IN VITRO PROPAGATION STUDIES IN *Momordica dioica* Roxb.

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in

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CERTIFICATE

This is to certify that the thesis entitled "***IN VITRO* PROPAGATION STUDIES IN *Momordica dioica* Roxb.**" submitted by **Ms. REKHA B.** for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **HORTICULTURE** to the University of Agricultural Sciences, Dharwad is a record of research work done by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis for the award of any other degree, diploma, associateship, fellowship or other similar titles.

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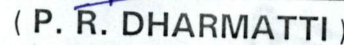

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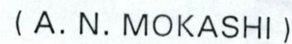

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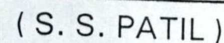

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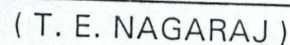

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Affectionately Dedicated

To

Beloved Parents,
Brothers
&
Sister

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Introduction

I. INTRODUCTION

Spine gourd (*Momordica dioica* Roxb.) is an under exploited cucurbitaceous perennial vegetable of Indo-Malayan origin (Rashid, 1976; Singh, 1990). It belongs to the family cucurbitaceae. Area under production of this crop is not available because of its sporadic cultivation, especially in forest areas. In India it is mainly grown in Orissa, Bihar and West Bengal. It is naturally growing in the forest areas of Karnataka, Maharashtra, Tamilnadu and Kerala. Tribal people collect the fruits during monsoon and sell in the metropolitan cities at a premium price.

In south India spine gourd is not cultivated on farmers field, and it is grown in forest areas of Karnataka during the onset of monsoon. After one month of emergence it will bear flowers and fruits. Flowers start and continue up to the senescence of vines (Hussain and Rashid, 1974). Fruits are small, dark green in colour, tough, contain false spines and non-bitter in taste. After the end of monsoon, the senescence of vine will begin and the tubers will remain dormant until the next monsoon season and repetitions of sprout from tubers will start during the pre-monsoon showers (last week of May to first week of June).

Spine gourd is a cross-pollinated crop and cross pollination ranges between 80-100. The fruits are rich in vitamin C, proteins (3.1 g per 100g), Calcium (33mg per 100g), Carotene (162mg per 100g) and minerals (1.1g per 100g). (Maharana and Tripathy, 1996). Slices of unripe fruit are served in different types of curries and fried forms. Spine gourd is also called as *Kakrol* in Hindi, *Adavihagalakai* in Kannada, it is cultivated in the tropical and sub-tropical countries. Among the cucurbits, Spine gourd is gaining lot of importance in the metropolitan cities because of high nutritive value.

Fruits of spine gourd are used in the preparation of pickles. It has several medicinal values, *viz.,* good healing power and wormicidal effect in the body. The fruit extraction are also used for curing blood diseases, rheumatism, asthama, diarrhea, diabetes, sugar and acidity. Spine gourd has very good demand in the market and it costs about Rs 60-80 per Kg and rarely available in metropolitan cities. Although it is a remuenerative crop, but farmers are not ready to cultivate on large scale due to lack of scientific information on cultivation of crop, non-availability of seeds etc. Germination of the seeds is very difficult or impossible because of hard seed coat (*Rashid,* 1976). It is dioecious in nature. Tubers will remain dormant and emerge only in rainy season. Moreover, it is impossible to predict the sex of seed produced plants. Systematic investigation followed by popularisation of the crop is very much important. Hence the study on *in vitro* propagation is very much needed.

Tissue culture is a commonly used collective term to describe all types of *in vitro* plant culture. Tissue culture can be defined as the art of growing isolated plant parts aseptically on appropriate media. A tissue culture method of propagation could produce numerous plants from a single seed and helps in breakdown the dormancy of seed. The main advantage of *in vitro* propagation studies are rapid multiplication of plants, free from pest and diseases, and for the improvement of crop as mentioned by Withers and Alderson (1986) in the following ways.

1. Induction of genetic variability (Somoclonal Variation) and its application in breeding.
2. *In vitro* mutagenesis on haploid and diploid basis.
3. *In vitro* selection for disease resistance, cold resistance and herbicide tolerance.
4. *In vitro* fertilization.

5. Circumvention of cross barriers between different species and genera by protoplast fusion.

6. Transfer of important genes

7. Cultivation of non-viable embryos occuring *in vivo*.

The practice of propagation through cuttings is slow, inconvenient and uncertain.

Hence, there is a need to standardize methods for the *in vitro* multiplication of *Momordica.* With this background, the present study was undertaken with following objectives.

a) To standardize the explant for rapid multiplication.

b) To study the effect of growth regulators on shoot growth from auxillary shoot buds using cytokinins.

c) To study the effect of growth regulator on rooting of *in vitro* shoots with various auxins.

d) To standardize the conditions for hardening of regenerated plantlets.

Review of Literature

II. REVIEW OF LITERATURE

Spine gourd is one of the most important unexploited cucurbitaceous perennial vegetables seen in forest areas. It has become an important nutritious medicinal vegetable crop in metropolitan cities. Its availability is very much restricted and many vegetable consumers are not aware of its importance. Till today very limited literature has been generated in spine gourd. Therefore the various aspects of spine gourd production and its scientific cultivation is not known to the vegetable growers. The information on production, varieties, Agro-techniques and mode of propagation is very much meager in spine gourd. The progress made to derive such information on method of propagation in spine gourd is very meager. Therefore the work on other cucurbitaceous vegetables are reviewed in this chapter.

The concept of plant cell, tissue and organ culture was reported by Schleiden (1838) and Schwann (1839). However, the concept of totipotency, which means that all cells containing a normal complement of chromosomes should have the ability of regenerating the entire plant. Haberlandt (1902) was the first person to attempt the culture of plant cells *in vitro* on a nutrient media.

White (1934) obtained the successful culture of tomato roots continuously *in vitro.* Further, Gautheret (1939), Nobecourt (1939) and White (1939) reported that cells in culture can be made to proliferate continuously and also to undergo differentiation.

The main application of *in vitro* plant culture in crop production are in micro propagation and production of disease free plants (Morel and Martin, 1952).

Tissue culture is currently being applied for the improvement of large variety of vegetable crops on different methods of propagation, effect of growth regulator, source of explant and hardening technique. Research work carried on "*In vitro* propagation of *Momordica*" is reviewed below. The work on this crop is very less, hence the literature on other cucurbitaceous crops are reviewed and presented under four stages of micropropagation.

1. Establishment stage
2. Multiplication of shoot
3. Induction of root
4. Hardening and Acclimatization

2.1 ESTABLISHMENT OF CULTURE

2.1.1 Type of Explant

Plants grown under both controlled condition (greenhouse or growth room) and field grown plants can be used for *in vitro* studies (Pierik, 1987). The type of explant, its size, position and physiological age have great influence on their morphological activity and degree of differentiation on *In vitro*. These points have great influence while selecting a suitable explant for micro propagation.

In shoot tip and auxillary bud culture, genetic stability is maintained to a large extent, callus mediated organogenesis and somatic embryogenesis are not recommended for clonal propagation, since there is a possibility of producing aberrants (Murashige, 1974).

Rajasekaran *et al.* (1983) reported that hypocotyl explants of cucumber produced callus on MS medium with 0.5 or 1.0 μM BAP and 1.5 or 5.0μM 2,4-D and also observed that somatic embryos and adventitious buds were formed when transferred to medium composed of growth regulators, with flowers of either staminate or pistillate.

Sang and Joung (1984) reported that explants of seedling cotyledon of *Cucumis sativus* produced the callus and regenerated number of plants through subcultures with no loss of regeneration potentiality.

Jeffrey *et al.* (1990) Showed the production of tetraploid in watermelon and muskmelon and regenerated from immature cotyledons on MS medium containing 10 μM BA.

Kageyama *et al.* (1991) reported that from mature seeds of melon, larger number of normal somatic embryoids were obtained from 7-day old cultures on MS medium containing 0.5% activated charcoal.

Chee (1992) observed that in squash embryogenic callus tissues were initiated with cotyledons of excised mature seeds and cultured on MS medium supplemented with either 2,4-D or BA and Kinetin.

Szymanska and Molas (1995) obtained seedlings from cucumber seeds and leaf explants on MS medium.

Zhang *et al.* (1995) reported that cotyledons, leaves and stems of *Cucumis sativus* used as explants to induce regenerated plants.

The percentage of cotyledons with shoots was 1.7 fold greater for cotyledons derived from seedlings incubated in darkness for 7 days than those germinated in light (Michael, 1997).

Kintzios and Taravira (1997) reported that cotyledonary explants of melon were cultured on MS medium with 2,4-D and Kinetin for the induction of shoots.

Molina and Nuez (1997) noticed that the cotyledon explants from seedlings of *Cucumis melo* had good regeneration capacity on MS medium with a high auxin : cytokinin ratio.

Shoots were regenerated on NAA free media with high cytokinin and ABA. Both NAA and BAP were more effective in inducing multiple shoots of culturing cotyledons of mature seeds of melon (Suh and Suh 1998).

Mythili and Thomas (1999) reported the rapid *in vitro* multiplication of pointed gourd, shoot tip and nodal explants on MS medium of 2 female cultivars Swarna Alaukik and Swarna Rekha.

Zhao and Zhao (1999) observed the high frequency *in vitro* regeneration of adventitious buds from cotyledon explants of *cucurbita moschata* on medium containing 4.0 mg/l BA and IAA.

Venkateshwaralu *et al.* (2001) reported that multiple shoot induction was achieved on stem and cotyledon explants from aseptically grown seedlings of *Coccinia indica.*

Kintizios *et al.* (2002) noticed the formation of somatic embryos when explants of squash and melon pre-treated with kinetin and 2, 4-D for 48 hours respectively.

Nabi et al. (2002) reported that out of four types of explant *viz.,* node, shoot tip, leaf and cotyledon of Teasle gourd cultured *in vitro* cotyledon showed best performance.

Curuk *et al.* (2003) reported that regeneration from the hypocotyl explants of cucumber resulted 100% diploid shoots, whereas from cotyledon resulted in 40.70% on MS medium provided with 4.45M BAP.

