is one of the most widely used techniques of estimating plant growth. Even though media – growth regulator interaction was found to be non-significant in terms of RGR, significant difference was observed between media as well as between growth regulators. Both the media containing vermicompost and coir pith compost were identical in their response to RGR but were superior to other two media. Among the growth regulators, BA 100 ppm recorded the highest RGR (0.00674) at the end of the experiment. Relative Growth Rate is closely associated with dry matter production and as a result the same growth regulator showed relatively higher values for dry matter production in all the four media at the end of the experiment.

The shoot- root ratio is an indication of relative growth of shoot and root portions and media – growth regulator interaction was found to be significant throughout the experiment. The influence of all the media on shoot – root ratio was found to be uniform. In general, at six month stage, the values were low and by twelve months the ratio showed higher values. This might be probably due to the

faster shoot growth compared to root production in the seedlings after a period of twelve months of imposing treatments. With respect to influence of growth regulators, no particular trend was noticed in shoot – root ratio in all the four media.

Chlorophyll content of the leaves influences the photosynthetic rate and ultimately plant growth to a considerable extent. The media - growth regulator interaction was found to be significant in the content of chlorophyll at all stages of the experiment. Medium containing vermicompost was found to be the most significantly superior treatment with respect to chlorophyll 'a', 'b' and total content. Even though a definite pattern could not be observed with respect to influence of growth regulators on chlorophyll content, its content was comparatively higher in all concentrations of BA. BA is said to have the property of retarding chlorophyll degradation in several plants (Kao, 1980; Yu and Kao, 1981) and this may be the reason for higher chlorophyll content in the treatments containing BA.

5.1.1.3 Nutrient content and uptake

Nutrient concentration of plants is a good indication of the overall growth of the plant, assimilate accumulated in the sink of the plant body and the potential of that plant to effectively utilize the nutrient sources provided. The nutrients applied, if properly absorbed and assimilated, only can contribute to the development of any plant. Hence it is essential to study whether treatments have any role in enhancing the absorption of nutrients from various media. Therefore nutrient content in the plant parts as well as the uptake are also of great importance.

The study revealed that media- growth regulator interaction was significant with respect to content of all macro and micro nutrients. Among the four media compared, vermicompost showed higher foliar nutrient contents of K, Ca and micronutrients such as Cu, Mn and Zn whereas cow dung medium was superior only

in N, P and Mg. Media containing coir pith compost was found to be superior only in terms of foliar Fe content.

Among the four media, vermicompost is having a higher nutrient content (1.8 % N, 1.9 % $P_2 O_5$, 1.6 % $K_2 O$) compared to well rotten cow dung (1.0 % N, 0.5 % $P_2 O_5$, 1.0 % $K_2 O$), poultry manure (1.2 – 1.5 % N, 1.4 – 1.8 % $P_2 O_5$, 0.8 – 0.9 % $K_2 O$) and coir pith compost (1.26 % N, 0.06 % $P_2 O_5$, 1.2 % $K_2 O$). In addition to plant nutrients, vermicompost also contains enzymes, vitamins and plant hormones (KAU, 2007). Moreover, seedlings grown in vermicompost also recorded highest values for primary, secondary, tertiary and total number of roots. The growth of these roots was also best in terms of length and spread in vermicompost. It may be inferred that the enhanced production of roots in vermicompost medium might have contributed to the higher nutrient uptake by the seedlings. So the increased availability of plant nutrients in vermicompost medium and large root system of seedlings might have resulted in the enhanced uptake by plants finally resulting in higher foliar nutrient content in this medium. In the case of growth regulator application, a definite pattern of foliar nutrient content was not observed between the treatments and content varied among treatments differently in all the media.

A very similar trend was observed with respect to uptake of nutrients as well with vermicompost recording highest uptake of all macro and micro nutrients among the media. With respect to growth regulators, in vermicompost medium all concentrations of BA were found to be superior compared to other growth regulators in terms of uptake of majority of nutrients. In the case of medium containing well rotten cow dung and poultry manure, all the treatments were found to be uniform with respect to uptake of majority of nutrients. A definite pattern of nutrient uptake was not observed between different growth regulator treatments in the medium containing coir pith compost and uptake of nutrients varied among treatments differently in the media.

Normally mangosteen seedlings are kept in the nursery for a minimum period of two to three years before transplanting to the main field (Diczbalis, 2009). By this time, they attain a height of about 25 cm with sufficient canopy growth. If the seedlings are transplanted before this stage, growth of the plants as well as establishment in the field will be poor. In the present study, seedlings raised in vermicompost medium alone and along with the application of GA and BA recorded a height above 25 cm with vigorous growth even by ninth month of experiment. Considering the fact that age of the seedlings was six month at the beginning of the experiment, the treated seedlings were ready for transplanting to the main field when they are 15 months old, at least nine months before the normal time required for transplanting. Hence a long period of nine months can be saved in the nursery by giving proper growth medium and growth regulator application. Seedlings raised in coir pith compost medium also attained this height when seedlings were 18 months old. Control plants did not attain this height even at the end of the study when seedlings were 21 months old, once again underlining the effect of growing media and growth regulators in promotion of growth of mangosteen seedlings. So with the use of suitable media and growth regulator application, seedlings can be transplanted to the main field much early by enhancing seedling growth in the nursery (Fig. 12 to 15).

On considering the overall growth of the seedlings for a period of fifteen months in four media, it may be suggested that, vermicompost medium was found to be the most superior in terms of all the growth and physiological parameters (Plate 16), foliar nutrient content and uptake of nutrients followed by coir pith, poultry manure and well rotten cow dung.

Considering the overall effect of various growth regulators studied, it was observed that, in general, all concentrations of BA were more effective than other

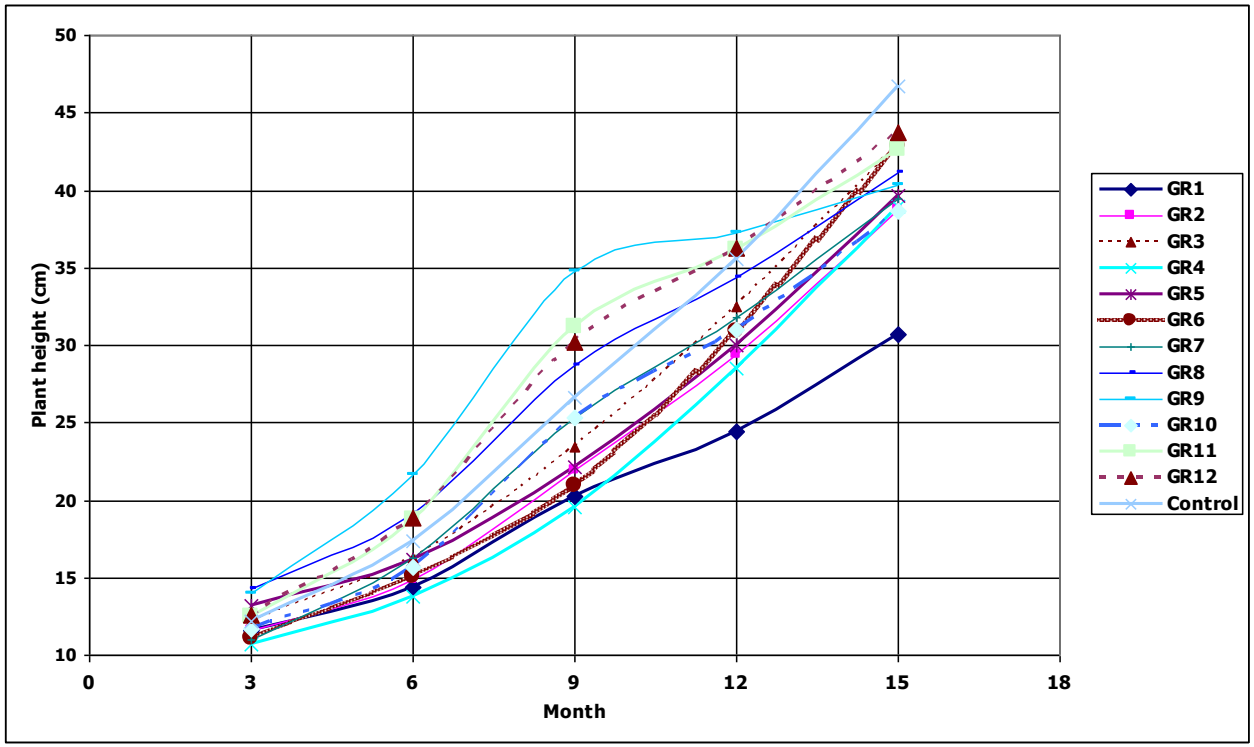


Fig. 12. Influence of growth regulators on plant height of mangosteen seedlings in vermicompost medium during different periods

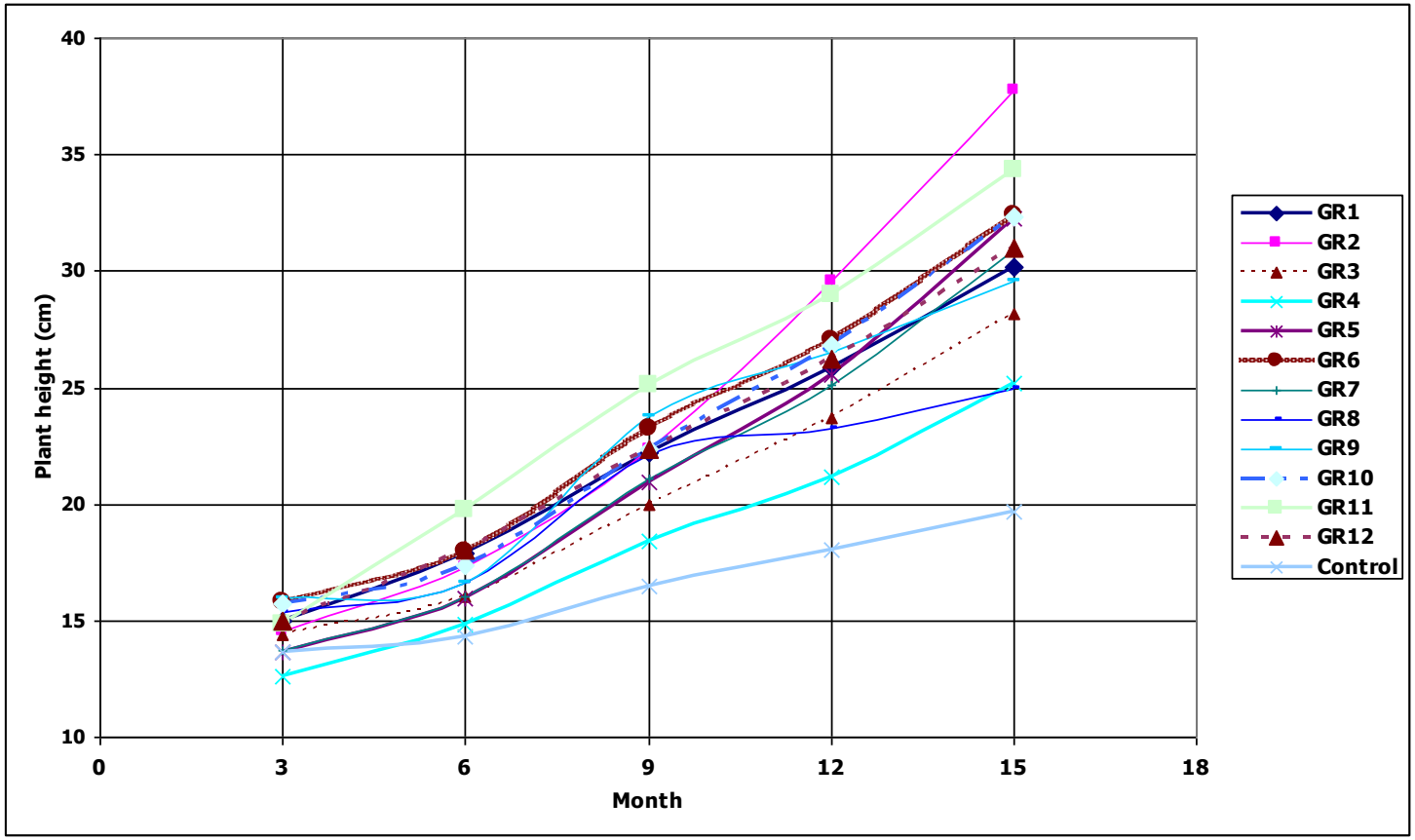


Fig. 13. Influence of growth regulators on plant height of mangosteen seedlings in coir pith compost medium during different periods