Mala and Raka (2004) reported that regeneration from shoot and leaf explants of Momordica when cultured aseptically on MS medium.

2.2 SHOOT MULTIPLICATION

The production of shoots that may eventually become new plant is called shoot proliferation.

The formation of adventitious shoots or roots was first determined by Skoog and Miller (1957) through discovery of the regulation of organ formation (Shoots and roots) by changing the ratio of Cytokinin / auxin when the ratio of cytokinin / auxin is high. It favours the formation of shoot but root formation is inhibited. These cytokinins are often used to stimulate growth and development. Most commonly used cytokinins include kinetin (KIN), 6-Benzyl amino purine (BAP) and 2(ip) isopentyl adenine. Several attempts were made to establish protocols for efficient plant regeneration and production of number of shoots in woody perennial crops.

The major break through in tissue culture of plant cells was the discovery of Auxins and Cytokinins. Auxins were discovered by Went (1926).

The first known growth regulator namely IAA was identified and purified by Kogl *et al.* (1934) to regulate plant cell growth in a medium.

The discovery of kinetin was reported by Skoog and Miller in 1957. Murashige and Skoog (1962) incorporated cytokinins to MS medium and proved that the combination of Auxin and cytokinin had a vital role in

affecting growth and development of cells in plant cell culture. Murashige (1974) was instrumental in giving the technique of *in vitro* culture and the status of a viable practical means for rapid and mass propagation of horticultural species.

Shoot tip culture is an important way to establish virus free plants. The demonstration of the practical utility of this important technique must be credited to Morel and Martin (1952) and first time recovered virus free dahlia plants from infected individuals by excising and culturing the apical shoot tips *in vitro.*

The pioneer work on *in vitro* propagation of cucumber was carried out by Handley and Chambliss (1979). They reported that auxillary buds of gynoecious cucumber on MS medium with 0.1mg/litre of NAA and Kinetin produced both shoots and roots.

Hisajima (1982) reported that multiple shoots were induced from the seeds of cucumber cultured on MS medium containing BAP.

Roberto (1982), obtained that meristem tips of *Castanea sativa* seedling explants cultured on MS medium with 1 mg per litre of BAP and 0.01 mg per litre of IAA, initiated multiple shoot buds.

Pink and Walkey (1984) noticed the rapid propagation of *Cucurbita pepo.* With meristem tips on MS medium containing 2.56 mg per litre of Kinetin and 8 mg per litre of IAA.

Lee and Thomas (1985) obtained 4 to 9 multiple shoots from the apical shoot tips and auxillary buds of buffalo gourd on MS medium within four weeks.

Kathal *et al.* (1988) noticed maximum shoot differentiation, when leaf explants of *Cucumis melo* were cultured on MS medium containing BAP and 2 mg of isopentyl adenine at 1 μM each.

Custers and Verstappen (1987) obtained normal plants in cucumber, when shoot tips and nodal explants, taken from *in vitro* seedlings were cultured on MS medium.

Hisajima *et al.* (1989) induced multiple shoot buds in cucumber, when seeds were cultured *in vitro* with 5 μM BAP, and shoot multiplication was observed in the combination of BAP and IBA.

Neidz *et al.* (1989) observed the maximum initiation of shoot buds when cotyledonary explants of 4 days old *Cucumis melo* cultured on MS medium supplemented with 5μM of IAA and BAP

Cade *et al.* (1990 a) obtained the shoot formation from cucumber cotyledonary nodes. Shoot production was highest (60%) on MS medium consisting only 0.3 mg per litre of BAP.

Ali *et al.* (1991 a) obtained cucumber plants when cotyledons from old cucumber seeds were cultured on MS medium containing 1 mg/litre each of NAA and BAP.

Gambley and Dodd (1990) induced *de novo* production of multiple shoots in cotyledon explants of cucumber using various cytokinins like BAP, Kinetin and ip at 4 mg/litre.

Gambley and Dodd (1991) reported that in cucumber hypocotyl explants and the apical bud are capable of producing multiple auxillary buds

from seedling apex, and adventitious shoots from the hypocotyl base, in a medium containing 2.0 mg/litre of kinetin.

Chee (1991) obtained shoot regeneration in *cucumis melo* by culturing the cotyledons on MS medium with NAA at 0.01 mg/litre.

Dong and Jia (1991) on culturing five days old cotyledons of watermelon on MS medium with 5.0 mg/litre of BAP and 0.5 mg/litre of IAA noticed high frequency of shoot regeneration (60-92%).

Compton and Gray (1992) observed the development of 2.3 to 5.2 shoots per explant from shoot tip of watermelon in MS medium with 1mg BAP.

Josekutty *et al.* (1993) reported successful regeneration of *Coccinia indica* plants, by culturing nodal explants on MS medium supplemented with 2.5 mg BAP + 0.5 mg Kinetin + 0.1 mg IBA per litre.

Compton and Gray (1994) obtained large number of shoots from 2 day old cotyledon explants of watermelon compared to 4,6,7 or 10 day old seedlings.

The leaf explants of 5 μM size of cucumber seedling of 14 days old gives shoot differentiation (Misra and Bhatnagar, 1995).

Shoot induction in muskmelon was better from Cotyledon petiole explants compared to leaf explants in BAP 0.5 mg per litre (Dong *et al.*, 1996).

The direct induction of shoot buds was obtained from cotyledon halves of *Cucumis melo* cultured on MS medium with 1.0 mg BAP (Singh *et al.,* 1996).

Nabi *et al.* (2002) observed the highest number of multiple and tallest shoots in MS medium with 1 mg BAP and 0.1 mg NAA in Teasle gourd.

Mala and Raka (2004) reported that multiple shoot development in *Momordica* was obtained on MS media + BAP 0.5 mg per litre.

2.3 ROOTING AND HARDENING

One of the major obstacles in the application of tissue culture method of plant propagation has been the difficulty in successful transfer of plantlet from the lab to the field (Wardle *et al.,* 1983). The reasons for such a difficulty appears to be due to dramatic change in the environmental condition. The environment of the culture vessel is one of low light intensity, with very high humidity and poor root growth, while the greenhouse or field condition are typified by very high light intensity, low humidity and mycoflora (Desjardine *et al.,* 1987)

Handley and Chambliss (1979) They cultured auxillary buds on MS medium supplemented with 0.1 mg/litre of NAA and kinetin to get both shoots and roots and the plants were successfully weaned in the greenhouse.

Barnes (1979) Rooting of shoot tips in a medium containing 11.5μM IAA, and hardened under greenhouse in 1:1 peat and sand medium for 3 weeks.

Wehner and Locy (1981) noticed that, when hypocotyl explants taken from 7 day old seedlings were grown on MS medium containing 1 mg/litre each of BAP and NAA, shoots and roots were initiated from the explants.

Hisajima (1982) reported that the cucumber seeds induced the multiple shoots on MS medium containing BAP, and rooting of these shoots was achieved without any auxin, the plants were successfully transferred to soil.

Roberto (1982) studied the *in vitro* propagation of *Castanea sativa* through meristem tip culture. Rooting was achieved when the single shoots were transferred to a fresh medium supplemented with 1 mg/litre of IBA .

Rooting can be achieved by culturing meristem tips of pumpkin on MS medium containing 8 mg/litre of IAA (Pink and Walkey, 1984).

Lee and Thomas (1985) cultured apical shoot tips and auxillary buds of buffalo gourd on MS medium and obtained 4 to 9 multiple shoots within four weeks, and these shoots when subcultured onto another MS medium produced roots in four weeks, and the plants were successfully transferred in a greenhouse.

Kathal *et al.* (1988) noticed that maximum shoot differentiation with leaf explants of *Cucumis melo* in MS medium and these shoots were rooted in MS medium containing 0.5μM IAA.

Hisajima *et al.* (1989) reported that in cucumber plant regeneration was achieved by rooting single shoots in IBA, where in the rooting rate was 100 percent by taking seed as an explant.

Trajanowska and Malepsgy (1989) induced shoot regeneration directly on MS medium supplemented with 1.5 mg/ litre of IAA rooting was noticed.

Cade *et al.* (1990 b) conducted experiment on effect of explant age and growth regulator concentration and they concluded that root production was also influenced by NAA and BAP with many short roots being formed in media having NAA but lacking BAP.

Singh (1990) reported that hypocotyl explants and cotyledon halves of *cucumis melo* cv. Pusa Madhura cultured *in vitro* on basal media with 1μM IBA produced large number of roots.

Ali *et al.* (1991 b) obtained shoot regeneration in cucumber, further rooting was obtained on MS medium containing 1.5 mg NAA/ litre.

Dong and Jia (1991) reported that in watermelon elongation of multiple shoots was achieved on a medium with 0.2 mg/ litre of kinetin, and rooting was accomplished on MS medium with 0.1 mg /litre of NAA.

Compton and Gray (1992) developed a protocol in watermelon, the *in vitro* shoots can be rooted and acclimatized in greenhouse for one week.

Leaf explants of 5 μM size taken from 14 day old cucumber seedlings gave good shoot differentiation which were rooted on MS medium with 1.0μM of IBA. (Raharjo *and* Punja ,1993)

Misra and Bhatnagar (1995) obtained roots on MS medium with 1.0μm of IBA from the leaf explants of 14 days old cucumber seedling.

Highest rooting was achieved in cucumber on MS medium with 1.0 μM IBA and the plants were successfully hardened by planting in a mixture of sand and garden soil in the greenhouse (Singh 1990).

Rapid organogenesis of callus obtained from leaf explants of *Coccinia indica* on MS medium. Further, rooting was completed when transferred to half strength MS medium (Josekutty *et al.,* 1993).

Shoots were regenerated from the cotyledon explants of squash when cultured on MS + 1 mg per litre BAP. These shoots were rooted and transferred to the greenhouse (Ananthakrishnan *et al.*, 2003).

Singh *et al.* (1996) reported that cotyledon halves of melon were cultured on basal medium with cytokinins. BAP proved most effective cytokinin for direct induction of shoot buds from petiolar end of explant. Best rooting was observed on basal medium + 1.0 μM IBA. Finally plantlets were transferred to the field.

Islam *et al.* (1997) found high frequency of plant regeneration from mature zygotic embryos of watermelon and the regenerated shoots were rooted on MS medium supplemented with 1 mg of NAA/litre.

Pious and Mythili (1998), they reported that diploid and tetraploid Arka Manik showed good rooting, triploid appeared shy in rooting. Incorporation of rooting hormones in the medium did not show much beneficial effect while quick dip treatment of micro-cuttings with high concentration of IBA (500-1000 ppm) has given some promising outcome 20-40 percent rooting. The rooted plantlets could be acclimatized.

In *Cucurbita moschata*, the cotyledon explants inoculated on MS medium supplemented with 0.25 – 0.5 mg BA/litre used for shoot elongation. The shoots were rooted on half strength MS medium (Zhao and Zhao, 1999).

Mythili and Thomas (1999) conducted experiment on rapid *in vitro* multiplication by culturing shoot tip and nodal explants of pointed gourd on

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MS medium containing 1.0μm IAA and 0.2μM IBA. The rooted plantlets from one week of culture were acclimatized and established *ex vitro* with 75 - 95 percent.

Hongwen *et al.* (1999) obtained plantlets from shoot tips or auxillary buds of cucumber and these rooted plantlets survived in vermiculite soil (96.7% survival) compared to other soil.

Nabi *et al.* (2002) observed that cotyledon explants of Teasle gourd were rooted in half MS with IBA and concluded that IBA prooved better than IAA.

By culturing shoot tip on MS medium with BAP and NAA for the study of shoot induction. The shoots were rooted most effectively in 1mg /litre IBA supplemented on MS medium (Sarowar *et al.,* 2003).

Mala and Raka (2004) observed that media containing 3 mg per litre IBA, most of the shoot were rooted and 40 percent of plants survived successfully when transferred to field.

2.4 INDIRECT ORGANOGENESIS

Indirect organogenesis means development of organ or plantlet from intermediary callus phase.

Hadler and Gadgil (1982) reported that, long term callus cultures derived from cotyledons formed adventitious buds and embryoids in case of *Momordica charantia* and *Cucumis melo* respectively.

(Lazarte and Sasser, 1982) Seedling was obtained on Nitsch and Nitsch medium without any growth regulator from the callus of cucumber anther explants.

Callus was induced from the hypocotyl explants of cucumber when cultured on MS medium with 1.0 μM BAP and 1.5 or 5.0 μ 2,4-D, and further this callus transferred to medium without any growth regulators. Flowers were also formed, which were either staminate or pistillate (Rajasekaran *et al.,* 1983).

Callus obtained from cotyledon explants of cucumber, when transferred to MS medium containing 0.5 μM NAA and 5 μM BAP resulted in shoot formation, while subsequent rooting occurred on MS medium with 0.1μM NAA (Kim and Jang, 1984).

Moreno *et al.* (1985) reported that more than 90 percent of the calli obtained from melon cotyledon, when cultured on MS medium with 1.5 mg of IAA and 6.0 mg / liter of kinetin, produced well developed shoots.

Hooymans *et al.* (1988) obtained callus from protoplasts of cucumber leaves, which when subcultured regenerated to form plants.

Kim *et al.* (1988) obtained callus from cotyledonary explants of cucumber and these regenerated to form shoots when cultured on to MS medium with 0.5 μM NAA and 5 μM BAP.

Rapid organogenesis of callus obtained from leaf explants of *Coccinia indica* on MS medium supplemented with 1.5 mg BAP + 0.5mg kinetin + 0.1 mg IBA / litre (Josekutty *et al.,* 1993)

Callus obtained from hypocotyl explants of cucumber showed the highest regenerative ability via shoot formation in MS medium with kinetin and IAA (Mukhamedhkanova *et al.,* 1995).

When the cucumber leaf explans were cultured on 2μM 2, 4–D for 10 days the embryonic callus yield increased upto 40 percent (Kuijpers *et al.,* 1996).

2.4 SOMATIC EMBRYOGENESIS

Some cells in callus may differentiate into bipolar embryo like structure called somatic embryos. These somatic cells pass through various stages of embryogeny and develop in to complete plant without the fusion of gametes is called Somatic embryogenesis.

Embryogenic callus was induced from pumpkin hypocotyl explants cultured on MS medium with IBA, 2, 4 – D, kinetin and NAA, either alone or in combination. Later embryos developed into normal plantlets (Jelaska, 1974).

Oridate *et al.* (1986) obtained callus from mature seeds, callus was transferred to liquid MS medium, somatic embryos and plantlets developed within 2 – 3 weeks.

Normal plantlets was obtained from 14 –17 day old cucumber leaves in liquid medium with 5μM 2,4 –5TPA and μM BAP (Chee and Tricoli, 1988).

Callus derived from leaf explants of cucumber on solid medium with 0.5μM kinetin + 0.5μM NAA formed somatic embryos. However, these somatic embryos did not develop into plantlets because of the growth arrest and abnormal development (Bergervoet *et al.,* 1989).

Callus induced from Zygotic embryos of cucumber on MS medium. The embryo germination and rhizogenesis was accomplished in auxin free media with 88μM sucrose (Kim and Janick, 1989).

Cade *et al.* (1990 b) reported that MS medium with either 1 or 2 mg^{-}2, 4–D /litre and 0.5mg kinetin is best for somatic embryogenesis. Plantlets were obtained when the embryos were transferred to MS medium containing 1mg / litre of NAA and 0.5mg / litre of kinetin.

Chee (1990) induced somatic embryogenesis from cotyledon and hypocotyl explants of cucumber on MS medium with 2,4 – D and kinetin.

Lou and Kako (1994) reported that frequency of somatic embryos was high in the callus derived from cotyledon and young first leaf compared to the callus derived from internode explants of cucumber.

Lou *et al.* (1996) observed that cotyledon explants cultured on MS medium with 8μM 2,4 – D and 13μM sucrose resulted in the greatest frequency of somatic embryo genesis.

Guedes and Jennings, (1999) showed the influence of explants source on embryogenic callus formation, somatic embryogenesis and plant regeneration in cucumber.

Ficcadenti *et al.* (1999) obtained 82 gynogenic embryos and haploid plants of *Cucumis melo* var. by culturing ovary slices *in vitro.*

Kurtar *et al.* (1999) reported that haploid plants was obtained through anther culture of squash in solid medium with 120 and 150g / litre of sucrose.

Metwally *et al.* (1998) studied the effects of sucrose and 2,4 – D on the induction of haploid plants of summer squash by anther culture. The most plantlets resulted from MS medium supplemented with 150g sucrose and 5mg 2,4 –D /litre.

Nakagawa *et al.* (2001) studied the effect of sugars and ABA. Sucrose induced somatic embryogenesis, but mannitol did not, 200 milli molar sucrose treatments were optimum for somatic embryogenesis.

Table 1 : Research work carried out in *in vitro* production of shoots of *Cucurbits*

Species	Explant	Media	Growth regulator	Response	Reference
Cucumber	Axillary bud	MS	NAA (0.1 mg/l), KIN	+	Handley and Chambliss, 1979
Cucumber	Seeds	MS	BAP	+	Hisajima, 1982
Castanea Sativa	Seedling explant	MS	BAP 1mg/l + 0.01 1BA	+	Roberto, 1982
Cucurbita pepo	Meristem tips	MS	2.56 mg/l KIN + 8mg/l IAA	+	Pink and Walkey, 1984
Cucurbita melo	Leaf explant	MS	1µm BAP + 2mg 1P	+	Kathal *et al.*, 1988
Cucumber	Seeds	MS	2.5 – 5 µM BAP	+	Hisajima *et al.*, 1989
Cucurbita melo	Cotyledon	MS	5µm 1AA and BAP	+	Neidz *et al.*, 1989
Water melon	Immature cotyledon	MS	10 µm BA	+	Jeffrey *et al.*, 1990
Melon	Seeds	MS	NAA and BAP	+	Suh and Suh, 1998
Cucurbita moschata	Cotyledon	MS	4 mg/l BA and 1AA	+	Zhao and Zhao, 1999
Pointed gourd	Nodal explant, shoot tip	MS	1AA 1mg/l + 0.2µmIBA	+	Mythili and Thomas, 1999
Coccinia indica	Hypocotyl	MS	3 mg/l KIN	+	Venkateshwaralu *et al.*, 2001
Melon	Hypocotyl	MS	100% shoots 4.45 BAP	+	Curuk *et al.*, 2003

Table 2 : Research work carried out in *in vitro* rooting of cucurbitaceous vegetables

Species	Explant	Media	Growth regulator	Response	Reference
Water melon	Shoot tip	MS	11.5 µM 1AA	+	Barnes, 1979
Cucumber	Axillary bud	MS	0.1 mg/l NAA + KIN	+	Handley and Chambliss, 1979
Cucumber	Seeds	MS	BAP	+	Hisajima, 1982
Watermelon	Cotyledon	MS	0.1 mg/l NAA	+	Dong and Jia, 1991
Cucumber	Cotyledon	MS	1.5 mg/l NAA	+	Ali *et al.*, 1991b
Melon	Cotyledon	MS	1µm IBA	+	Singh *et al.*, 1996
Watermelon	Mature zygotic embryos	MS	1 mg/l NAA	+	Islam *et al.*, 1997
Watermelon		MS	500-1000ppm IBA	+	Pious and Mythili, 1998
Pointed gourd	Shoot tip	MS	10 µm 1AA + 0.2µm IBA	+	Mythili and Thomas, 1999
Cucurbita moschata	Cotyledon	MS	½ MS	+	Zhao and Zhao *et al.*, 1999
Coccinia indica	Leaf explant	MS	½ MS	+	Josekutty *et al.*, 1993
Cucurbita	Shoot tip	MS	1mg/l	+	Sarowar *et al.*, 2003
Cucurbita pepo	Cotyledon	MS	-	+	Ananthakrishnan *et al.*, 2003

Material and Methods

III. MATERIAL AND METHODS

The "*In vitro* propagation studies in *Momordica dioica*" was carried out at the tissue culture laboratory of the Department of Horticulture, college of Agriculture, Dharwad during 2003 – 04. The details of experimental materials and the methodology followed are presented in this chapter.