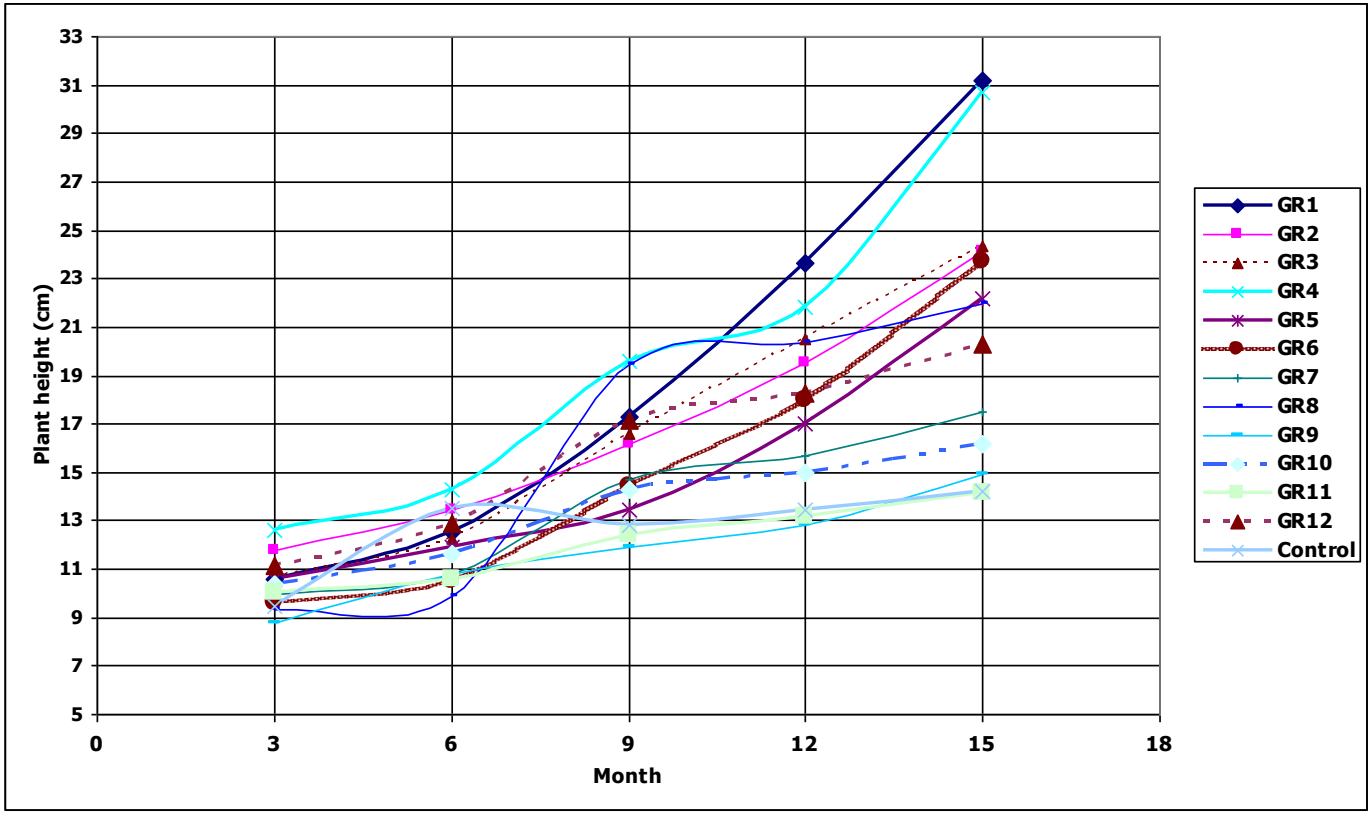


Fig. 14. Influence of growth regulators on plant height of mangosteen seedlings in poultry manure medium during different periods

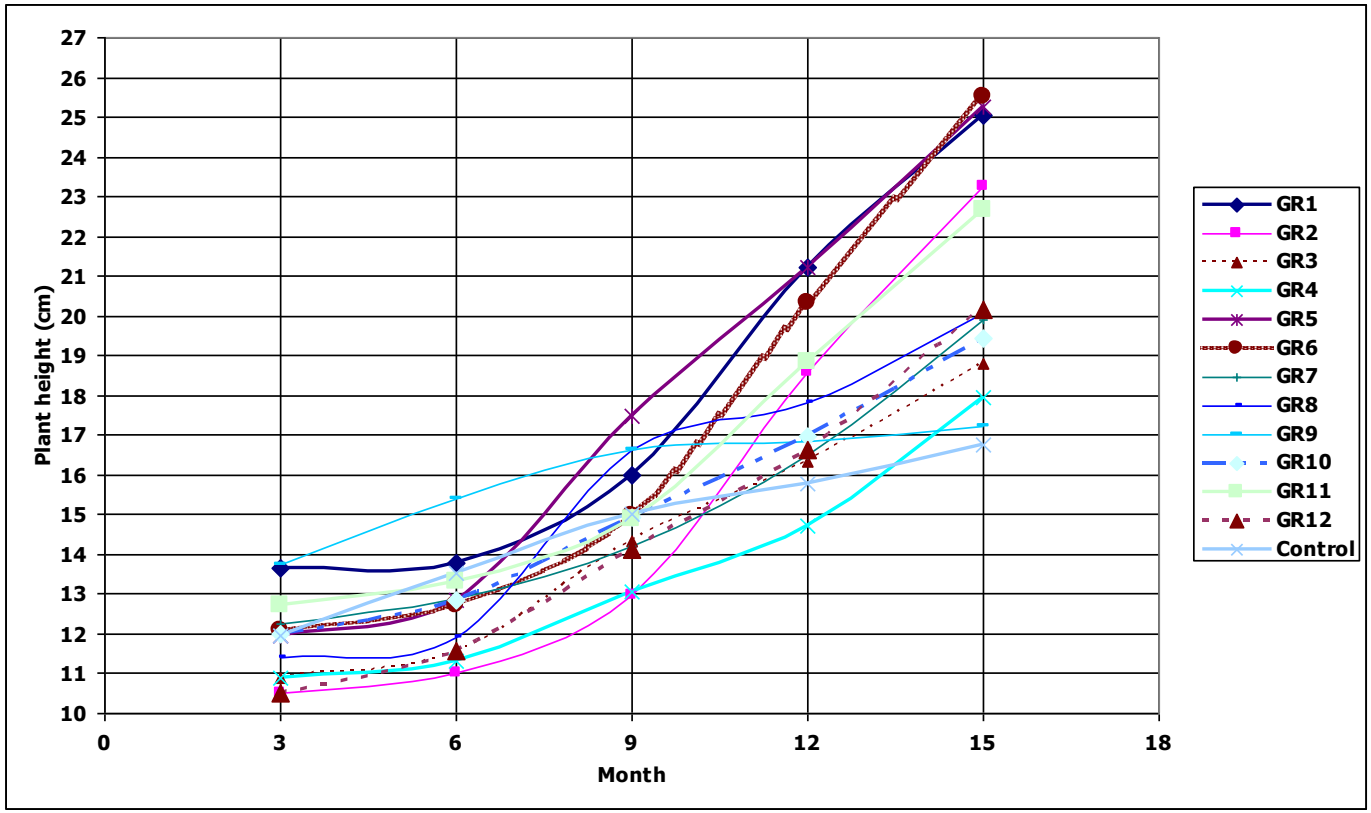


Fig. 15. Influence of growth regulators on plant height of mangosteen seedlings in cow dung medium during different periods

Plate 16. Superior treatments in vermicompost medium

growth regulators in improving growth parameters like height, production of branches and leaves, plant spread, plant biomass production, physiological parameters and nutrient uptake. Influence of IAA and IBA was more evident in the case of height, leaf area and root parameters while effect of GA was more pronounced in the case of plant height and internodal length.

Considering the combination effect of media and growth regulators, a definite pattern of interaction could not be observed. Still it was interesting to note that certain growth regulators in vermicompost put forth a poor performance compared to vermicompost alone. Therefore it can be inferred that even without additional application of any growth regulator, good seedling growth can be attained if vermicompost is used as the growing medium for mangosteen.

In the absence of vermicompost, coir pith compost, poultry manure or well rotten cow dung can be used along with suitable growth regulators. For media containing coir pith compost, IAA 300 ppm, BA 200 ppm and BA 300 ppm are ideal while IAA 150 ppm, IBA 150 ppm and BA 300 ppm are the superior growth regulators for poultry manure medium that can enhance seedling growth significantly. For the normal potting mixture, use of IBA 450 ppm, BA 200 ppm, IBA 300 ppm can accelerate seedling growth than use of media alone (Plate 17 to 19).

5.1.2 *Effect of growth promoting substances*

The present study revealed that different growth promoting substances have a significant influence on plant characters, physiological parameters and nutrient content and uptake in mangosteen seedlings. Treatments were imposed on seedlings grown on potting mixture (2:1:1 soil, sand and cow dung) as medium.