3.1 PLANT MATERIAL

Spine gourd *Momordica dioica* Roxb. was taken for the study. The plant material required for the study was collected from the Olericulture Unit, Department of Horticulture, Dharwad.

3.1.1 Source of Explants

3.1.1.1 Shoot tips

The shoot tips of 0.5 to 1.0 cm were excised from the main field during September – October month.

3.1.1.2 Auxillary buds

Auxillary buds of two to three cm. Were taken from the vine.

3.1.1.3 Tubers

Underground tubers were collected from the matured plant.

3.1.1.4 Leaf

Fresh and healthy leaf segments of 0.5 – 1 cm were excised from the main plant (Plate 1).

1. Shoot tip   2. Auxillary bud   3. Leaf   4. Tubers

Plate 1: Source of different explants used for experimentation

3.1.1.5 Seed

Viable and healthy seeds of spine gourd are used for experiment.

3.1.2 Preparation of explants

The various plant parts like shoot tips, auxillary bud, Tubers were directly collected from field. Shoot tips were cut into 0.5 – 1 cm length, Auxillary buds were cut into 2 to 3 cm. The explants were thoroughly washed and then treated with a detergent, Teepol for 10 minutes as a surfactant. After thorough washing the explants were surface sterilized with 0.1 percent (W / V) aqueous mercuric chloride for 10 minutes followed by treating the explants with 70% alcohol for 1 sec. Then thoroughly wash the explants with sterile water for 3 – 4 times. The explants were individually cultured on solidified MS medium containing 3 percent sucrose and 0.8 percent agar. The cultures were incubated in dark at 25 $\pm$ 2°C within 15 days of culture, new shoots arises from the explants which can be used for further multiplication.

The seeds were washed 3-4 times in tap water with detergent followed by soaking the seeds in fungicide after thorough washing with 3-4 times in distilled water, then surface sterilize by using 70% alcohol for 1 sec. Then with 0.1% mercuric chloride for 15 minutes followed by thorough washing with sterilized water. The seeds were individually cultured on solidified half MS medium containing 3 per cent sucrose and 0.8 percent agar.

3.2 MEDIA

The nutrient media used for the study was Murashige and Skoog (MS) medium.

3.2.1 Preparation of Stocks

Murashige and Skoog (MS) medium was commonly used for all the experiments. Five stock solutions (8x) were prepared with double distilled water, poured into well stoppered bottle and were stored in refrigerator.

3.2.1.1 Mineral and Vitamin stock

Stock A: Macro elements - 1000 ml (10x)

Component		Amount
$NH_4 NO_3$	-	16.5 g
KNO_3	-	19.0 g
$Mg SO_4 7H_2O$	-	3.7 g
KH_2PO_4	-	1.7 g

Stock B: Micro elements - 1000 ml (10x)

Component		Amount
$COCl_2 6H_2O$	-	0.25 g
$CuSo_4 5H_2O$	-	0.25 g
$MnSo_4 4H_2O$	-	223 mg
KI	-	8.3 mg
$Na_2Mo_4 2H_2O$	-	2.5 gm
$Zn SO_4 7H_2O$	-	86 mg
H_3BO_3	-	62 mg

Stock C: Calcium chloride stock 1000 ml (10x)

Component		Amount
$CaCl_2 2H_2O$	-	4.4 g

Stock D: Iron stock - 1000 ml (10x)

Component		Amount
$FeSO_4 7H_2O$	-	273 mg
Na_2EDTA	-	373 mg

Details of stock solutions prepared for this work are given below.

Murashige and Skoog stock	–	**1000ml (8x)**
NH_4NO_3	-	13.2g
KNO_3	–	15.2 g
$MgSO_4 \cdot 7H_2O$	–	2.96 g
$ZnSO_4.7H_2O$	–	86.4 mg
$MnSO_4.4H_2O$	–	178.4 mg
H_3BO_3	–	49.6 mg
$Na_2MO_4.2H_2O$	–	2.0 mg
$CuSO_4$ $5H_2O$	–	0.2 mg
$COCl_2$. 6 H_2O	–	0.2 mg
KI	–	6.6mg
$CaCl_2.2H_2O$	–	3.53 g
KH_2PO_4	–	1.36 g
Na_2 EDTA	-	372 mg
$FeSO_4$. $7H_2O$	–	279 mg

Preparation of Iron Stock

Na_2EDTA (372 mg) in 50 ml of distilled water was boiled to which 279 mg of $FeSO_4.7H_2O$ was added gently. From this 40ml was taken and finally made volume up to 1000 ml.

Vitamin stock	–	**100 ml (50x)**
Thiamine HCl	–	0.5 mg
Pyridoxine HCl	–	2.5 mg
Nicotinic acid	–	2.5 mg
Glycine	–	10 mg
Mesoinositol	–	500 mg

3.2.1.2 Preparation of growth regulator stock

Stock solutions of 6 benzyl amino purine (BAP), (KIN) kinetin, naphthalene acetic acid (NAA), indole butyric acid (IBA), gibberellic acid (GA) and indole acetic acid (IAA) 100 ppm each was made upto 100 ml with distilled water to make 100 ppm stock.

3.2.2 Quality of chemicals

All chemicals used in the experiments were of analytical grades.

3.2.3 Preparation and Sterilization of media

The stock solutions were mixed in the required proportion along with growth regulators and sucrose 3%. Then the volume was made up by adding double distilled water. The P^H of medium was adjusted between 5.6 to 5.8 by using either 0.1 N HCl or NaOH with the help of a digital P^H meter. The volume was finally adjusted and agar (0.8%) was added into medium. Agar in the medium was melted by gentle heating and the medium of 15.0ml was poured into sterilized glass test tubes and plugged with sterile non-absorbent cotton. The media was autoclaved at 121°C at 15 lb /square inch pressure for 20 minutes.

3.3 CULTURE ESTABLISHMENT

3.3.1 Inoculation

Before inoculation of explants, the ultra violet light of laminar air flow was switched on for 20 minutes for area sterilization. Inoculation was carried out under aseptic condition under laminar air flow chamber.

3.3.2 Culture Condition

The culture tubes were incubated in culture room having control over temperature and light. The temperature of culture room was maintained at $25 \pm 2°C$ with light intensity of 16 hours light and 8 hours dark.

3.3.3 Subculture

Micro shoots were taken out from the test tubes after 5–6 weeks of inoculation. The shoots were separated by dissecting them in the sterile environment of laminar air flow cabinet with sterile needle and forceps. Then they were placed in the test tubes containing fresh media.

3.3.4 Rooting

The micro shoots which were taken out and were inoculated in the tubes containing Auxin media with different concentrations of IBA and NAA for rooting.

3.3.5 Hardening

Young rooted plantlets were taken out of the test tubes, washed with distilled water and planted in pots containing common potting mixture, soilrite, perlite, vermiculite and peat. These plants were maintained in polyhouse. The plants were watered, then plantlets were transferred to mist chamber, after one month again transferred to green house.

3.4 EXPERIMENTAL DETAILS

3.4.1 Experiment I : Studies on *in vitro* regeneration of shoot by different explants

For micro-propagation, different explants were used to study the response of different parts of shoot regeneration and multiplication. The explants used are as follows.

3.4.1.1 Treatment details

Shoot tips

Auxiliary buds

Tubers

Leaf

Seed

Design		CRD
Number of treatments	:	5
Number of replications	:	5
Number of tubes/treatment	:	10

3.4.1.2 Observations recorded

The inoculated tubes were observed for their response and it was noted as positive response – " + " and no response = "-".

a. Percent survival: The number of plants survived out of total inoculated was converted into percentage.

b. Days taken for initiation of growth : The number of days taken to show initial sprout from date of inoculation of various explants was recorded mean number of days.

c. Number of multiple shoots / explant : The number of shoots produced from single explant after sub culturing was noted.

3.4.2 Experiment II : Effect of media composition on shoot multiplication

3.4.2.1 Treatment details

I. Effect of cytokinin on shoot proliferation

T_1 – MS + BAP 0.5mg /litre

T_2 – MS +BAP 1.0mg / litre

T_3 – MS +BAP 1.5mg /litre

T_4 – MS +BAP 3.0mg /litre

T_5 – MS + Kinetin 0.5mg/ litre

T_6 – MS + Kinetin 1.0mg / litre

T_7 – MS +Kinetin 1.5mg /litre

T_8 – MS + Kinetin 3.0mg / litre

Design : CRD

No. of treatments : 8

No of replications 3

No. of tubes/treatment 10

MS – Murashige and Skoog medium

II. Effect of cytokinin and NAA on shoot proliferation

T_1 – MS + BAP 0.5mg + NAA 0.1mg/litre

T_2 – MS +BAP 1.0mg + NAA 0.1mg/litre

T_3 – MS +BAP 1.5mg + NAA 0.1mg/litre

T_4 – MS +BAP 3.0mg + NAA 0.1mg/litre

T_5 – MS + Kinetin 0.5mg + NAA 0.1mg/litre

T_6 – MS + Kinetin 1.0mg + NAA 0.1mg/litre

T_7 – MS +Kinetin 1.5mg + NAA 0.1mg/litre

T_8 – MS + Kinetin 3.0mg + NAA 0.1mg/litre

MS – Murashige and Skoog

3.4.2.2 Observations recorded

a. **Days taken for initial sprout :** As mentioned in experiment I

b. **Number of shoots / explants :** As mentioned in experiment I

c. **Number of leaves /shoot :**The number of leaves produced from single shoot was counted.

d. **Shoot length:** Shoot length was measured from base to tip of the apex and average was expressed in centimeters.

e. **Shoot dry weight :** The shoots were dried in hot air oven at 60°C for 48 hours and the dry weight of each shoot were recorded in milligrams.

3.4.3 Experiment III : Studies of auxin levels on rooting of shoot.

3.4.3.1 Treatment details

T_1 – MS + IBA 0.5mg /litre

T_2 – MS + IBA 1.0mg / litre

T_3 – MS + IBA 1.5mg /litre

T_4 – MS + IBA 3.0mg /litre

T_5 – MS + NAA 0.5mg/ litre

T_6 – MS + NAA 1.0mg / litre

T_7 – MS + NAA 1.5mg /litre

T_8 – MS + NAA 3.0mg / litre

Design : CRD

Number of treatments : 8

Number of replications : 3

Number of tubes per treatment : 10

3.4.3.2 Observations recorded

a. **Number of days taken for rooting**: The number of days taken for rooting from date of inoculation was noted and average was worked out.

b. **Number of roots:** The plantlets were taken out from the culture medium and mean number of roots produced per micro-shoot was recorded.

c. **Root length:** The root length was measured from base to the tip of the root and expressed in centimeters.

3.4.4 Experiment IV: Hardening of *in vitro* rooted plantlets.

3.4.4.1 Treatment details

T_1 – Common potting mixture (Soil: Sand: FYM)

T_2 – Soilrile (pertile : peat)

T_3 – Perlite + vermiculite (1:1)

T_4 – Peat

Design : CRD

Treatment : 4

No. of replication : 5

No.of tubes/treatment : 10

3.4.4.2 Observations recorded

a. **Percentage survival of plantlets:** The number of plants survived out of total plants transferred to greenhouse was converted into percentage.

b. **Plant height:** The plant height was measured from base to the tip of the plant and expressed in centimeters

c. **Number of shoots:** The shoots produced per plant was recorded

d. **Number of leaves:** The number of leaves produced from shoot was counted

3.5 STATISTICAL ANALYSIS

Completely randomized design was employed. The data in percentages were transformed to arc sin values for statistical analysis. The data was subjected for analysis as suggested by Panse and Sukhatme (1967). Critical difference values were tabulated at one per cent probability where 'f' test was significant.

Experimental Results

IV. EXPERIMENTAL RESULTS

The experiment on "*In vitro* propagation studies in *Momordica dioica*" was conducted to standardize the explant type and culture media with the growth regulator concentrations for rapid multiplication of shoots and roots and evaluation of plantlets under different rooting media, was carried out at the tissue culture laboratory of the Department of Horticulture, College of Agriculture, Dharwad during 2002-04. The results of the investigation are presented in this chapter.

4.1 RESPONSE OF DIFFERENT TYPES OF EXPLANTS OF *MOMORDICA* IN *IN VITRO* CONDITION (Cf. Table 3)

In an attempt to standardize explants for tissue culture of *Momordica,* different types of explants were tried for *in vitro* shoot regeneration and multiplication. The response observed from each explant is presented in table 3. Different types of explants were cultured in media and it was observed that explants exhibited differential response to the *in vitro* conditions. The explants used for the present investigation were shoot tip, auxillary bud, tubers, leaf and seed.

All kinds of explants produced whitish brown friable callus on MS medium followed by the formation of shoot proliferation, shoot multiplication, root formation and plantlet formation except in tubers and leaf explants.

Table 3: Response of different types of explants of *Momordica* in *in vitro* condition

Explant		Callus	Shoot proliferati on	Shoot multiplica tion	Plantlet formation	Root formation
T_1	Shoot tip	+	+	+	+	+
T_2	Auxiliary bud	+	+	+	+	+
T_3	Tubers	-	-	-	-	-
T_4	Leaf	+	-	-	-	-
T_5	Seed	+	+	+	+	+

\+ Positive response
\- No response

4.2 STUDIES ON *IN VITRO* REGENERATION OF SHOOT BY DIFFERENT EXPLANTS

4.2.1 Days taken for initiation of growth (Cf. Table 4)

Significant differences were noticed with different explants for initiation of growth. Auxillary buds took minimum number of days (4.00) for initiation of growth followed by shoot tip (5.00) (Plate 2) Where as, seed took maximum number of days (14.6) for initiation of growth. While no initiation of growth was observed in the explants of tuber and leaf.

4.2.2 Number of multiple shoots per explant

Significant differences were noticed among the different explants for number of shoots per explant. Auxillary buds produced maximum number of shoots (4.66), followed by seed (4.52). lowest number of multiple shoots were noticed from shoot tip explant. Where as tuber and leaf explants failed to produce the shoots.

4.2.3 Survival percentage

Significantly highest percentage of survival was noticed from auxillary bud explants (5.74) followed by seeds (4.8) and shoot tip (3.14). Where as in tuber and leaf explants survival percentage was zero.

4.3 EFFECT OF MEDIA COMPOSITION ON SHOOT MULTIPLICATION

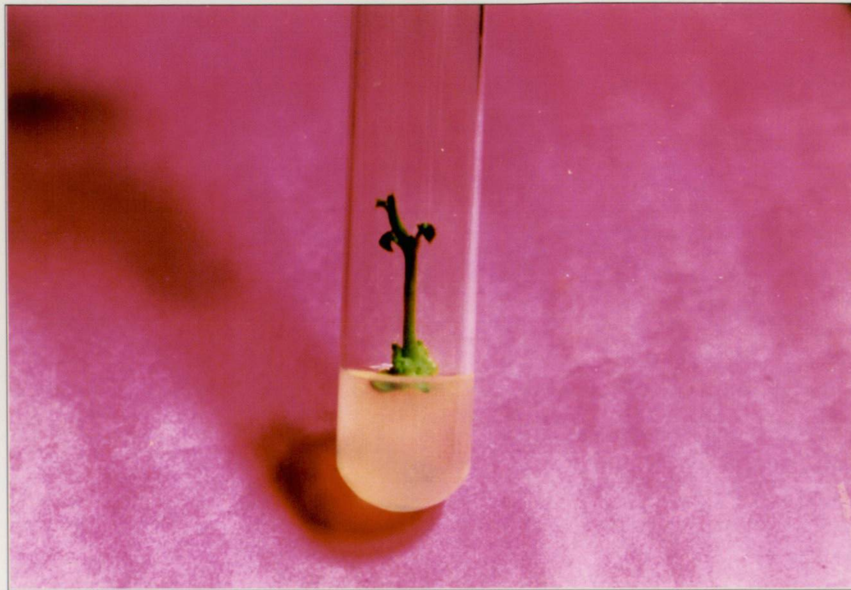
4.3.1 Effect of cytokinin on shoot proliferation.

In all the experiments the auxillary bud used as a explant.

Table 4 : Studies on *in vitro* regeneration of shoot by different explants

Treatment	Days taken for initiation of growth	No.of multiple shoot / explant	% survival
Shoot tip	5.0	0.80	0.30 (3.14)*
Auxillary bud	4.0	4.66	1.00 (5.74)
Tubers	0.0	0.00	0.00 (0.00)
Leaf	0.0	0.00	0.00 (0.00)
Seed	14.6	4.52	0.70 (4.8)
SEm±	0.109	0.042	0.08
CD (1%)	0.395	0.152	0.290

*Figures in Parenthesis indicate arc sin values



Germinating seed

Callus growth

Plate 2: Germination of seeds in MS medium

4.3.1.1 Number of days taken for initial sprout (Cf. Table 5)

There was non significant difference among the treatments with respect to days taken for initial sprout. However, MS medium containing BAP (1.0), BAP (1.5) and Kinetin (1.5) required only four days for initiation of sprout compared to other treatments.

4.3.1.2 Number of shoots per explant

Statistically significant differences were observed among the treatments for number of shoots produced by auxillary bud explants cultured on MS medium with different cytokinin concentrations.

Maximum number of shoots were recorded in MS medium containing BAP 1.0 mg per litre (14.7) followed by 3.0mg per litre BAP (12.2) and 3.0mg per litre of kinetin (11.6). Where as MS medium containing Kinetin 0.5mg per litre recorded lowest number of shoots (3.2) per auxillary bud.

4.3.1.3 Number of leaves per shoot

Significant differences were observed for the number of leaves per shoots among the treatments. Highest number of leaves (5.2) were recorded in MS medium containing 3 mg per litre of Kinetin and it was on par with 0.5 mg per litre of kinetin (5.0), 1.0 mg per litre of kinetin (4.9) and 1.5 mg per litre of Kinetin (5.0). While the least number of leaves (2.4) was recorded in 3.0 mg per litre BAP.

4.3.1.4 Shoot length (cm)

Significant differences were noticed for shoot length with increasing trend observed among the treatments. MS medium containing Kinetin 0.5mg

Table 5: Effect of cytokinin on shoot proliferation

Treatment		Days taken for shoot initiation	Shoot / explant	Shoot length (Cm)	No. of leaves	Shoot dry wt. (gm)	Callus
T_1	BAP (0.5mg/litre)	5.0	9.2	2.2	3.1	63.66	+
T_2	BAP (1.0 mg/litre)	4.0	14.7	2.1	3.1	80.00	+
T_3	BAP (1.5 mg/litre)	4.0	10.7	1.4	3.0	71.33	+
T_4	BAP (3.0 mg/litre)	5.0	12.2	0.9	2.4	98.33	++
T_5	Kinetin (0.5 mg/litre)	5.0	3.2	7.9	5.0	75.33	+
T_6	Kinetin (1.0 mg/litre)	5.0	5.3	6.3	4.9	125.33	+
T_7	Kinetin (1.5 mg/litre)	4.0	6.6	5.6	5.0	85.33	+
T_8	Kinetin (3.0 mg/litre)	5.0	11.6	3.2	5.2	67.66	+
	SEm±	-	0.06	0.08	0.13	2.28	
	CD (1%)	-	0.218	0.29	0.472	8.28	

per litre recorded the maximum shoot length (7.9cm), followed by 1.0mg per litre Kinetin (6.3), and minimum shoot length (0.9) was noticed in 3.0mg per litre of BAP.

4.3.1.5 Shoot dry weight (g)

There was significant difference obtained among the treatments. The highest shoot dry weight of (125 gm) was obtained when cultured on MS medium contaning Kinetin 1.0mg per litre followed by BAP 3.0mg per litre (98). The lowest dry weight was recorded in medium containing BAP at 0.5mg per litre (63.66).