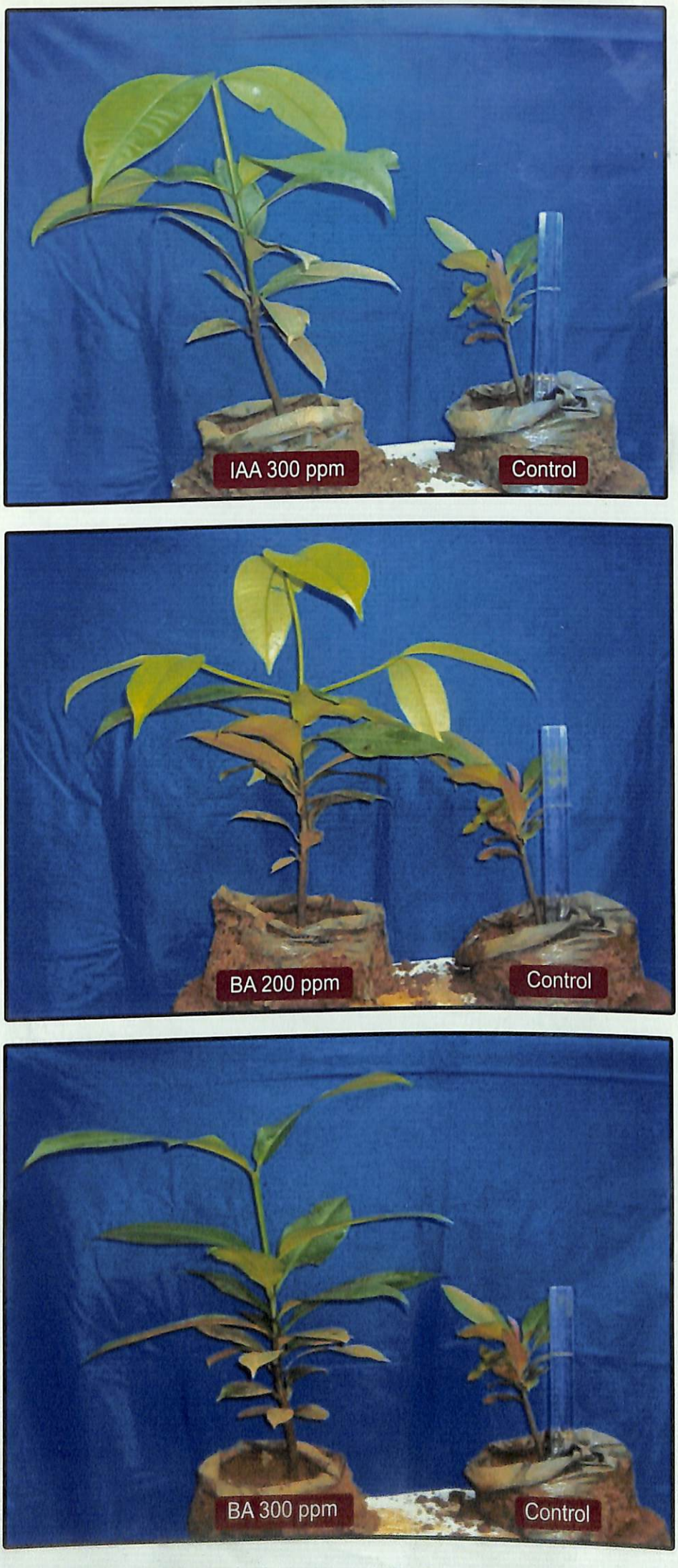


Plate 17. Superior treatments in coir pith compost medium

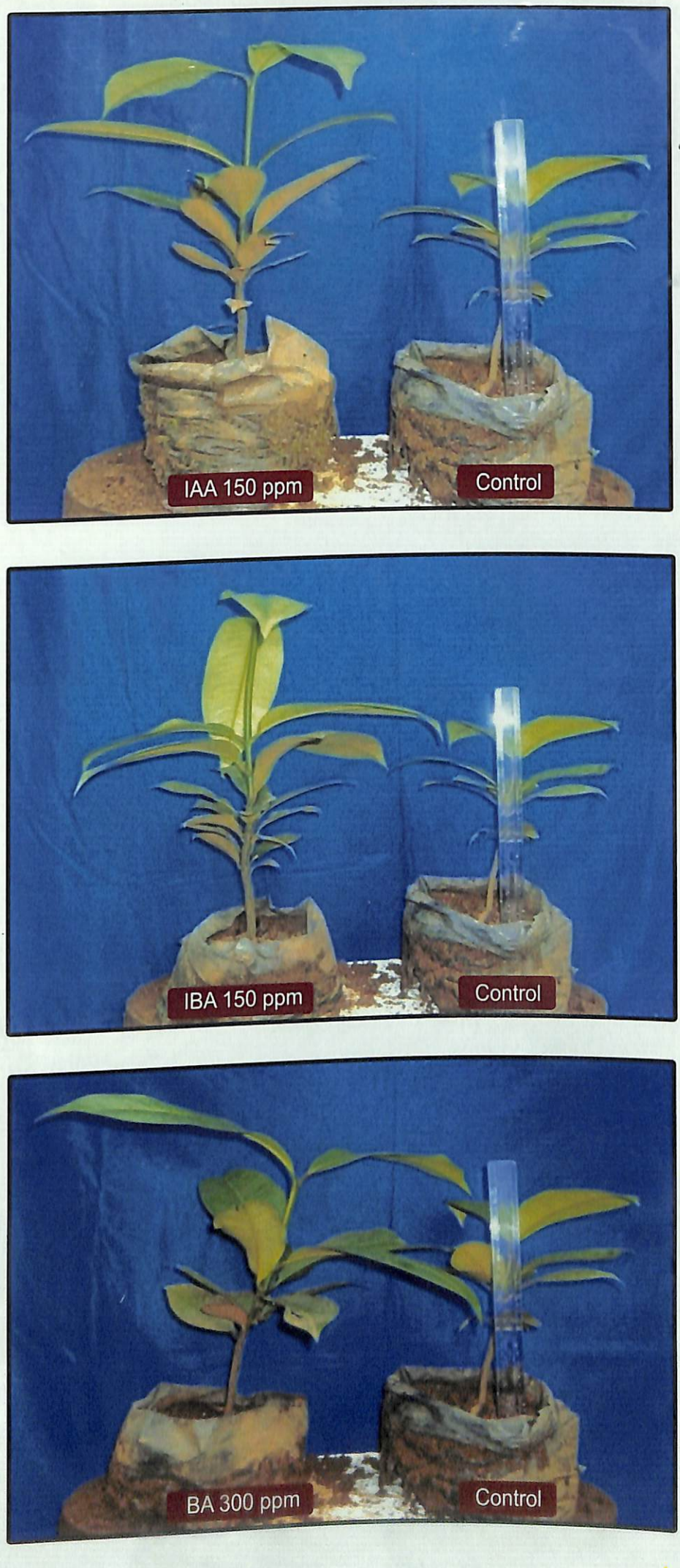


Plate 18. Superior treatments in poultry manure medium

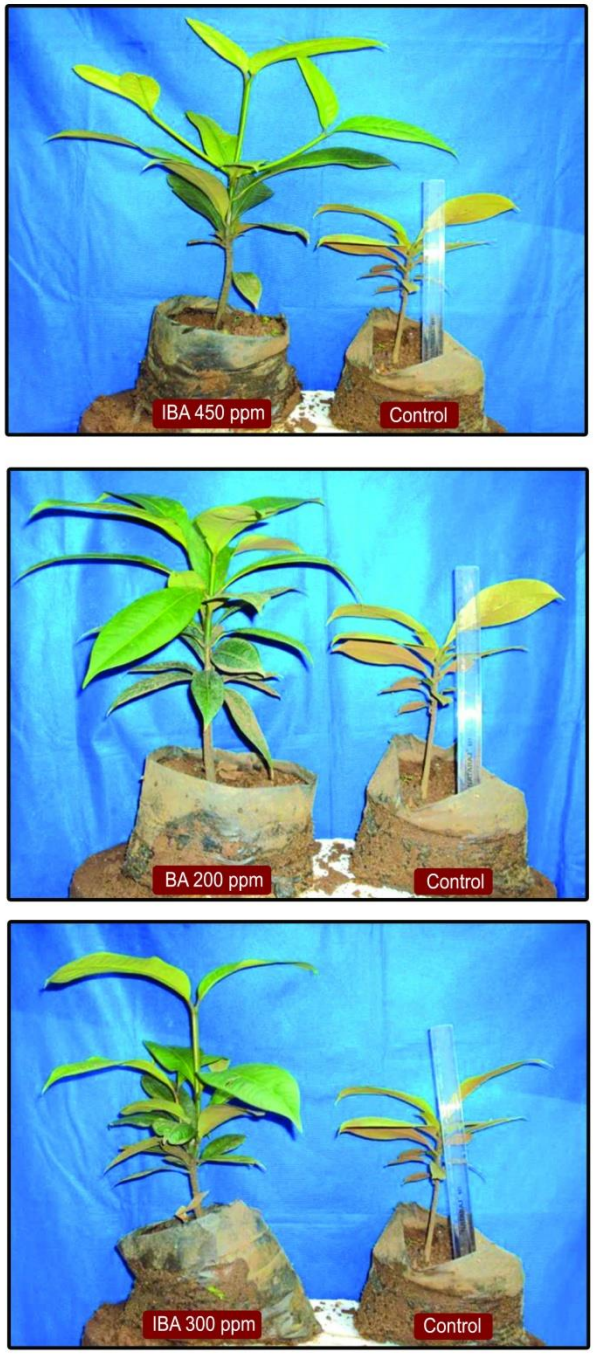


Plate 19. Superior treatments in cow dung medium

5.1.2.1 Plant, leaf and root characters

Plant height, one of the prime criteria describing the overall growth of a plant was significantly influenced by various growth promoting substances. The significant effect of various treatments on plant height started manifesting by sixth month and continued till the end of the experiment. The treatment B11 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm] was significantly superior throughout the experimental period and recorded a maximum height of 37.04 cm at the end of 15 months. This is in conformity with the report of Osman and Milan (2006) that application of gibberellic acid could accelerate the early growth of mangosteen seedlings. Gibberellins characteristically increase growth by cell elongation and enhance the internodal length (Hull and Lewis, 1959; Davies and Holmes, 1962; Ak *et al.*, 1995). Normally mangosteen seedlings attain an average height of 25 cm by two to three years (Osman and Milan, 2006). In the present study, in addition to B11, treatments B9 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm], B13 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm + BA 100 ppm] and B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm] were able to surpass this value by fifteenth month of study underlining the effectiveness of these treatments in enhancing height of the plants in the nursery.

Internodal length, a parameter related to height of the plant also showed a similar trend. The significant effect of treatments manifested by sixth month and B11 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm] which showed the highest influence on height was the most superior treatment (3.56 cm) and it differed significantly with all other treatments. This result again confirms the role of gibberellins in increasing growth by elongation of the internode.

None of the treatments in the potting mixture could induce branching in mangosteen during the entire period of study.

During the early stages of growth, the influence of growth promoting substances on plant spread was not evident. The treatments started showing its significance only by twelfth month. B2 [*Azospirillum* sp. (10 g per plant)] was the superior treatment influencing plant spread. These results are in conformity with the findings of Rao and Dass (1989) that soil inoculation with pure cell suspension of *Azospirillum brasilense* resulted in growth enhancement in pomegranate and ber. They claimed that the growth enhancement could be due to production of growth regulators and nitrogen fixation.

Mangosteen is slow growing with a long juvenile phase. Faster growth of seedlings can be achieved by promoting leaf production thereby enhancing total leaf area so that a larger photosynthetic area is available to the plant. It has also been reported that assimilate production rather than translocation may be a major factor limiting the growth of mangosteen seedlings (Wiebel *et al.*, 1995). So any attempt to enhance the growth of the seedlings should primarily aim at production of more leaves with a larger leaf area at a greater pace.

In the present study, treatments showed a significant response to number of leaves after sixth months and its influence was more pronounced from ninth month onwards. During ninth month, B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] was the most superior treatment. By twelfth month, B12 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm] surpassed B8 and recorded the highest number of leaves (19.20). Though B12 maintained the superiority in leaf number (22.80) by the end of the study, B8 (22.60) was equally superior.

B12 evoked a similar response in the case of total leaf production for a period of fifteen months. This treatment recorded the highest leaf production (14.40) and lowest senescence (1.60) during the entire period of study. It is a well known fact that nitrogenous fertilizers promote vegetative growth and BA is reported to delay

senescence (Lassoie and Hinckley, 1991). This might be the possible reason for the higher production and retention of more leaves in the plant by the treatment containing both nitrogen and BA.

The major leaf parameters like leaf length, breadth, mean and total leaf area and petiole length were significantly influenced by the treatments while leaf thickness did not respond much. Influence of treatments on petiole length was evident from third month onwards. Rest of the characters showed significant increase from ninth month onwards. B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] and B2 [*Azospirillum* sp. (10 g per plant)] were the two most superior treatments that influenced all the leaf parameters. Throughout the study these treatments proved their superiority on overall growth of the seedlings.

An unexpected observation during the study was the reduction in leaf length and breadth in all the treatments involving GA. The reduction in leaf length and breadth was more drastic when GA was applied in combination with BA. The exact reason for this reduction in photosynthetic area due to BA, GA combination is yet to be investigated.

With respect to root number both at six and twelve month stages, all the treatments showed significant effect. B2 [*Azospirillum* sp. (10 g per plant)], B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] and B10 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + BA 100 ppm] were the three superior treatments with respect to number of roots. B2 recorded maximum values for primary, secondary and total number of roots at twelfth month of the study. But with respect to number of tertiary roots, B8 recorded the highest values during sixth and twelfth months. The highest number of secondary and total roots at six month stage was recorded by B10. Towards the end of the study, B1 [*Pseudomonas* sp. (2 %)], B4 [Fresh cow dung solution (3g/ l)], B8 and B10 were on par with B2 with respect to total number of

roots. These results are in line with the report of Kapulnik *et al*. (1983) who observed enhanced root elongation, root hair development and branching in a number of crops by *Azospirillum* inoculation. Hence this biofertilizer is recommended for root induction in polybag raised seedlings of plantation and orchard crops and also for vegetables (KAU, 2007).

Root length and spread also varied significantly between the treatments. B5 [cow's urine (25 times dilution with water)] and B9 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm] were the two most superior treatments. B5 showed the maximum root length at twelve months and maximum root spread both at six and twelve months while B9 recorded highest root length at six months. Cow's urine is widely used in many crops and its promotional effects on growth are well known. The effect of GA on root growth observed in this study is in agreement with the report of Ross *et al.* (1983) who also suggested the positive effects of GA on root growth.

Plant weight is an indication of growth of the plant. In the present study, treatments showed significant difference in both fresh weight and dry weight of different plant parts at twelve month stage. B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] was the most superior treatment in terms of fresh and dry weight of all the plant parts both at six and twelve months.

B8 was also superior in terms of several growth and physiological parameters such as size of the leaf, mean and total leaf area, petiole length, LAI, RGR and uptake of several nutrients. This superiority of B8 over other treatments has also reflected on fresh and dry weight of different plant parts.

5.1.2.2 Physiological parameters

Physiological parameters indicate the efficiency of the plant in terms of growth and yield. The present study took into consideration the physiological parameters like, Leaf Area Index (LAI), Specific Leaf Weight (SLW), Relative Growth Rate (RGR), shoot – root ratio, dry matter production and chlorophyll content of the plants.

LAI is a factor that influence crop growth rate. The rate of dry matter production by a crop will increase as the LAI increases. In the present study, effect of treatments on LAI was not evident till sixth month and thereafter treatment showed a significant effect till end of the experiment. B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] which was superior in terms of mean leaf area and total leaf area recorded the highest values of LAI during the entire period of study. This higher LAI is effective in increasing the photosynthetic efficiency thereby enhancing growth of seedlings (Yoshida, 1972).

Specific Leaf Weight was positively correlated to Transpiration Efficiency in many crop species (Brown and Byrd, 1996). Thicker leaves are usually having higher photosynthetic capacity and plants with a higher SLW are able to synthesize more photo assimilates than those with lower SLW values. Treatments showed significant difference with respect to SLW both at six and twelve month stages and B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm] recorded the highest value in both the stages. In general, treatments with GA as one of the components recorded higher values compared to rest. In this experiment, GA induced reduction in leaf area and this may be the probable reason for high SLW values for the treatments involving GA.

Relative Growth Rate (RGR) is one of the most widely used techniques for estimating plant growth. The highest relative growth rate was recorded in treatment

B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] which was also superior in terms of LAI. Relative Growth Rate is closely associated with dry matter production and as a result the same treatment recorded the highest dry matter production at the end of the experiment.

The shoot- root ratio which is an indication of relative growth of shoot and root portions varied significantly both at six and twelve month stages. At six month stage, the values were low and by twelve months the ratio showed higher values probably due to a faster shoot growth with production of more leaves with large leaf area. B12 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm] which also recorded highest leaf production and also having superior mean leaf area recorded the maximum shoot – root ratio which was followed by B11 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm] which had the maximum height among the treatments.

Chlorophyll content of the leaves influences the photosynthetic rate and ultimately plant growth to a considerable extent. The treatments showed significant difference in the content of chlorophyll in all the periods of study. B1 (*Pseudomonas* sp. - 2 %) was significantly superior in terms of chlorophyll ‘a’, ‘b’ and total content at four month stage. At twelve month stage also the same treatment had significantly superior values for the three types of chlorophyll except for chlorophyll ‘a’. But at eight month stage, B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm] recorded higher values for all the types of chlorophyll. BA is said to have the property of retarding chlorophyll degradation in several plants (Kao, 1980; Yu and Kao, 1981).

5.1.2.3 Nutrient content and uptake

Nutrient concentration of plants is a good indication of the overall growth of the plant, assimilate accumulated in the sink of the plant body and the potential of

that plant to effectively utilize the nutrient sources provided. The nutrients applied if properly absorbed and assimilated only can contribute to the development of any plant. Hence it is essential to study whether the supplied nutrients are absorbed and the different treatments have any role in enhancing the absorption of nutrients by plants. Therefore nutrient content in the plant parts as well as the uptake are also of great importance.

The study revealed that treatments differed significantly with respect to content of all macro and micro nutrients both at six and twelve month stages. But a definite pattern of nutrient content was not observed between the treatments and content varied among treatments differently. Considering all the treatments, B3 [AMF (10 g per plant)] recorded highest content of more elements (two each) both at six month (P and Zn) and twelve month (Fe and Cu) stages. The ability of mycorrhizae to absorb phosphorous from insoluble and sparingly soluble P sources such as rock phosphate has been reported in several crops (Barlett and Lewis, 1973; Zhenyao and Kaihen, 1984). They demonstrated that such plants showed higher leaf P nutrient status but lower leaf N and K concentrations than non- mycorrhizal seedlings. In the present study also, B3 recorded relatively low leaf N and K concentrations compared to rest of the treatments.

Several studied show that AM fungi directly enhance the uptake of micronutrients viz. Zn, Cu and Fe (Gildon and Tinker, 1983; Pacovsky *et al.,* 1986; Kucey and Tanzen, 1987; Rai, 1988). It was observed that AM fungi association resulted in higher uptake of micronutrients in various plants, which was brought about by selective uptake of minor nutrients (Sukhada, 1988; An *et al.,* 1993; Singh and Sharma, 1993). This is in conformity with the results obtained in the present study that the highest content of Zn (six months) and Fe and Cu (twelve months) were recorded in the treatment B3 [AMF (10 g per plant)].

The leaf nutrient concentrations recorded in the present study matched only with N, P, K, Ca and Mg contents reported by Poowarodom *et al*. (2002). According to him, N, P, K, Ca and Mg concentrations were 1.33, 0.09, 1.27, 1.01 and 1.05 g/100g (dried weight) respectively. The values for Fe, Mn, Cu and Zn concentrations were comparatively high in the present study as against the values reported by him.

Treatments showed significant difference in the uptake of N, Fe, Cu and Mn. In the case of other nutrients even though effect of all the treatments was uniform, some of the treatments evoked a better response than others. The most notable observation of this study is that only three treatments recorded highest values with respect to uptake of all the nutrients. B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] recorded the highest uptake in the case of N, P, Ca, Mg and Mn though significant difference was observed only in the case of N and Mn. B2 [*Azospirillum* sp. (10 g per plant)] was next most superior treatment having highest values in the case of K, Fe and Zn having significant influence in the case of Fe. The highest uptake of Cu was recorded by B3 [AMF (10 g per plant)] which differed significantly from all other treatments. Control treatment recorded the lowest uptake in the case of all the nutrients except P, K, Cu and Mn. The findings of this study are in line with the reports that AM fungi directly enhance the uptake of micronutrients viz. Zn, Cu and Fe (Gildon and Tinker, 1983; Pacovsky *et al.*, 1986; Kucey and Tanzen, 1987; Rai, 1988).

Moraes *et al.* (2006) reported a low Fe absorption efficiency of mangosteen seedlings despite a high concentration of Fe in the substrate of surface soil. In the present study, significant difference was observed between treatments with respect to uptake of Fe. The different treatments might have generated a differential response in the uptake of Fe which may be the probable reason for this variation in uptake.

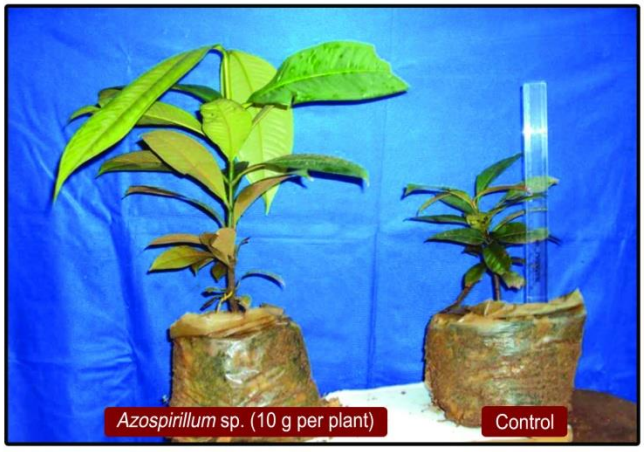
Among all the treatments, B8 was found to be the most superior in terms of uptake of majority of nutrients. Higher uptake of nutrients in the treatment B8 explains the superior growth characteristics of the plants like size of the leaf in terms of length and breadth, mean and total leaf area, petiole length, LAI, dry matter production, RGR etc. in this treatment.

In the present study a comparison of influence of different growth promoting substances on various growth parameters of mangosteen seedlings was investigated. The study indicated that the various nutrient solutions, bio-agents and bio-regulators used in this study showed a greater response on plant growth compared to bio-products. Further among the bio-regulators, application of GA and BA alone was found to be more effective than their combined application.

Based on the influence of various growth promoting substances on plant height, leaf characteristics, canopy development, root production and overall growth and vigour of the seedlings, B8 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %] followed by B2 [*Azospirillum* sp. (10 g per plant)] were found be the superior treatments that can enhance growth of seedlings significantly (Plate 20). Though response of other treatments did not follow a definite pattern, B11 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm] followed by B14 [Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm] also showed greater influence on plant height.

B8 is a foliar grade nutrient solution containing NPK (3:1:1 – 0.50 %). The study revealed that growth of mangosteen seedlings in nursery can be enhanced significantly with this nutrient solution without any additional growth regulators. So this treatment is cost effective and can be adopted by farmers as well as nurserymen. Commercial formulations of *Azospirillum* are also available locally at a reasonable price and hence this can also adopted easily.

Plate 20. Superior growth promoting substances influencing seedling growth



Comparison of the effectiveness of growing media, growth regulators and growth promoting substances on the growth parameters of mangosteen seedlings revealed that media such as vermicompost and coir pith compost and use of growth regulators are more effective in enhancing the growth of seedlings compared to use of growth promoting substances alone in normal potting mixture. All the growth parameters recorded higher values in the media – growth regulator experiment compared to the experiment with growth promoting substances. Even then, all the treatments involving growth promoting substances recorded higher values for all the growth parameters compared to control indicating that they are also effective in accelerating seedling growth in the nursery when ordinary potting mixture is used as the medium.

5.2 Accelerating plant growth in the main field

The major constraint to the spread of mangosteen cultivation is its slow growing nature and long gestation period. Trees may take up to 10 to 15 or even more years to start bearing. Though the long gestation period can be reduced by resorting to vegetative propagation, the problem of slow growth becomes all the more conspicuous and the resultant grafts exhibit extremely stunted growth together with non-upright shoot development. Unless vegetative growth of these grafts are promoted by some means, yield from such grafts are usually very low in the initial years. In the present study it was observed that growth regulators have a significant role in breaking bud dormancy and enhancing growth of grafted plants. They also had a positive and significant effect on reducing the juvenile period and improving yield and yield attributes of grown up trees.

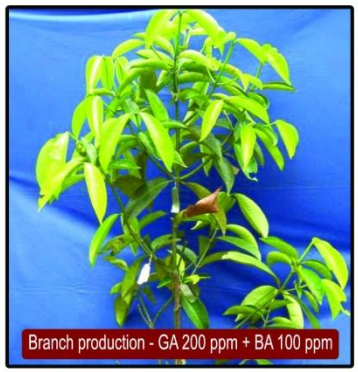
5.2.1 *Two year old grafted plants*

5.2.1.1 Plant characters

The overall growth of the plant is assessed in general in terms of height of the plant which in turn is determined by extension of shoot in the main stem as well as branches. One of the possible reasons for the slow growth of mangosteen plants is attributed to its prolonged bud dormancy. Any attempt to promote the growth of the plants should therefore aim at breaking this dormancy thereby enhancing the growth of the plant. This will in turn result in production of more leaves, branches and thus extending its canopy further resulting in a faster growth. With this objective, various growth regulators in different combinations were tried and it has been observed that they had a definite role in accelerating the growth of the grafts.

Plant height and the factors contributing to height like extension growth of main stem and branches were significantly influenced by the growth regulators. The treatments C1 (GA 100 ppm), C5 (GA 100 ppm + BA 100 ppm) and C7 (GA 200 ppm + BA 100 ppm) showed significant influence on these characters over control though their effect was varying in the main and lateral branches. The extension growth of main stem for one year period was highest (46.37 cm) in C1 followed by C5 and C7. But in the case of branches, C7 recorded the highest mean extension of growth per year (26.55 cm) followed by C1 and C5 (Plate 21). In all these three treatments, GA was the common constituent and effect of GA in growth promotion is well established (Jones and Macmillan, 1984; Takahashi *et al.,* 1986). Gibberellins characteristically increase growth by cell elongation and increasing the internodal growth (Hull and Lewis, 1959; Davies and Holmes, 1962; Ak *et al.,* 1995). BA is also known to promote growth mainly by cell enlargement and delay of senescence (Lassoie and Hinckley, 1991). The findings of the present study is in conformity with the report of Wiebel *et al*. (1992b) that treatments with gibberellins were effective in overcoming bud dormancy in mangosteen, but only when application was made directly to the bud. In their experiment, GA_{4+7} + BA gave the best result of 100 per

Plate 21. Influence of growth regulators on extension of shoot, production of leaves and branches in two year old grafts of mangosteen



cent bud break within a week when applied onto the buds of four year old field grown trees and significantly increased the number of new flushes as well as leaf area.

The most superior treatments which resulted in maximum extension growth of shoot (C1, C5 and C7) significantly influenced plant girth also. The effect was evident from third month onwards and lasted throughout period of study with C7 (GA 200 ppm + BA 100 ppm) having maximum influence among all treatments.

GA, BA combination was highly significant in the case of production of branches in grafted plants of mangosteen. When their influence was evident from third month onwards in the case of production of primary and total number of branches, their impact on production of secondary branches manifested by twelfth month only. C7, C5 and C1 were found be the most superior treatments influencing these characters also. When C7 (GA 200 ppm + BA 100 ppm) showed highest influence on production of primary and total number of branches, C5 (GA 100 ppm + BA 100 ppm) was more effective in inducing secondary branch production. These results are in line with the report of Wiebel *et al.* (1992b) that GA_{4+7} + BA in four year old mangosteen trees produced significantly more shoots. Franchlet (1981) also reported that cytokinins are responsible for multiple shoot production in many plant species.

GA and GA, BA combinations which showed significant effect on production of branches (C1, C5 and C7) had its influence on plant spread as well. Production of more number of branches will extend the canopy area and ultimately result in more plant spread. C1 (GA 100 ppm) was the most superior treatment affecting plant spread and this followed by C5 and C7 were the only other treatments showing significant difference from the control with respect to plant spread.

Effect of growth regulators on leaf production was manifested after three months and showed its significant effect throughout the experiment. C7 (GA 200 ppm + BA 100 ppm) which showed maximum influence on extension of shoot and production of branches was the most significantly superior treatment with a leaf production of 7.59 per shoot per year. The effect of GA and BA in increased shoot development is well established (Franchlet, 1981) and this may be the reason for the higher leaf production for the treatment which also had maximum effect on shoot extension. Wiebel *et al.* (1992b) also observed that GA_{4+7} + BA significantly increased the number of leaves in four year old mangosteen trees.

Normally shoot growth and leaf production in mangosteen coincide with flushing season and production of two (Alex, 1996) to three (Bourdeaut and Moreuil, 1970) vegetative flushes per year are reported in the crop. But in the present study, leaf production and further vegetative growth were continuous all through the year without a definite period of bud break and flushing thereby ensuring continuous and faster growth round the year.

Growth regulators showed significant influence on leaf area with C1 (GA 100 ppm) recording highest values till ninth month and C6 (GA 100 ppm + BA 200 ppm) with significantly superior values compared to all other treatments at the end of one year period of study. C7 which had the highest leaf production among all treatments showed a slight decline in leaf area from third month onwards till the end. But its leaf area was on par with all other treatments except C6 at the end of one year. A similar trend in average leaf size was reported by Wiebel *et al.* (1992b) who observed production of significantly smaller sized leaves in the induced flush following application of GA_{4+7} + BA.

The results of the present investigation have emerged with successful growth regulator combinations for accelerating growth of two year old mangosteen grafts.

GA and combinations of GA and BA have significantly influenced the various growth parameters *viz*., extension of shoot, girth, plant spread, production of leaves and related parameters. C7 (GA 200 ppm + BA 100 ppm) has dominated with respect to majority of parameters studied followed by C5 (GA 100 ppm + BA 100 ppm) and C1 (GA 100 ppm) all having significant influence on several growth characteristics (Plate 22). So GA and BA are the growth regulators which could be successfully employed to enhance the growth of field grown mangosteen grafts which are extremely slow growing.

5.2.2 *Five year old orchard trees*

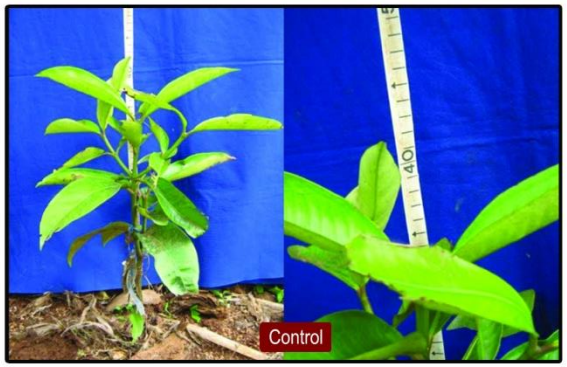
5.2.2.1 Plant characters

Unlike in the previous experiment, the growth parameters of five year old orchard trees were not significantly influenced by the external application of various growth regulators. Still certain treatment combinations evoked positive response in branch production and shoot extension.

Even though height of the trees was not significantly influenced by the treatments, D4 (BA 200 ppm) induced a better response than other treatments. When the percentage increase in plant height for one year period was 34.22 in D4, Paclobutrazol 2.0 g a.i./tree (D10) induced a retarding effect (8.31 %). This is in line with the report that Paclobutrazol reduces shoot elongation thereby leading to reduction in tree height (Voon *et al.*, 1991).

Treatments involving GA and paclobutrazol had a positive response with respect to production of primary, secondary and total branches during one year period though significant difference was observed only in the case of total number of branches at ninth and twelfth months. Maximum production of primary branches (9.67 per year) was noted in the treatment involving higher concentration of GA (GA 200 ppm). Termination of bud rest by GA sprays is a practise in peach and apple

Plate 22. Superior growth regulators influencing growth of two year old mangosteen grafts



orchards (Ross *et al.,* 1983) and in the present study GA application might have activated the dormant buds of the tree and induced production of more primary branches. D10 (Paclobutrazol 2.0 g a.i./tree) showed a greater effect on production of secondary as well as total branches compared to other treatments though the significant influence of the treatments on total branches manifested only by ninth month of treatment.

Among the treatments, GA, BA combinations were found to be more effective in extension of shoot in the primary branches. Treatments D5 (GA 100 ppm + BA 100 ppm) and D6 (GA 100 ppm + BA 200 ppm) induced a better response with a mean extension of 28.46 and 27.19 cm respectively compared to 22.60 cm of untreated trees. Alex (1996) has reported an extension growth of 6.91 cm in a year in old mangosteen trees. Age of the tree may be one of the factors leading to this huge difference in growth rate between the two studies. The difference in growth between the most superior treatment and control in the present investigation is almost comparable to the total extension growth reported in the early report. This also points to the possible influence of growth regulators in enhancing shoot growth which is in line with the report of Wiebel *et al.* (1992b) that application of GA_{4+7} + BA in lanolin paste applied directly onto the bud is effective in overcoming bud dormancy. A similar trend was noted in the action of growth regulators in extension shoot in secondary branches also with D9 (Paclobutrazol 1.5 g a.i. per tree) and D6 (GA 100 ppm + BA 200 ppm) recording a better response than others.

Another notable observation was the very low extension growth in the primary and secondary branches (9.32 and 4.68 cm per year respectively) and short internodes of the trees treated with higher dose of paclobutrazol (2.0 g a.i per tree) which underlines the fact that paclobutrazol is most efficacious in reducing shoot elongation and growth reduction is by reducing the intermodal length of new shoots (Voon *et al.,* 1991). But lower dose of paclobutrazol (1.5 g a.i per tree) did not induce

this response and this treatment also had high values (20.03 and 14.66 cm respectively) in both the type of branches. The lower dose of paclobutrazol used in this study may not be sufficient enough to evoke the response of reduction in tree vigour. A similar response was noticed in induction of flowering as well.

Plant spread is directly related to extension of shoot in the branches. In the present study, effect of treatments on extension of shoot was found be non-significant and this also reflected in less response of treatments on plant spread and the treatments were found to be uniform in its effect. The same statement also applies to leaf production as all the treatments were found to be homogeneous with respect to production of leaves in primary as well as secondary branches. But the treatments which showed highest response to extension of shoot in primary and secondary branches (D5 and D9 respectively) also had the maximum leaf production of 21.06 and 7.79 respectively.