4.3.2 Effect of cytokinin and NAA on shoot proliferation

4.3.2.1 Number of days taken for shoot initiation (Cf. Table 6)

Non significant difference was noticed among the treatments with respect to number of days taken for shoot initiation. However except on MS medium contaning BAP 1.5 + NAA 0.1 mg per litre and Kinetin 0.5 + NAA 0.1 mg per litre rest showed early initiation of shoot.

4.3.2.2 Shoot dry weight (grams)

Significant difference was obtained among all the treatments. The highest shoot dry weight of 183.0 was obtained in MS medium with Kinetin 1.0 mg per litre + NAA 0.1 (123.3). The lowest dry weight was recorded in BAP 1.5 + NAA 0.1 mg per litre (61.0).

Table 6 : Effect of different cytokinin levels and NAA on shoot proliferation

Treatment		Days taken shoot initiation	Shoot dry weight (gm)
T_1	BAP *(0.5) + NAA (0.1)	4	75.33
T_2	BAP (1.0) + NAA (0.1)	4	98.33
T_3	BAP (1.5) + NAA (0.1)	5	61.00
T_4	BAP (3.0) + NAA (0.1)	4	123.3
T_5	Kinetin (0.5) + NAA (0.1)	5	81.0
T_6	Kinetin (1.0) + NAA (0.1)	4	183.0
T_7	Kinetin (1.5) + NAA (0.1)	4	74.66
T_8	Kinetin (3.0) + NAA (0.1)	4	64.0
	SEm±	–	0.89
	CD (1%)	–	3.23

* mg/litre

4.3.2.3 Number of shoots per explant (Cf. Table 7)

Significant differences were noticed for number of shoots per explant. The treatment combination of MS medium with BAP (3.0) + NAA (0.1) recorded highest number of shoots per explant at 10 days after inoculation (4.7), 25 DAI (6.1) and 40 DAI (8.6) followed by BAP (1.0) + NAA (0.1), where as least was noticed by BAP (1.5) + NAA (1.0) at 10 DAI (0.2), Kinetin (1.5) + NAA (1.0) at 25 DAI (0.9) and 40 DAI (1.7).

4.3.2.4 Extent of callus

In all the treatments the callus was noticed. But the green type of friable callus was observed in MS medium containing 3 mg per liter of BAP

4.3.2.5 Effect of cytokinin on number of leaves. (Cf. Table 8)

The data on the effect of cytokinin on the number of leaves produced differed significantly when inoculated after 10, 25 and 40 days after inoculation(DAI). Among the different treatments the MS medium supplemented with Kinetin 0.5mg + NAA 1.0mg per litre recorded significantly highest number of leaves at 10,25 and 40 DAI (6.2, 6.5 and 8.5) followed by BAP at 1.0mg per litre at 10 DAI (5.6), where as, the cases when inoculation was done after 25 and 40 DAI these were on par with each other (6.4 and 8.4) respectively. The least number of leaves 0.6 and 3.4 at 10 and 40 DAI respectively, was recorded by BAP 1.5mg per litre + NAA 0.1 mg per litre. At 25 DAI the lowest number of leaves was recorded in 3.0mg per litre BAP + 0.1mg per litre NAA (2.8).

4.3.2.6 Shoot length (Cf. Table 9)

There was a significant differences among the different treatments with respect to average shoot length when supplemented with cytokinins at 10, 25 and 40 DAI.

Table 7 : Effect of different cytokinin levels and NAA on number of shoots

Treatment		10 DAI*	25 DAI	40 DAI
T_1	BAP *(0.5) + NAA (0.1)	2.3	3.3	5.8
T_2	BAP (1.0) + NAA (0.1)	4.5	5.6	6.7
T_3	BAP (1.5) + NAA (0.1)	0.2	1.3	2.2
T_4	BAP (3.0) + NAA (0.1)	4.7	6.1	8.6
T_5	Kinetin (0.5) + NAA (0.1)	1.2	1.6	1.7
T_6	Kinetin (1.0) + NAA (0.1)	3.1	3.5	5.0
T_7	Kinetin (1.5) + NAA (0.1)	0.8	0.9	1.7
T_8	Kinetin (3.0) + NAA (0.1)	2.0	4.0	5.4
	SEm±	0.09	0.115	0.079
	CD (1%)	0.32	0.41	0.28

* mg/litre
DAI - days after inoculation

Table 8: Effect of different cytokinin levels and NAA on number of leaves

Treatment		10* DAI	25 DAI	40 DAI
T_1	BAP *(0.5) + NAA (0.1)	3.6	4.1	7.2
T_2	BAP (1.0) + NAA (0.1)	5.6	4.9	4.4
T_3	BAP (1.5) + NAA (0.1)	0.6	5.7	3.4
T_4	BAP (3.0) + NAA (0.1)	2.7	2.8	4.9
T_5	Kinetin (0.5) + NAA (0.1)	3.4	5.5	7.2
T_6	Kinetin (1.0) + NAA (0.1)	6.2	6.5	8.5
T_7	Kinetin (1.5) + NAA (0.1)	1.8	3.1	3.5
T_8	Kinetin (3.0) + NAA (0.1)	3.9	6.4	8.4
	SEm±	0.091	0.125	0.135
	CD (1%)	0.33	0.454	0.49

* mg/litre
DAI - days after inoculation

Table 9 : Effect of different cytokinin levels and NAA on shoot length(cm)

Treatment		10* DAI	25 DAI	40 DAI
T_1	BAP* (0.5) + NAA (0.1)	1.8	4.4	9.8
T_2	BAP (1.0) + NAA (0.1)	2.0	1.8	2.3
T_3	BAP (1.5) + NAA (0.1)	0.3	1.7	3.7
T_4	BAP (3.0) + NAA (0.1)	1.5	1.7	3.0
T_5	Kinetin (0.5) + NAA (0.1)	1.9	4.3	8.5
T_6	Kinetin (1.0) + NAA (0.1)	2.3	2.4	4.9
T_7	Kinetin (1.5) + NAA (0.1)	0.7	0.9	6.6
T_8	Kinetin (3.0) + NAA (0.1)	1.8	4.2	9.9
	SEm±	0.09	0.112	0.077
	CD (1%)	0.32	0.40	0.279

* mg/litre
DAI - days after inoculation

The treatment supplemented with MS+ Kinetin (1.0) + NAA (1.0) mg per litre was recorded the highest average shoot length (2.3) and it was on par with BAP (1.0) + NAA (0.1) mg per litre (0.3). At 25 DAI the maximum shoot length was (4.4) obtained in MS medium with BAP (0.5) + NAA (0.1) mg per litre and it was on par with kinetin (3.0) + NAA (0.1) mg per litre (4.2). The lowest shoot length (0.9) was obtained in MS medium containing Kinetin (1.5) + NAA (0.I) mg per litre At 40 DAI MS+ Kinetin 3mg + NAA 0.1mg per litre was recorded the highest shoot length (9.9) and it was on par with BAP 0.5 + NAA 0.1 mg per litre (9.8). The lowest (2.3) shoot length was observed in BAP 1.0 + NAA 0.1 mg per litre.

4.4 STUDIES OF AUXIN LEVELS ON ROOTING OF SHOOT. (Cf. Table 10)

4.4.1 Number of days taken for rooting

Significant differences were obtained among different auxin treatments. The treatment MS + IBA at 1.0 mg per litre recorded early root initiation (12 days) followed by MS+ IBA at 0.5 mg per litre and IBA at 3.0 mg per litre. Where as, delayed (15 days) rooting was noticed in NAA 3mg per litre.

4.4.2 Effect of auxin on number of roots per shoot

Significant differences were noticed for number of roots per shoot. IBA at 1.0 mg per litre recorded the maximum number of roots (27.16) followed by IBA at 3mg per litre (12.5). The lowest number of roots (2.6) was recorded in NAA at 2.0 mg per litre.

Table 10 : Effect of auxin on rooting of *in vitro* shoots

Treatment	No. of days for rooting	No. of roots	Root length (Cm)	Percentage of rooting	Shoot length (Cm)	Leaves / shoot	Root dry wt. (gm)
MS + IBA* (0.5)	15.0	4.16	0.433	100	1.60	3.03	51.0
MS + IBA (1.0)	12.0	27.16	1.133	100	3.90	4.70	71.0
MS + IBA (2.0)	14.6	11.50	0.733	100	4.50	4.77	53.66
MS + IBA (3.0)	15.0	12.50	1.167	100	4.50	3.50	70.33
MS + NAA (0.5)	15.0	7.30	0.467	100	5.50	4.50	36.33
MS + NAA (1.0)	14.0	6.50	0.633	100	4.03	3.03	26.66
MS + NAA (2.0)	14.6	2.60	0.600	100	1.17	3.70	46.66
MS + NAA (3.0)	15.0	3.00	1.467	100	3.50	3.03	57.33
SEm±	0.166	0.101	0.036		0.08	0.08	1.040
CD (1%)	0.603	0.366	0.130		0.29	0.29	3.778

*mg/l

4.4.3 Effect of auxin levels on root length (cm)

The root length was differed significantly among the treatments. The treatment MS+NAA at 3mg per litre recorded maximum root length (1.46), followed by IBA at 3.0 mg per litre (1.16) and IBA at 1.0 mg per litre (1.13). The lowest root length (0.4) was observed in NAA at 0.5mg per litre.

4.4.4 Percentage of rooting

100 per cent of rooting was obtained in all the treatments with different auxins such as NAA and IBA

4.4.5 Effect of auxin on shoot length (Cm)

The auxin treatment differed significantly for length of shoot. The maximum shoot length of (5.5cm) was obtained on MS medium with NAA 0.5 mg per litre. Where as minimum shoot length (1.16) was noticed in NAA 2.0 mg per litre.

4.4.6 Effect of auxin on number of leaves per shoot

Significant differences were noticed for number of leaves. The MS medium containing IBA 2.0mg per litre (4.76) recorded the highest number of leaves followed by IBA 1.0mg per litre (4.7) and NAA 0.5mg per litre (4.5) which were on par with each other.

The lowest number of leaves (3.03) was observed in MS+ NAA 3.0 mg per litre, NAA 1.0mg per litre and IBA 0.5mg per litre (3.0) which were on par with each other.