Similarly no significant difference was observed between the treatments in leaf area. But application of paclobutrazol seemingly produced slightly twisted leaves. A similar response to paclobutrazol application though for a higher dose (5.0 g/plant) was reported by Sdoodee and Saelim (1991).

Generally mangosteen trees put forth its vegetative growth in distinct flushes of one to two during April – June and October – November. In the present study, combination of GA and BA could induce continuous growth and leaf production in addition to normal flushing. Out of the eleven treatments tried, seven treatments induced a second flushing resulting in additional growth of the trees. So it proves that it is possible to enhance the growth of field grown mangosteen trees during their pre-bearing age by inducing additional flushing per year with the use of growth regulators such as GA and BA.

Prolonged bud dormancy is attributed as one of the reasons for the slow growth of mangosteen especially in the early years. Any attempt to enhance the growth of the trees should primarily aim at breaking this dormancy thereby leading to enhanced shoot growth and leaf production. In the present study to accelerate the growth of the trees in the main field, some of the treatments showed a better response than others. Considering the combined effect on shoot growth and leaf production, treatments D5 (GA 100 ppm + BA 100 ppm), D6 (GA 100 ppm + BA 200 ppm) and D7 (GA 200 ppm + BA 100 ppm) were found to be more effective than others. In future trials, a higher dose of these growth regulators may be tried in order to realize an enhanced effect of these bio-regulators for accelerating growth of juvenile mangosteen trees.

5.2.2.2 Flowering, yield and yield attributes

The major constraint to the spread of mangosteen cultivation is its slow growing nature and long gestation period. Trees may take up to 10 – 15 or even more years to produce their first fruits (Hume, 1947; Gonzales and Anoos, 1951). In the present study it was observed that growth regulators have a significant influence in reducing the long juvenile period and improving the yield and fruit characters.

Growth regulators had a significant influence on early induction of flowering and reducing the long gestation period of the crop. Treatments D7 (GA 200 ppm + BA 100 ppm), D8 (GA 200 ppm + BA 200 ppm) and D10 (Paclobutrazol 2.0 g a.i./tree) were the most superior treatments and were equally effective resulting in the induction of flowering in five year old orchard trees. When all the plants in the control treatment failed to flower in the first year of treatment (five year old plants), combinations of GA and BA and paclobutrazol induced flowering in two out of three trees treated in each treatment indicating their superiority over other treatments. The effect of growth regulators were more pronounced in the second year of application (six year old plants) where all the treatments except control induced flowering in all

the replications. Among the control trees, only one started flowering in the sixth year and the period of flowering was much delayed, as late as last week of February. In majority of other treatments, flowering commenced between January last week and middle of February.

Sdoodee and Saelim, (1991) conducted similar studies in five year old mangosteen at Thailand to induce early bearing. The five treatments they employed included control and paclobutrazol application at four rates: 2.0, 3.0, 4.0, and 5.0 g/plant. In their study, bearing was found only in the treatments of 2.0 and 3.0 g/plant, with limited fruit-setting of only 1 and 2 fruits per plant, respectively. Therefore they concluded that 5-year mangosteen trees were not suitable to induce early bearing by paclobutrazol application. Contrary to the above, the present study revealed that paclobutrazol was effective in early induction of flowering in five year old mangosteen. The early flowering obtained in the present study might be due to better and faster development of sufficient canopy size within five years. All the trees which started flowering in fifth year had a better canopy size compared to unflowered trees. The trees flowered in the fifth year had an average height of 2.94 m and an average canopy diameter of 2.81 m compared to 2.78 m height and 2.35 m canopy diameter of unflowered trees. It may be inferred that more than the age of the tree, the size of the canopy might be a deciding factor in induction of early flowering. The effect of paclobutrazol in early induction of flowering in crops like mango is well established (Davenport and Elisea, 1997) and as a result, paclobutrazol is being promoted to control flowering and vegetative growth in commercial mango orchards of Indo-China, Australia and South Africa (Voon *et al.*, 1991).

Wiebel *et al.* (1992b) reported that combination of GA_{4+7} + BA in four year old mangosteen trees produced significantly more shoots and they are of the view that it may be possible to enhance the growth of field- grown trees during their pre-bearing age by inducing one or two extra flushes per year. Probably overcoming of

this bud dormancy with GA, BA combinations and thereby enhancing the growth of the trees might have resulted in early flowering of five year old orchard trees in the present investigation.

Growth regulators also showed a significant influence on yield and yield attributes. D10 (Paclobutrazol 2.0 g a.i./tree) was the most superior treatment in terms of yield (1.977 kg/tree and 6.45 kg/tree in first and second year respectively) and number of fruits per tree (29.50 and 114.33 in first and second year) in both the year followed by D2 (GA 200 ppm). Control recorded the lowest value for both these parameters. In the case of paclobutrazol treatment, fruit size was also medium in the first year compared to small sized fruits produced by majority of other treatments, though size of the fruits reduced slightly in the second year probably due to a large increase in yield compared to previous year. This is in agreement with the report of Sdoodee *et al.* (2008) who observed a similar reduction in fruit size in trees which yield more number of fruits per tree. Considering its significant influence on early induction of flowering by the fifth year and its superior performance with respect to yield and yield attributes, it can be concluded that application of paclobutrazol (2.0 g a.i /tree) is the most superior among all the treatments in the induction of early flowering as well as for obtaining maximum yield and highest number of fruits per tree (Plate 23 to 26). Similar results were reported by Omran and Semiah (2006) where application of paclobutrazol combined with potassium nitrate or bicomine enhanced flowering and fruiting of mangosteen. Chalumpuk *et al*. (1998) also reported that paclobutrazol application three months before flowering reduced shoot length, promoted earlier flowering and gave the highest yield.

Plate 23. Influence of growth regulators on fruit size and number of segments

Plate 24. High yielding five year old mangosteen trees

Plate 25. High yielding five year old mangosteen trees

GA 200 ppm

Plate 26. High yielding five year old mangosteen trees

5.2.2.3 Floral and fruit characters

Similar to its effect on early flowering, yield and yield attributes, growth regulators had a significant effect on various floral and fruit characters of mangosteen.

D10 (paclobutrazol 2.0 g a.i./tree) which recorded the highest yield and maximum number of fruits per tree also produced the maximum number of flowers (8.53) per 0.5m x 0.5 m canopy area which is an important character related to yield. Another notable observation of this treatment was that trees of this treatment produced flowers in clusters of 2 to 3 per cluster compared normal production of flowers singly (Steenis, 1981). But this clustering habit and large number of flower production also resulted in highest flower drop in D10 (65.65 per cent) among all the treatments. But it should be considered as a normal physiological mechanism of the plant to reduce extra crop load as this has not adversely affected yield and D10 recorded the highest yield among all the treatments. This is in conformity with the report of Chalumpuk *et al*. (1998) that paclobutrazol application three months before flowering resulted in the highest number and weight of fruits per tree without affecting fruit development and fruit quality. They also reported that application of nitrogen and paclobutrazol together increased the total number and fruit weight per tree and fruit quality in terms of sweetness.

Treatments also showed significant difference in important fruit characters studied. Lower dose of paclobutrazol (1.5 g a.i/tree) recorded the maximum weight of pulp (29.41 g) but its higher dose (2.0 g a.i/tree) also had comparable value (27.70 g). The higher dose also showed the highest percentage of edible portion (37.96 %) with more number of big sized segments (1.57 per fruit) which are highly desirable fruit characters. TSS was also high (18.87 ° brix) compared to rest of the treatments though significantly not different. Gamboge is a major physiological disorder in

mangosteen resulting in spoilage of large number of fruits and one of the most noteworthy findings of this study is the extremely low incidence of gamboge in paclobutrazol (2.0 g a.i/tree) applied trees. When D1 (GA 100 ppm) recorded a very high incidence of gamboge (49.52 %) paclobutrazol treatment recorded a very low value of 5.56 per cent (Plate 27).

Maximum seed weight was also recorded in paclobutrazol treated trees. In mangosteen, the number of seeds per fruit and seed viability is extremely poor. The size of the seed is an important factor governing germination and seedling growth (Wester, 1916). In this context, production of large number of bold seeds with the help of paclobutrazol treatment may be of great help in large-scale propagation of mangosteen.

Evaluation of all the parameters such as floral and fruit characters, yield and yield attributes and effect on early induction in flowering, paclobutrazol (2.0 g a.i/tree) is found to be the most superior treatment. Performance of D2 (GA 200 ppm), D5 (GA 100 ppm + BA 100 ppm) and D9 (Paclobutrazol 1.5 g a.i./tree) was also comparable with paclobutrazol with respect to yield and fruit characters. Even though paclobutrazol is a costly chemical, the additional income generated due to the increased yield realized will nullify the extra cost involved in its application as the value of the produce is also high even in the local markets.

Taking into consideration, growth parameters, induction of flowering, yield and fruit characters, the following inference can be drawn from the present experiment.

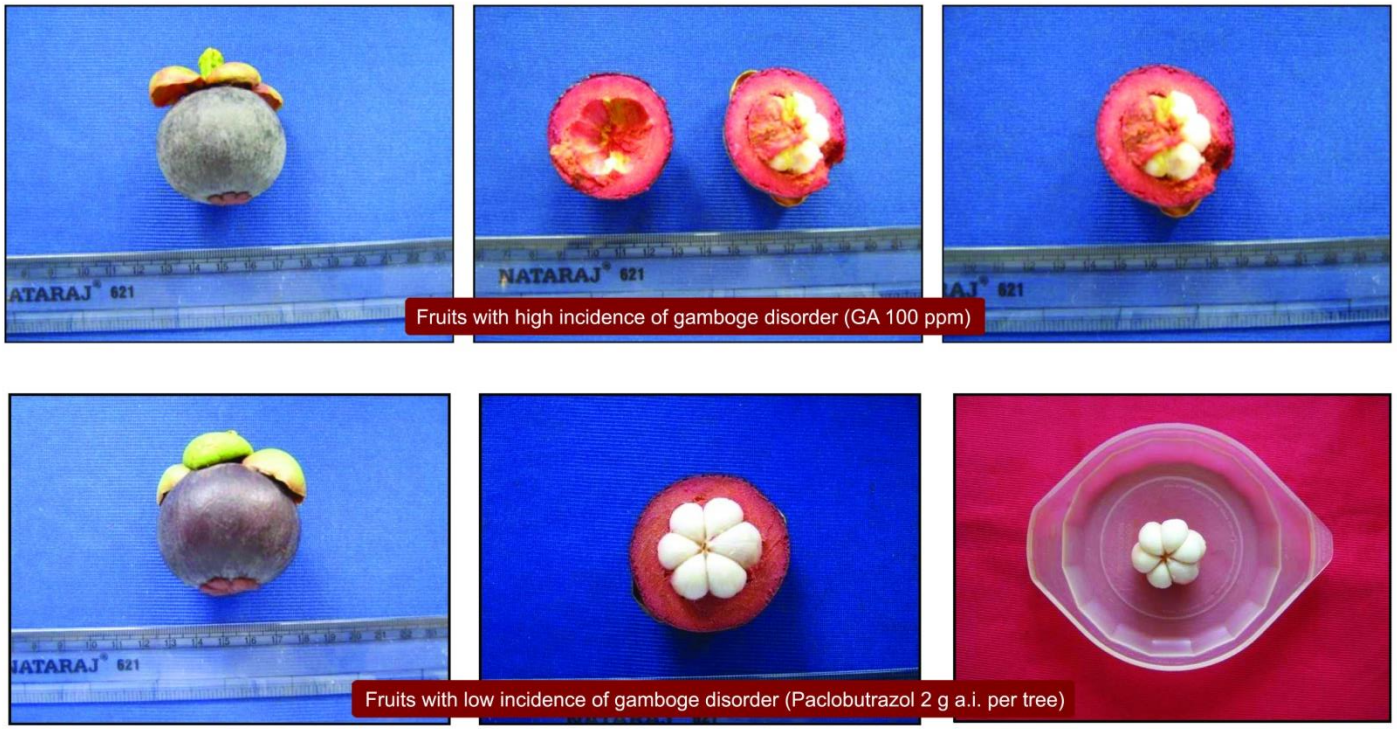


Plate 27. Influence of growth regulators on incidence of gamboge disorder

1. In mangosteen, more than the age, canopy size is the factor deciding early flowering. So in very young trees, where sufficient canopy has not developed, the primary objective is to promote growth so that a canopy sufficient for flowering is attained as early as possible. To achieve this objective, application of D5 (GA 100 ppm + BA 100 ppm), D6 (GA 100 ppm + BA 200 ppm) and D7 (GA 200 ppm + BA 100 ppm) were found to be most effective.
2. In trees which have developed sufficient canopy but not yet flowered, early flowering can be induced with the use of D7 (GA 200 ppm + BA 100 ppm), D8 [GA 200 ppm + BA 200 ppm) and D10 (Paclobutrazol 2.0 g a.i./tree).
3. Yield, yield attributes and fruit characters can be significantly improved in trees which already started flowering, with the use of D10 (Paclobutrazol 2.0 g a.i./tree), D2 (GA 200 ppm) and D5 (GA 100 ppm + BA 100 ppm).

Thus by the systematic application of specific growth regulators suited to each situation, growth, early flowering, yield, yield attributes and fruit characters can be effectively managed in field grown mangosteen trees.

Comparison of the effectiveness of growth regulator applications on the growth parameters of two year old grafts and five year old juvenile orchard trees revealed that growth regulators had a significant influence in two year old plants than orchard trees. This indicates that growth regulators are more effective in early stages and need to be applied in early growth stages itself so that growth can be promoted significantly. This will help in faster development of sufficient canopy resulting in early flowering.

5.3 Use of nurse stocks and rootstocks to enhance growth

5.3.1 Nurse stock

In the present study, additional nurse stocks namely seedlings of *Garcinia mangostana, G. indica, G. hombroniana, G. gummi-gutta, G. xanthochymus and G. cowa* were inarched to two year old mangosteen seedlings to provide a double root system in order to enhance growth and to shorten the long juvenile period. Generally all *Garcinia* species except mangosteen can adapt well to drought and semi-arid zones since they have good root systems, which develop horizontally (soil surface) and vertically (deep beneath the surface). At their early growth stage, they require no shade and frequent watering is not needed. Because of these characteristics, evaluation of related species is essential to use these as rootstocks for mangosteen especially for dry areas (Te-chato and Lim, 2005).

Various nurse stocks showed a considerable effect on growth characters of mangosteen seedlings. With regard to increase in plant height, leaf production, average leaf area, total leaf area, canopy spread, internodal length and number of branches, mangosteen seedlings having its own seedlings as nurse stock recorded the highest values after eighteen months period compared to seedling with other nurse stocks. Its incompatibility with other related species may be the reason for the poor performance of such grafts compared to seedlings with mangosteen as nurse stocks.

The growth of the mangosteen seedlings grafted with different nurse stocks was compared with that of seedlings (control) of same age. In the case of majority of characters, seedlings without nurse stock showed intermediary values compared to seedlings with nurse stocks. But seedlings with *G. mangostana* nurse stock was most superior to all other treatments for all the parameters under study indicating that seedling growth can be promoted considerably by nurse stock grafting with *G.*

mangostana seedlings. Similar results are reported by Poerwanto (2002) though with a different set of nurse stock plants.

G. hombroniana is considered as a partially compatible species with mangosteen and growth performance of seedlings using *G. hombroniana* nurse stock was superior to all the characters studied except for increase in height and intermodal length compared to control. Control treatment showed higher values for all the parameters studied compared to seedlings with *G. cowa* as nurse stock except in the case of mean number of branches which was less in seedlings compared to the latter. This indicates that, *G. cowa* is the least compatible among the various nurse stocks studied.

It can be concluded that the use of nurse stock plants can be considered as a new technique to enhance growth of mangosteen seedling. Giving an additional nurse stock plant to the mangosteen seedling provides the tree with a double root system. Therefore to improve the root system of mangosteen and to enhance growth, a nurse stock plant technique can be adopted.

5.3.2 Use of Garcinia species as rootstock

In the present study, in addition to seedlings of *Garcinia mangostana*, its related species *viz. G. indica, G. hombroniana, G. gummi-gutta, G. xanthochymus and G. cowa* were used as rootstocks in order to enhance growth and to shorten the long juvenile period.

Among the various rootstocks used, the least time for sprouting (24.40 days), highest percentage of sprouting (93.33 %) and maximum success of grafting (83.33 %) were recorded in grafts using *G. mangostana* as rootstocks. These grafts also recorded highest values for growth parameters like mean leaf production, mean leaf area, mean total leaf area and scion growth for a period of 18 months after grafting.

George *et al.* (1998) reported that softwood grafting using sweet or sour mangosteen as rootstocks and scion from one year old shoots with current season's sprout gave promising results with an initial success of 60 – 80 per cent and final success of 50 per cent in mangosteen. Javier (1989) reported that cleft grafting of mangosteen using matured scions onto two year old stocks was 100 per cent successful after one to two months after grafting.

Other rootstocks showed varying degrees of graft success ranging from 0.00 to 55.26 per cent. *G. hombroniana* showed the next highest percentage of grafting success (55.26 %) which is also in conformity with the reports of John *et al.* (2008). They reported that *G. hombroniana* is an ideal rootstock for mangosteen by virtue of its well developed root system and fast growth. Graft compatibility is good and success rate is also high in polythene mist houses. They recommended that by grafting to a highly adapted rootstock like *G. hombroniana*, the cultivation of mangosteen can be extended to diverse soil types especially laterite uplands of Kerala. But in the present study, even though percentage of success was 55.26, the resultant grafts showed stunted growth compared to grafts using *G. mangostana* seedling as rootstock.

In the case of *G. gummi-gutta* and *G. cowa*, no grafts survived after a period of two year of study. In both these cases, all the sprouted grafts showed no further growth after sprouting and dried completely within a period of 50 days (*G. gummi*-gutta) and 31 days (*G. cowa*) of grafting. In the case of *G. indica* also, sprouted grafts showed stunted growth and 97.78 per cent grafts dried within a period of 77 days of grafting. Fairchild (1915), Ochse *et al.* (1961) and Mathew *et al.* (2004) also reported that most *Garcinia* species are not compatible with mangosteen. The difference in plant survival and scion growth among the different genotypes may be due to the built-in mechanism and inherent potential or physiological condition of the rootstock

genotypes for the survival and growth of the sprouts after grafting (Dubey *et al.*, 2002).

These results indicate that among the rootstocks studied for enhancing growth of mangosteen plants, mangosteen is compatible with its own rootstock (*G. mangostana*) only and all other rootstocks showed various degrees of incompatibility in terms of success of grafting and growth rate. Further, on comparison of growth of grafts with seedlings, seedlings had a slightly better growth rate with respect to shoot growth, leaf production and mean leaf area during the period of study. Winters (1953) also reported that mangosteen seedlings themselves are not necessarily good rootstocks because of their slow growth. Though grafting can reduce juvenile period (Chong, 1992), grafts exhibit extremely stunted growth, usually together with non-upright shoot development. In spite of several attempts made to identify other rootstocks that are fast growing with vigorous root systems, results were not promising because of incompatibility problems (Osman and Milan, 2006). Hence new combinations need to be developed to induce faster growth and early bearing since grafts on its own seedlings had a very slow growth rate.

5.3.3 Rooting of softwood cuttings

Though several attempts on rooting of cuttings have failed in mangosteen (Gonzales and Anoos, 1951; Campbell 1967), in the present study, promising results were obtained with respect to rooting of cuttings. Softwood cuttings from juvenile trees were used and 86 per cent of cuttings rooted within a period of two months even without the use of growth regulators. Slow growth of mangosteen seedlings is attributed to its poor root system with limited lateral roots. Contrary to this, the rooted cuttings had profuse root growth with large number of lateral roots which may be helpful in overcoming the slow growth of plants. If this method is standardized with proper growth regulator application, the technique may facilitate large scale

production of planting materials in a limited time where seed production is minimum in this crop.

5.4.1 Induction of variability through induced mutation and polyploidy

Mangosteen is considered as a crop with limited genetic variability and this has become a major constraint in the conventional breeding of the crop. Mutation and polyploidy breeding can play an important role in the induction of variability in such crops where inherent variability is low. In the present study, it is envisaged to induce variability in mangosteen by various mutagenic treatments.

5.4.1.1 Gamma irradiation

5.4.1.1.1 Seed irradiation

Freshly harvested bold seeds were used for irradiation purpose. Based on LD_{50} value, the dosage was fixed between 5 Gy and 50 Gy. The growth characters of seedlings were observed for a period of one year. Treatments showed wide variation in days required for germination as well as percentage of germination. As the dosage of irradiation increased, days required for germination also increased progressively ranging from 19.20 days (5 Gy) to 133.60 days (30 Gy). Similarly germination percentage showed a gradual decline from 100 per cent (5, 10 and 15 Gy) to 50 per cent (30 Gy). Doses beyond 30 Gy completely prevented germination.

Morphologically seedlings of all the treatments were similar with respect to leaf length, breadth, mean leaf area and root length during the entire period of study. But seedlings irradiated with 30 Gy dose had the lowest values for plant height (5.87 cm), leaf production (3.33), total number of leaves (3.33), total leaf area (26.23 cm^2), intermodal length (0.13 cm) and root length (5.75 cm). Sobir and Poerwanto (2007) also reported that seeds irradiated with gamma rays at four different doses i.e. 0 Gy, 10 Gy, 20 Gy and 30 Gy affected seedling growth and their rooting system, several leaf anatomy parameters such as upper cuticle thickness, spongy mesophyll thickness and leaf thickness. They observed that higher gamma ray doses inhibited seedling

growth. Rostini *et al.* (2003) also reported that gamma ray irradiation of seeds with 1 kR and 2 kR resulted in variations in growth rate, plant height, leaf size, leaf colour, chlorophyll content, number of lateral roots and root length of resultant seedling. The findings of the present study is in conformity with these reports and to confirm that such a variation was originated due to changes at genomic level, molecular studies were taken up utilizing the seedlings irradiated with two higher doses of gamma ray irradiation (30 Gy and 25 Gy) the results of which are discussed separately in the ensuing pages.

5.4.1.1.2 Scion irradiation

In addition to irradiation of seeds, scions were also irradiated with 5 Gy to 50 Gy gamma irradiation and these were grafted onto mangosteen rootstocks. As in the case of seed irradiation, the higher doses of gamma rays had an adverse effect on days required for sprouting of scions, percentage of sprouting and final graft success. Scions without any irradiation (control) took minimum time for sprouting (16.00 days) and the maximum time (36.00 days) was noted in 40 Gy dosage. Initial doses of irradiation up to 20 Gy and the control recorded a higher percentage of sprouting up to 90.00 per cent and as the dose progressed, the sprouting percentage also declined to as low as 40.00 per cent. All doses above 25 Gy resulted in poor sprouting of 40 - 50 per cent indicating that these doses are lethal. Hence doses below 25 Gy may be able to induce some variation in treated scions. But in the case of doses above 15 Gy, no grafts survived after a period of 180 days of grafting. Among the treated scions, the maximum survival of grafts was noted in 10 Gy (20 per cent) where as control recorded a success rate of 80 per cent. Similar results are reported in the case of mango also (Sharma *et al.*, 1983). They found that LD_{50} for Neelum, Dashehari and Amrapali was between two and four kR of gamma rays and in most cases dosages beyond 5 kR of gamma irradiation were found to be lethal.

All the treated scions showed a stunted growth after sprouting and those survived after one year presented an underdeveloped appearance. The leaf production for one year was limited to production of just two undersized leaves without any further extension growth of the shoot. But the control treatment showed a normal growth with a mean production of 3.75 leaves and mean extension growth of 2.12 cm for one year period.

As the grafts with irradiated scions were of stunted growth without any leaf production and shoot growth for one year period, they could not be utilized for any further variability studies.

5.4.1.2 Colchicine application

In the case of seed treatment using 0.1 to 3.5 per cent colchicine, all the resultant seedlings were uniform in morphological characteristics indicating that the concentrations used were not sufficient enough to induce any visible variations in the seedling.

But in the case of bud application, two seedlings treated with higher dose of colchicine (3.0 and 3.5 per cent) showed vigorous growth and distinct variation in plant height, extension of shoot, internodal length, canopy spread, size of the leaf, leaf area and leaf production compared to rest of the seedlings indicating that this may be due to some genetic change induced due to colchicine application. Te-chato and Sujaree (1999, 2000) also reported the effect of colchicine on induction of polyploidy and mutation of adventitious shoot buds and callus. The results showed that leaves of regenerated shoots contained more chlorophyll ‘b’ than those of control. Some of the intact leaves produced a large number of shoots. They could not count the number of chromosomes following colchicine treatment due to their small size. However peroxidase and esterase markers indicated that there were differences between treated and non-treated plantlets. Morphological abnormalities of plantlets

induced from treated callus included the production of new shoots on intact leaves, a large number of roots and three leaves per whorl.

In the present study, to confirm that morphological changes observed as a result of colchicine application originated at genetic level, two seedlings identified with distinct morphological variation were subjected to RAPD analysis and the results of which are discussed separately in the ensuing pages.

5.4.2 Assessment of induced variability through Random Amplified Polymorphic DNA (RAPD) Analysis

Molecular marker based genetic diversity studies are currently getting prominence (Virk *et al*., 1996). Techniques such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat) etc. have been utilised successfully to study diversity of germplasm/ varieties (Te-chato and Prommee, 1999; Raghunathachari *et al*., 2000; Ravi *et al*., 2003).

In the present study an attempt was made to evaluate the variability / relatedness of five mangosteen variants using RAPD analysis in order to assess the extent of variability created by mutagenic treatments. Out of the eleven primers tried in the primary screening, two primers were selected and were used for screening of five mangosteen variants in order to assess variability between the genotypes. Of the total 29 scorable amplified products, 14 (48.28 per cent) were polymorphic and 15 bands were monomorphic (51.72 per cent). Rostini *et al.* (2003) screened five primers to differentiate the wild and mutant mangosteen types. Out of the five only two primers were able to differentiate the wild and mutant as the other three showed only monomorphism. In another study to assess the genetic variability of mangosteen population in Java Island utilizing RAPD analysis, five selected primers produced a

total of 51 bands of which 42 bands (82.40 %) were polymorphic (Sobir and Poerwanto, 2007). A study with 23 accessions of mangosteen using ISSR markers revealed only a few number of polymorphic bands with an average of 3.82 bands per primer probably due to the apomictic origin of the species (Mansyah *et al.,* 2010). Wittayawannakull *et al.* (2010) reported that *Garcinia mangostana* accessions analysed by them had low genetic variation and different *Garcinia* species can be clearly distinguished by combined peroxidise, RAPD and gene sequence specific amplification polymorphism.

In order to assess the variability between the genotypes, cluster analysis was performed based on dendrogram. Clustering of five variants using the dominant scoring (presence or absence) of bands based on UPGMA Dendrogram separated them into two groups at Jaccard's similarity coefficient of 0.46. Group I comprised of three variants namely S2 (Colchicine 3.5 %), S3 (γ irradiation 30 Gy) and S4 (γ irradiation 25 Gy). The remaining two variants namely S1 (Colchicine 3.0 %) and S5 (Control) formed Group II. In another study on mangosteen population, trees were separated into two main clusters at dissimilarity level of 27 per cent, the first of which was dominated by genetically identical trees and the second consisted of trees which showed genetic variability (Sobir and Poerwanto, 2007). Mansyah *et al.* (2007) conducted RAPD analysis of eight mangosteen accessions using two primers OPH-13 and OPN-16 and results showed that the accessions divided into two main groups with genetic similarity coefficient of 0.78 to 1.0. Grouping of accessions based on RAPD bands were not in accordance with morphological characters. Wittayawannakul *et al.* (2010) constructed dendrogram using UPGMA-SAHN cluster analysis based on peroxidise, RAPD and GSS amplification and showed that *Garcinia* species clustered into five groups at mean similarity coefficient of 0.54.