4.4.7 Effect of auxin on root dry weight

Significantly higher dry weight (70.33) of roots was recorded in MS medium containing IBA 3.0 mg per litre and the lowest dry weight (26.66) noticed in NAA at 1.0 mg per litre.

4.5 HARDENING OF *IN VITRO* ROOTED PLANTS

The *in vitro* rooted plantlets were subjected to different potting media like common potting mixture (Sand : Soil : FYM) , Soilrite, perlite + Vermiculite (1:1) and peat to study the effect of potting media on the hardening of plantlets, the survival percent, average plant height (cm), number of leaves and number of shoots are presented in table 11.

4.5.1 Effect of potting media on percent survival(Cf. Table 11)

The per cent survival of plantlet in different treatments differed significantly after planting in poly house. The peat media recorded the highest per cent of survival at 30 Days after planting (5.7%) followed by the soilrite media (5.1%). Where as in common potting mixture and perlite + vermiculite (1:1) media none of the plants survived.

4.5.2 Effect of potting media on plant height

Significant differences were observed among the treatments. The peat media recorded the highest plant height of (28.2 cm) followed by the soilrite media (15.6).

4.5.3 Effect of potting media on number of shoots.

The data revealed significant difference among the treatment with respect to number of shoots. Highest number of shoots were produced in

Table 11 : Hardening of *in vitro* rooted plantlets

Treatment	% survival	Plant height (cm)	No. of shoots	No. of leaves
Common potting mixture	0.00	0.00	0.00	0.00
Soilrite	5.13	15.60	2.80	14.2
Perlite + Vermiculite	0.00	0.00	0.00	0.00
Peat	5.70	28.20	4.40	24.2
SEm±	0.014	0.353	0.204	0.273
CD (1%)	0.050	1.282	0.74	0.992

peat media, followed by soilrite media. No shoots were observed in common potting mixture and perlite + vermiculite media (1.1).

4.5.4 Effect of potting media on number of leaves

Significant differences were noticed among the treatments. The peat media is the best potting medium, with the production of maximum number of leaves followed by soilrite media (14.2). In case of common potting mixture and perlite + vermiculite (1.1) media no leaves were observed.

4.6 EFFECT OF HARDENING TREATMENTS ON SURVIVAL PERCENTAGE OF *Momordica dioica* PLANTLETS UNDER DIFFERENT CONDITIONS

4.6.1 Survival percentage(Cf. Table 12)

The percent survival of plantlet differed significantly after transferred to field. The highest survival percentage noticed in net house (60.00) compared to field.

Table 12 : Effect of hardening treatments on survival percentage of *Momordica dioica* plantlets under different conditions

Hardening treatments	Survival percentage
Plantlets transferred to net house	60.00
Plantletes transferred to field	40.50

Discussion

V. DISCUSSION

Traditionally, cucurbits are propagated through seeds. Spine gourd (*Momordica dioica* Roxb.) is an unexploited protein rich vegetable crop. The fruits having several medicinal values. It has got good healing power and wormicidal effect in body. Many farmers are experiencing difficulties in getting good planting material. Because the germination of the seeds is very difficult or impossible due to hard seed coat dormancy (Rashid, 1976). Seeds have very low multiplication rates of plantlets and because of dioecious nature. Till today no attempt has been made for propagation of this crop through tissue culture. Therefore the current investigation has been taken up to standardize the type of explants, and nutrient media to increase the rate of multiplication, growth regulator combination for shoot proliferation and to standardize the conditions for hardening of regenerated plantlets. The results obtained in the experiment are discussed in this chapter.

The cultivation of spine gourd is a lucrative business, because of very high cost in the market. During monsoon naturally growing Vines of Spine gourd are seen in the forest areas. It is less popularised due to propagation method. The vegetable consumer get the fruits only once in a year. Spine gourd is a cucurbitaceous vegetable crop and easily subjected to several tissue cultural aspect. Most of herbaceous plants can be vegetatively propagated with relative ease by either conventional propagation with micro propagation methods (George, 1993;George and Sherrington, 1984; Murashige, 1974).

5.1 STANDARDIZATION OF THE EXPLANT FOR RAPID MULTIPLICATION

In general various explants *viz.,* meristems, shoot tips, auxillary buds, hypocotyl, epicotyl, seeds, single nodal cutting, leaf disc, tuber disc, roots etc., can be used for micro-propagation. The success of the *in vitro* culture could largely depend on the selection of explant. Murashige (1974) recognized several factor, that should be considered in an explant selection including the organ that is to serve as tissue source, the physiological and ontogenetic age of the organ and the size of the explants. In the present investigation, five different explants *viz.,* shoot tip, auxillary bud, tuber, leaf and seed were used for standardization of rapid multiplication of plantlets (Fig. 1). It was evident from results that among all the explants, auxillary buds gave more number (4.66) of shoots per explants (Plate 3). The highest survival percentage (5.74) noticed in auxillary bud followed by seeds(4.8). However, survival percentage from other explants was very poor. Where as, leaf explants and tuber explants did not survive because of bacterial and fungal contamination. Among all these auxillary bud was found to be better for regeneration of shoot. Although seed was next best for the propagation, it should be discouraged because of dioecious nature of plant. Handley and Chambliss (1979) also reported that auxillary buds were best for *in vitro* culture and for increasing the multiplication rate. Because *in vitro* propagation of cucumber plants using auxillary buds offers a greater chance of tissues obtaining desirable selection from the field than the traditional method. Bud culture has a 5 fold advantage.

Nutritional requirement for optimal growth of a plant *in vitro* may vary with the species, even tissues from different plant parts. Media with high cytokinin to auxin ratio leads to caulogenesis (induction of shoot development

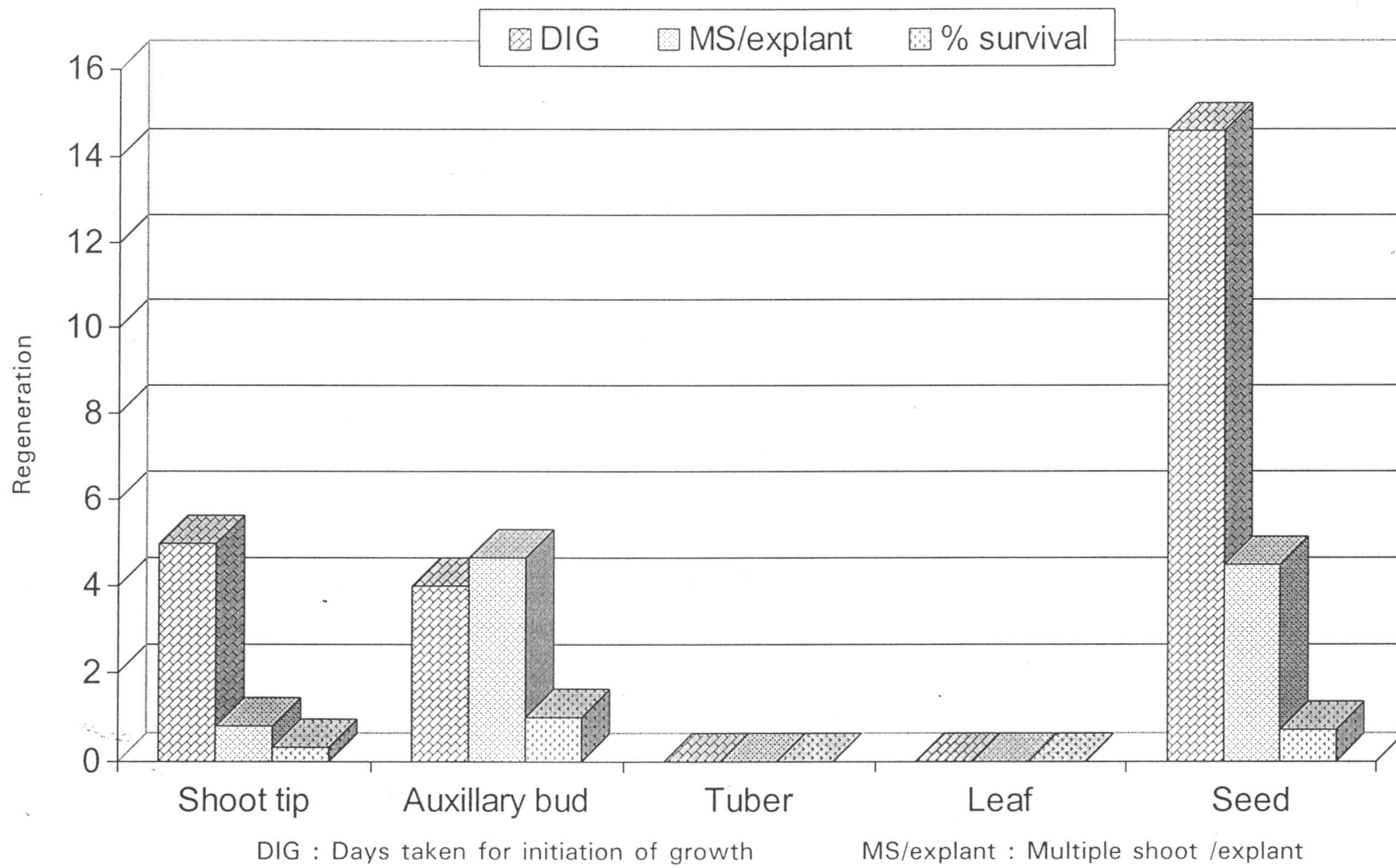
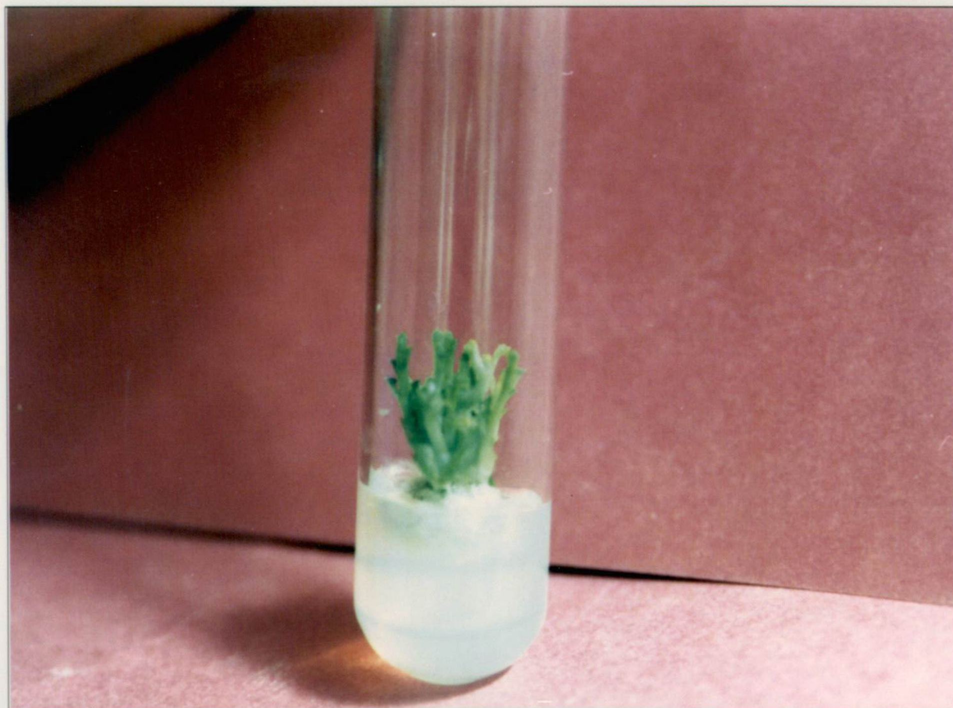