In the present study, the group I comprised of two clusters. The highest dose of gamma ray irradiation (30 Gy) formed one cluster and the other cluster was formed

with lower dose of gamma ray irradiation (25 Gy) along with colchicine (3.5 percent). S3 (γ irradiation of 30 Gy) showed some amount of variability in terms of reduction in biometric parameters like plant height, leaf production, total number of leaves, total leaf area, intermodal length and root length compared to plants irradiated with lower doses of γ irradiation or colchicine treated plants implying that some genetic changes were induced by this treatment. In another study, RAPD analysis on several irradiated mangosteen seedlings using five random primers resulted in 24 polymorphic bands and separation among mutants showed that genetic distance between irradiated seedlings based on dissimilarity level of Dice was 0.62 which was higher than the genetic variability of mangosteen accessions in Java Island of 0.27. Further a dendrogram based on UPGMA function showed that clustering among irradiated seedlings was not associated with gamma ray dose and effects of gamma ray irradiation was at random (Sobir and Poerwanto, 2007).

Physical and chemical mutagens are widely used to induce variability in many horticultural crops (Ray, 1999). The clustering pattern of the genotypes in the present study indicates that colchicine and gamma rays are able to induce some amount of variability in mangosteen. Prommee *et al.* (1999), Te-chato and Prommee (1999), Rostini *et al.* (2003) and Sobir and Poerwanto (2007) after carrying out RAPD analysis on several irradiated plantlets of mangosteen also reported that gamma ray irradiation is successful in increasing the genetic variability in the crop as well as in improving the desired mangosteen traits.

Further field evaluation is necessary to ascertain whether the mutagenic treatments have induced any desirable traits. Treated seedlings are planted in the field for further investigation.

Summary

6. SUMMARY

The present investigation on "Stimulation of growth and induction of variability in mangosteen (*Garcinia mangostana* L.)" was undertaken in the Department of Pomology and Floriculture, College of Horticulture, Vellanikkara during 2006 - 2009 with the objective of developing techniques for accelerating seedling growth, reducing gestation period and inducing variability through mutation and polyploidy in mangosteen.

The study comprised of four experiments involving enhancing seedling growth in the nursery, accelerating plant growth in the main field, use of nurse stocks and rootstocks to enhance the growth and induction of variability through induced mutation and polyploidy. Observations on growth and physiological parameters, flowering and yield attributes, foliar nutrient content and uptake of nutrients were recorded at different stages.

The salient findings of the study could be summarised as follows:

1. Study on the effect of different growing media and growth regulators on enhancing seedling growth in the nursery revealed that interaction between media and growth regulators was significant with respect to all the characters studied, except for petiole length, number of branches, Relative Growth Rate and uptake of magnesium and iron.

2. Among the four media compared, medium containing vermicompost was most superior in terms of plant height (46.70 cm), plant spread (52.90 cm), number of leaves (27.00), leaf length (23.73 cm), leaf breadth (8.21 cm), mean leaf area (121.37 cm^2), total leaf area (3275.98 cm^2), internodal length (3.46 cm), leaf thickness (0.379

mm), fresh and dry weight of all the plant parts, number of roots, length and spread of roots and chlorophyll content at the end of fifteen months of study.

3. In the case of vermicompost medium, effect of medium alone without application of any growth regulator was homogeneous with the superior growth regulator treatments in that medium in the case of characters like plant height, spread, leaf production, leaf length and breadth, mean and total leaf area, internodal length, leaf thickness, uptake of all macro nutrients and foliar nutrient content of P, K, Ca, Mg, Cu, Mn and Zn. This indicates that use of vermicompost medium itself without any additional use of growth regulators is sufficient to improve the growth of mangosteen seedlings.

4. In media other than vermicompost, use of several growth regulators had a profound influence on enhancing the growth of the seedlings. Use of IAA (all concentrations) and IBA (150 and 450 ppm) could enhance plant height in poultry manure. All concentrations of BA, two lower concentrations (150 and 300 ppm) of IAA, two higher concentrations (300 and 450 ppm) of IBA and GA 100 and 300 ppm were found to be effective in enhancing seedling growth significantly in coir pith compost medium. All the growth regulator treatments were homogeneous in cow dung medium with respect to plant height.

5. In medium containing well rotten cow dung, use of IBA 450 ppm was found to enhance internodal length significantly compared to use of media alone. IBA 150 ppm and all concentrations of GA (100, 200 and 300 ppm) were more effective than medium alone in improving internodal length in poultry manure medium. Two higher concentrations of IAA (300 and 450 ppm), IBA 300 ppm, GA 100 and 300 ppm were the treatments inducing a similar effect in medium containing coir pith compost.

6. In all the media, in general, treatments involving BA were able to induce a greater response compared to other growth regulators in the production of leaves.

With respect to total leaf area, in poultry manure medium, use of IAA 150 and 300 ppm and IBA 150 ppm was found to be effective in enhancing total leaf area significantly compared to use of media alone. In medium containing coir pith compost, in addition to IAA 150 and 300 ppm, all concentrations of IBA and BA were found to be effective in significantly improving total leaf area compared to use of media alone.

7. In all the media, growth regulators had a significant influence on root production compared to use of media alone, but their effect varied widely among media without following a definite pattern.

8. Plant biomass production was also significantly influenced by growth regulator application. BA 100 ppm in vermicompost medium, IBA 300 ppm in poultry manure medium and IAA 150 ppm in coir pith compost medium were superior to media alone in enhancing fresh weight of plant parts.

9. In the case of foliar nutrient content, a definite pattern of influence of growth regulators was not observed between the treatments and content varied among treatments differently in all the media.

10. With respect to nutrient uptake, in vermicompost medium all concentrations of BA were found to be superior compared to other growth regulators in terms of uptake of majority of nutrients. In the case of media containing well rotten cow dung and poultry manure, all the treatments were found to be uniform with respect to uptake of majority of nutrients. A definite pattern of nutrient uptake was not observed among different growth regulator treatments in the media containing coir pith compost.

11. In the present study, seedlings raised in vermicompost medium alone and along with the application of GA and BA were ready for transplanting to the main

field by fifteen months, at least nine months before the normal time required for transplanting. Hence a long period of nine months can be saved in the nursery by giving proper growth medium and growth regulator application. Seedlings raised in coir pith compost medium also attained this height by eighteenth month. Therefore with the use of suitable media and growth regulators, seedling growth can be accelerated in the nursery and seedlings attain transplanting size much earlier.

12. Considering the overall effect of various growth regulators studied, it was observed that, in general, all concentrations of BA were more effective than other growth regulators in improving growth parameters like height, production of branches and leaves, plant spread, plant biomass production, physiological parameters and nutrient uptake. Influence of IAA and IBA was evident only in the case of height, leaf area and root parameters while effect of GA was pronounced only in the case of plant height and internodal length.

13. Considering the overall growth of the seedlings for a period of fifteen months in four media, it may be concluded that, vermicompost medium was found to be the most superior in terms of all the growth and physiological parameters, foliar nutrient content and uptake of nutrients followed by coir pith, poultry manure and well rotten cow dung. In the absence of vermicompost, coir pith compost, poultry manure or well rotten cow dung can be used along with suitable growth regulators. For media containing coir pith compost, IAA 300 ppm, BA 200 ppm and BA 300 ppm are ideal while IAA 150 ppm, IBA 150 ppm and BA 300 ppm are the superior growth regulators for poultry manure medium that can enhance seedling growth significantly. For the normal potting mixture, use of IBA 450 ppm, BA 200 ppm, IBA 300 ppm can accelerate seedling growth than use of media alone.

14. In the experiment on the effect of growth promoting substances on enhancing seedling growth in the nursery showed that with respect to plant height, the treatment

combination nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm was significantly superior throughout the experimental period and recorded a maximum height of 37.04 cm at the end of 15 months. The treatment was most superior in terms of internodal length also.

15. *Azospirillum* sp. (10 g per plant) was the superior treatment influencing plant spread while nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm showed its superiority by recording the highest number of leaves (22.80) and lowest senescence (1.60) during the entire period of study.

16. Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % and *Azospirillum* sp. (10 g per plant) were the two superior treatments that influenced the major leaf parameters like leaf length, breadth, mean and total leaf area and petiole length.

17. *Azospirillum* sp. (10 g per plant) and nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % were the two superior treatments with respect to number of roots. *Azospirillum* sp. (10 g per plant) recorded maximum values for primary, secondary and total number of roots at twelfth month of the study. But with respect to number of tertiary roots, nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % recorded the highest values during sixth and twelfth months. Next to *Azospirillum* sp. and nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 %, nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + BA 100 ppm recorded the highest number of secondary as well as total roots.

18. Cow's urine (25 times dilution with water) and nutrient solution- foliar grade 3:1:1 (NPK) – 0.25 % + GA 100 ppm were the two superior treatments with respect to root length and spread.

19. Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % was the most superior treatment in terms of fresh and dry weight of all the plant parts, Leaf Area Index, Relative Growth Rate and dry matter production.

20. Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + BA 100 ppm followed by nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm were the two superior treatments with respect to shoot- root ratio.

21. *Pseudomonas* sp. - 2 % and nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % + GA 100 ppm + BA 100 ppm were the two superior treatments in terms chlorophyll 'a', 'b' and total content throughout the experiment.

22. A definite pattern of leaf nutrient content was not observed among the treatments. Considering all the treatments, AMF (10 g per plant) recorded highest content of more elements at different stages. But with respect to uptake of nutrients, only three treatments recorded highest values for the uptake of all the nutrients. Nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % recorded the highest uptake in the case of N, P, Ca, Mg and Mn while *Azospirillum* sp. (10 g per plant) recorded highest values for K, Fe and Zn. The highest uptake of Cu was recorded by AMF (10 g per plant).

23. Considering all the growth parameters, namely, plant height, leaf characteristics, canopy development, root production and overall growth and vigour of the seedlings, nutrient solution- foliar grade 3:1:1 (NPK) – 0.50 % followed by *Azospirillum* sp. (10 g per plant) were the superior treatments.

24. In the experiment with two year old grafted plants, plant height and the factors contributing to height like extension growth of main stem and branches were significantly influenced by the growth regulators. GA and GA, BA combinations showed significant effect on these characters. The extension growth of main stem for one year period was highest (46.37 cm) in GA 100 ppm followed by GA 100 ppm + BA 100 ppm and GA 200 ppm + BA 100 ppm. But in the case of branches, GA 200 ppm + BA 100 ppm recorded the highest mean extension of growth per year (26.55 cm) followed by GA 100 ppm and GA 100 ppm + BA 100 ppm. GA 200 ppm + BA 100 ppm also showed maximum influence on plant girth. GA 100 ppm followed by GA 100 ppm + BA 100 ppm and GA 200 ppm + BA 100 ppm were the superior treatments influencing plant spread.

25. GA, BA combination had significant effect on production of branches in grafted plants of mangosteen. GA 200 ppm + BA 100 ppm showed highest influence on production of primary and total number of branches and GA 100 ppm + BA 100 ppm was more effective in inducing secondary branch production.

26. With respect to leaf production, GA 200 ppm + BA 100 ppm was the most significantly superior treatment with a production of 7.59 leaves per shoot per year. In addition, leaf production and further vegetative growth were continuous all through the year without a definite period of bud dormancy thereby ensuring continuous flushing and faster growth round the year. GA 100 ppm and GA 100 ppm + BA 200 ppm were the superior treatments with respect to leaf area.

27. In short, in two year old grafted plants, GA and combinations of GA and BA significantly influenced the various growth parameters viz., extension of shoot, girth, plant spread, production of leaves and related parameters. GA 200 ppm + BA 100 ppm has dominated with respect to majority of parameters studied followed by GA 100 ppm + BA 100 ppm and GA 100 ppm. Therefore these growth regulators given

as lanolin paste at shoot tip at monthly interval could successfully be employed to enhance the growth of field grown mangosteen grafts which are extremely slow growing.

28. In five year old orchard trees, external application of growth regulators did not influence significantly on vegetative characters. Still BA 200 ppm influenced plant height better than other treatments. Treatments involving GA and paclobutrazol had a positive response with respect to production of primary, secondary and total branches. Among the treatments, GA, BA combinations were found to be more effective in extension of shoot in the primary branches.

29. Considering the combined effect on shoot growth and leaf production, treatments GA 100 ppm + BA 100 ppm, GA 100 ppm + BA 200 ppm and GA 200 ppm + BA 100 ppm were found to be more effective than others in accelerating the growth of juvenile orchard trees.

30. Treatments GA 200 ppm + BA 100 ppm, GA 200 ppm + BA 200 ppm applied as lanolin paste at shoot tip at monthly interval and drenching of paclobutrazol 2.0 g a.i./tree during September – October were superior and equally effective in induction of flowering in five year old orchard trees.

31. Growth regulators showed a significant influence on yield and yield attributes of 5 – 6 year old trees. Paclobutrazol 2.0 g a.i./tree was the most superior treatment in terms of yield (6.45 kg per tree), number of fruits per tree (114.33), number of flowers per unit canopy area (8.53 per 0.5 m x 0.5 m canopy), percentage of edible portion (37.96 per cent), higher number of big sized segments (2.00 per fruit) and least incidence of gamboge (5.56 per cent). Next to paclobutrazol, GA and GA, BA combinations also influenced yield.

32. In mangosteen, more than age, canopy size is the factor deciding early flowering. So in very young trees, where sufficient canopy has not developed, the primary objective would be promoting growth so that a canopy sufficient for flowering is attained as early as possible. To achieve this objective and to overcome juvenility, application of GA 100 ppm + BA 100 ppm, GA 100 ppm + BA 200 ppm and GA 200 ppm + BA 100 ppm as lanolin paste at shoot apex were found to be effective.

33. In trees which have developed sufficient canopy but not yet flowered, early flowering can be induced with the use of GA 200 ppm + BA 100 ppm, GA 200 ppm + BA 200 ppm and Paclobutrazol 2.0 g a.i./tree.

34. Yield, yield attributes and fruit characters can significantly be improved in mangosteen with the use of Paclobutrazol 2.0 g a.i./tree followed by GA 200 ppm and GA 100 ppm + BA 100 ppm.

35. Growth of the mangosteen seedlings grafted with different nurse stocks such as *Garcinia mangostana, G. indica, G. hombroniana, G. gummi-gutta, G. xanthochymus and G. cowa* was compared with that of seedlings (control) of same age. Various nurse stocks showed a considerable effect on growth characters of mangosteen seedlings. With regard to increase in plant height, leaf production, average leaf area, total leaf area, canopy spread, internodal length and number of branches, mangosteen seedlings having its own seedlings as nurse stock recorded the highest values after eighteen month period compared to seedlings with other nurse stocks. This indicates that seedling growth can be promoted considerably by nurse stock grafting with *G. mangostana* seedlings.

36. Graft incompatibility of related species with mangosteen resulted in the poor growth performance of seedlings grafted with these nurse stocks and these species are not recommended for nurse stock grafting with mangosteen seedlings.

37. Study on the performance of softwood grafts of mangosteen using seedlings of its own and related species as rootstock revealed that among the various rootstocks used, the least time for sprouting (24.40 days), highest percentage of sprouting (93.33 %) and maximum success of grafting (83.33 %) were recorded in grafts using *G. mangostana* as rootstocks. These grafts also recorded highest values for growth parameters like mean leaf production, mean leaf area, mean total leaf area and scion growth for a period of 18 months after grafting.

38. Grafts with rootstocks of related species showed varying degrees of graft success ranging from 0.00 to 55.26 per cent. But the resultant grafts showed stunted growth compared to grafts using *G. mangostana* seedling as rootstock. In the case of *G. gummi-gutta* and *G. cowa,* no grafts survived after a period of two years.

39. The results indicate that among the rootstocks studied for enhancing growth of mangosteen plants, mangosteen is compatible with its own rootstock (*G. mangostana*) only and all other rootstocks showed various degrees of incompatibility in terms of success of grafting and growth rate. Comparison of growth of grafts with seedlings, seedlings had a slightly better growth rate with respect to shoot growth, leaf production and mean leaf area during the period of study. Hence new combinations need to be developed to induce faster growth and early bearing since grafts on its own seedlings had a very slow growth rate.

40. Large-scale multiplication of planting materials could be achieved through rooting of softwood cuttings from juvenile shoots of mangosteen. Eighty six per cent of cuttings rooted within a period of two months even without the use of growth

regulators. The rooted cuttings also had profuse root growth with large number of lateral roots compared to seedlings.

41. Studies on induction of variability showed that seeds irradiated with 5 Gy to 50 Gy gamma irradiation showed wide variation in days required for germination as well as percentage of germination. As the dosage of irradiation increased, days required for germination also increased progressively ranging from 19.20 days (5 Gy) to 133.60 days (30 Gy). Similarly germination percentage showed a gradual decline from 100 per cent (5, 10 and 15 Gy) to 50 per cent (30 Gy). Doses beyond 30 Gy completely prevented germination.

42. Seedlings irradiated with 30 Gy dose showed the lowest values for plant height (5.87 cm), leaf production (3.33), total number of leaves (3.33), total leaf area (26.23 cm^2), intermodal length (0.13 cm) and root length (5.75 cm) indicating a possible variation originated due to genetic change through gamma irradiation (30 Gy).

43. Irradiation of scions with 5 Gy to 50 Gy had an adverse effect on days required for sprouting of scions, percentage of sprouting and final graft success. Scions without any irradiation (control) took minimum time for sprouting (16.00 days) and the maximum time (36.00 days) was noted in 40 Gy dosage. In the case of doses above 15 Gy, no grafts survived after a period of 180 days of grafting. Among the treated scions, the maximum survival of grafts was noted in 10 Gy (20 per cent) whereas control recorded a success rate of 80 per cent. All the treated scions showed a stunted growth after sprouting and those survived after one year presented an underdeveloped appearance. Further studies are required at molecular level to confirm any genetic change that has occurred in the irradiated scion.

44. Soaking of seeds with 0.1 to 3.5 per cent colchicine solution resulted in seedlings which were uniform in morphological characteristics without any visible variations.

45. Two seedlings treated with colchicine at 3.0 and 3.5 per cent concentrations in the apical bud showed vigorous growth and distinct variation in plant height, extension of shoot, internodal length, canopy spread, size of the leaf, leaf area and leaf production compared to rest of the seedlings. This indicated a possible genetic change induced due to colchicine application.

46. Five mangosteen seedlings with induced variations selected from irradiation and colchicine treatments were subjected to RAPD analysis. Clustering of five variants based on dendrogram separated the genotypes into two groups. Clustering pattern indicated that seed irradiation with 25 Gy and 30 Gy gamma rays and bud application of colchicine 3.5 per cent were effective in inducing variation in genomic DNA of mangosteen.

Suggested future line of work

1. Among the growth promoting substances used for enhancing seedling growth in the nursery, nutrient solution containing NPK (3:1:1 – 0.50 %), the highest dose used in the study, was the most superior treatment. Hence in future trials, a still higher dose of nutrient solution may be tried.

2. A combination effect of vermicompost medium along with nutrient solution containing NPK (3:1:1 – 0.50 %) may be studied as these were identified as the best medium and best growth promoting substance respectively.

3. In the present experiment, GA, BA combinations were applied directly to the apical bud as lanolin paste. This is a very cumbersome and time consuming process. Therefore a better method of application of these growth regulators such as periodic spraying along with some additives may be tried.

4. Extensive field trials may be taken up to evaluate the performance of mangosteen grafts with nurse stocks.

5. Studies may be taken up to evolve new rootstocks compatible with mangosteen to induce faster growth and early bearing since grafts on its own seedlings had a very slow growth rate and its related species were graft incompatible.

6. Possibility of introducing potential rootstocks such as *Garcinia malaccensis*, *G. speciosa* and *G. livingstonei* may be explored and grafting studies using these rootstocks may be conducted.

7. In five year old trees, the highest dose of GA and BA tried was 200 ppm each. Compared to two year old grafts, these doses did not influence much on growth parameters of five year old juvenile mangosteen trees. Therefore a higher dose of these growth regulators may be tried in future in order to assess their response.

8. Response to lower dose of paclobutrazol (1.5 g a.i/tree) towards induction of flowering was found to be less compared to its higher dose of 2.0 g a.i/tree. So in future trials, effect of dosages above 2.0 g a.i/tree may be investigated.

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Appendices

APPENDIX I

Weather data of the experiment site

Year: 2006

Month	Mean temperature (^{0}C)		Mean RH (%)	Rainfall (mm)	Mean sunshine (hrs.)	Mean wind speed (km/hr)
	Maximum	Minimum				
January	32.5	22.6	57	0.0	8.2	8.5
February	34.3	22.3	51	0.0	9.6	7.2
March	34.8	23.8	68	95.2	7.6	4.0
April	33.4	24.7	75	86.2	7.0	3.6
May	31.8	24.3	79	675.5	5.8	3.7
June	29.9	23.6	84	608.6	3.8	3.8
July	29.5	23.3	85	519.0	2.2	3.2
August	29.0	23.1	83	550.6	4.2	2.7
September	29.4	23.0	84	522.2	3.9	3.0
October	30.5	23.0	79	323.7	4.8	3.2
November	31.7	23.7	72	79.5	6.3	4.5
December	31.6	23.6	54	0.0	7.8	8.6

Year: 2007

Month	Mean temperature (^{0}C)		Mean RH (%)	Rainfall (mm)	Mean sunshine (hrs.)	Mean wind speed (km/hr)
	Maximum	Minimum				
January	32.5	22.0	54	0.0	8.7	9.2
February	34.0	22.2	55	0.0	9.8	4.9
March	36.0	24.4	63	0.0	8.2	4.3
April	35.1	25.0	69	61.0	7.7	4.3
May	32.8	24.6	76	240.5	6.6	3.7
June	30.1	23.5	84	826.5	3.5	3.8
July	28.4	22.9	88	1131.9	0.7	3.2
August	29.0	22.8	84	549.7	3.2	2.7
September	29.4	22.9	86	765.9	2.5	3.0
October	30.5	22.5	79	383.8	4.4	3.2
November	31.7	21.6	67	24.8	8.0	4.5
December	32.2	22.7	56	8.7	6.7	8.6

Year: 2008

Month	Mean temperature (^{0}C)		Mean RH (%)	Rainfall (mm)	Mean sunshine (hrs.)	Mean wind speed (km/hr)
	Maximum	Minimum				
January	32.3	21.7	59	0.0	9.4	7.0
February	33.6	22.9	61	29.7	8.2	4.5
March	33.2	23.4	64	205.3	6.9	4.8
April	33.2	24.9	75	65.6	6.3	3.2
May	33.0	24.7	73	11.5	6.1	3.5
June	29.9	23.5	85	636.7	2.0	3.0
July	29.3	23.2	84	416.3	2.7	3.1
August	29.8	23.6	82	321.9	3.4	2.9
September	30.6	23.6	80	314.2	5.4	2.4
October	31.7	23.4	76	380.8	5.7	3.3
November	32.2	23.1	70	21.7	6.0	4.0
December	31.6	22.5	60	2.6	7.7	7.1

Year: 2009

Month	Mean temperature (^{0}C)		Mean RH (%)	Rainfall (mm)	Mean sunshine (hrs.)	Mean wind speed (km/hr)
	Maximum	Minimum				
January	32.8	21.9	54	0.0	9.4	8.0
February	35.1	22.1	57	0.0	9.9	5.1
March	35.1	24.4	70	29.0	7.9	3.4
April	34.5	25.3	74	16.5	5.8	1.8
May	33.0	24.8	77	199.5	5.5	1.9
June	30.0	23.7	84	565.0	3.9	3.4
July	28.6	22.8	88	985.8	1.7	3.6
August	30.2	23.2	85	421.4	4.1	2.9
September	30.0	23.2	83	276.0	4.0	3.0
October	32.0	23.2	77	166.8	6.7	3.3
November	31.5	23.7	76	180.6	5.7	4.6
December	31.8	23.9	62	42.7	7.8	8.9

STIMULATION OF GROWTH AND INDUCTION OF VARIABILITY IN MANGOSTEEN (*Garcinia mangostana* L.)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Mangosteen (*Garcinia mangostana* L.) is identified as a fruit crop deserving priority attention with a potential for increased acceptability. It is recognized as the 'queen of tropical fruits' due to its instant visual and taste appeal and has recently been popularized for its medicinal benefits. It yields profusely and fits very well as a component in the homesteads of Kerala. Almost all trees are female and variability is meagre. Its slow growth and long gestation period limit its commercialization.

The present investigation on "Stimulation of growth and induction of variability in mangosteen (*Garcinia mangostana* L.)" was undertaken in the Department of Pomology and Floriculture, College of Horticulture, during 2006 - 2009 with the objective of developing techniques for accelerating seedling growth in the nursery, reducing gestation period and inducing variability through mutation and polyploidy.

The studies on different growing media and growth regulators on seedling growth in the nursery revealed that potting mixture containing vermicompost as organic component was the most superior medium in terms of all the growth and physiological parameters, foliar nutrient content and uptake of nutrients, followed by coir pith, poultry manure and well rotten cow dung. Use of vermicompost medium without any additional growth regulators was sufficient to accelerate seedling growth in the nursery. When coir pith compost, poultry manure or well rotten cow dung was used in the medium, growth regulators had specific effect. Along with coir pith compost, IAA 300 ppm, BA 200 ppm and BA 300 ppm were ideal, while IAA 150 ppm, IBA 150 ppm and BA 300 ppm were superior with poultry manure medium. In normal potting mixture using cow dung, IBA 450 ppm, BA 200 ppm and IBA 300 ppm showed superiority. With the use of suitable media and growth regulators,

seedling growth can be accelerated in the nursery and transplanting stage can be attained much earlier.

Evaluation of different growth promoting substances in normal potting mixture showed that foliar spray of nutrient solution 3:1:1 (NPK) – 0.50 % (50 ml per plant) and *Azospirillum* sp. (10 g per plant) applied at fortnightly interval were the superior treatments with respect to all the growth parameters.

In two year old grafted plants in the main field, a combination of GA 200 ppm + BA 100 ppm as bud application was the best with respect to majority of growth parameters, followed by GA 100 ppm + BA 100 ppm and GA 100 ppm. These growth regulators can successfully be used in early stages for promoting growth.

In five year old juvenile orchard trees also GA and BA combinations, namely, GA 200 ppm + BA 100 ppm, GA 100 ppm + BA 100 ppm and GA 100 ppm + BA 200 ppm were the best treatments in accelerating growth and improving flushing.

For the induction of flowering, soil drenching of paclobutrazol 2.0 g a.i. per tree, and bud application of GA 200 ppm + BA 100 ppm and GA 200 ppm + BA 200 ppm were superior and equally effective. For improving yield and yield attributes, paclobutrazol 2.0 g a.i. per tree was the most superior, followed by GA 100 ppm + BA 100 ppm and GA 200 ppm. Incidence of gamboge was minimum in paclobutrazol treatments, compared to GA, BA combinations.

Among the various rootstocks tried, mangosteen was most compatible with its own rootstock where as all other rootstocks showed varying degrees of incompatibility. On comparing the growth of softwood grafts with seedlings, the

latter showed faster rate of growth. Seedling growth could also be promoted by the use of nurse stocks.

Large-scale multiplication of planting materials could be achieved through rooting of softwood cuttings from juvenile shoots of mangosteen.

Seeds exposed to 5 Gy to 50 Gy gamma radiation showed wide variation in germination. Beyond 30 Gy, seeds failed to germinate. Seedlings from 30 Gy dose showed stunted growth indicating a possible genetic variation. Irradiation of scions with 5 Gy to 50 Gy had an adverse effect on days required for sprouting of scions, percentage of sprouting and final graft success. All the irradiated scions showed stunted growth even after one year. Two seedlings treated with colchicine at 3.0 and 3.5 per cent in the apical bud showed vigorous growth and distinct variation in growth characteristics.

Five mangosteen seedlings with induced variations selected from irradiation and colchicine treatments were subjected to RAPD analysis. Clustering of five variants based on dendrogram separated the genotypes into two groups. Clustering pattern indicated that seed irradiation with 25 Gy and 30 Gy gamma rays and bud application of colchicine 3.5 per cent were effective in inducing variation in genomic DNA of mangosteen.