Fig. 1: Regeneration of shoots in different explants

Auxillary bud showing initiation of shoot



Shoot multiplication from auxillary bud

Plate 3: Induction of multiple shoots from auxillary bud

from callus). Media with high auxin to cytokinin ratio leads to rhizogenesis (induction of root). Media with intermediate combination of auxin and cytokinin leads to callus formation.

In the present study, MS medium was used for *in vitro* propagation. The Murashige and Skoog media has been extensively used for a wide range of culture types and species, particularly for herbaceous plants. This is because MS media is rich in salts, relatively high concentration of ammonium in addition to higher nitrate level and calcium concentration when compared with others. In MS macro nutrients (with nitrogen provided by both NH4 and NO3) cells doubled in dry matter and protein with in 48 hrs (George *et al.,* 1988). The multiple shoot, shoot length and number of leaves produced was more on MS medium containing vitamins 2mg per litre and growth regulator. These results were in accordance with (Curuk *et al.,* 2003).

5.2 TO STUDY THE EFFECT OF GROWTH REGULATOR ON SHOOT GROWTH FROM AUXILLARY BUDS USING CYTOKININS

The growth of auxillary bud explants of shoot growth on media containing plant growth regulators has been studied with a view to obtain rapid shoot proliferation. The addition of growth regulators to the medium, the cultures responded differently to different growth regulator and their combinations. The main purpose of multiplication stage is to maintain the microculture in a stabilized state and multiply the microshoots to a large extent. The basic medium of shoot multiplication stage is similar to sub-culturing medium but often the growth regulators and mineral supplement levels are varied. The growth regulator are used to support a basic level of growth, it is also equally important to direct the developmental response of

propagules. Similar findings were represented by (Hartmann *et al.,* 1997). In *vitro* culture of higher plants with growth regulators auxins and cytokinins are very significant to get the large number of propagules.

The term direct adventitious shoot refers to only those buds that arise directly from the tissue or organ (explants) removed from the mother plant without an intervening callusing phase. The adventitious buds can be induced on sprout tip explants with the influence of the appropriate combination of growth regulator.

The effect of cytokinin was noticeable in tissue culture as they appear to be necessary in plant cell division, cytokinin added to shoot culture media is supported to overcome apical dominance and release lateral buds from dormancy. The low level of cytokinin is frequently required to be added to the culture media. The effect of cytokinin on tissue and organ culture can vary according to the particular compound used, the type of culture and the crop variety.

In the present study, the addition BAP in the culture medium increase the number of shoots (14.7) per explants with medium containing 1.0mg BAP per litre compared to Kinetin. Similar results were also obtained by (Singh *et al.,* 1996) where they obtained shoots from cotyledon halves of *Cucumis melo* cultured on MS medium with 1.0μM BAP. These results clearly indicate BAP as a superior source of cytokinin for shoot proliferation. It may be due to active cell division and differentiation and overcoming apical dominance. Several scientists have also reported that BAP is the most effective cytokinin for stimulation of shoot proliferation. Similar results obtained by (Jeffrey *et al.,* 1990, Cade *et al.,*1990a and Suh and Suh, 1998). The concentration of

cytokinin in the medium had a prominent effect on shoot multiplication and increasing concentration of cytokinin led to higher rate of shoot multiplication.

The maximum shoot length (7.9cm) was obtained from auxillary bud explants on MS medium containing Kinetin 0.5 mg per litre (Fig 2). The Kinetin in the culture medium increased the height of plantlets. Compared to BAP, Kinetin helped in elongation of shoot. The increased height of the plantlet become easy for separation of single nodal cuttings for further sub-culturing. Similar results were obtained by Pink and Walkey 1984 and Josekutty *et al.,* 1993.

In the present investigation involving various concentrations of Kinetin and BAP, the Kinetin showed maximum number of leaves compared to BAP.

Media supplemented with 3.0 mg per litre Kinetin, highest number of leaves (5.2) were obtained. This is in confirmity with the work done by Venkateshwaralu *et al.* (2001) reported that 3 mg per litre Kinetin induced shoot regeneration from stem explants.

5.3 EFFECT OF CYTOKININ AND NAA ON SHOOT PROLIFERATION

The callus formation by the cytokinin may be due to the presence of high concentration of endogenous auxin in the auxillary bud explant. The ratio of both auxin and cytokinin might have been about the same, resulting in callus more being produced (Bhojwani and Rajdan 1983). The cytokinin specificity also plays a role in shoot growth and this may be the cause for the difference in shoot growth response induced by Kinetin and BAP (Vietez and Vietez, 1980).

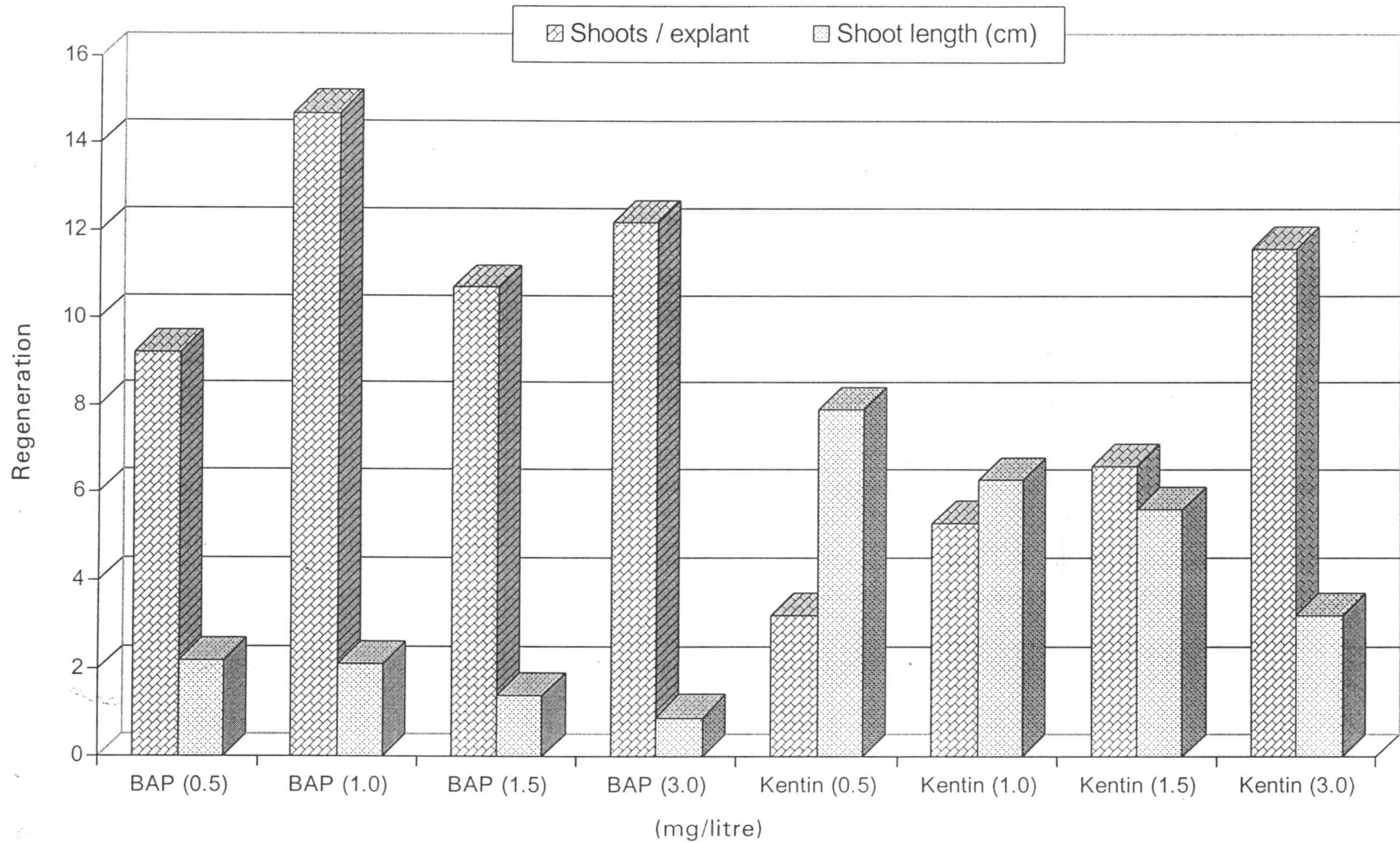


Fig. 2: Effect of cytokinin on shoot length and number of shoots per explant

The MS medium containing BAP 3 mg per litre + NAA 0.1mg per litre was produced the maximum number of multiple shoot per explants (8.6) at 40 days after inoculation and 6.1 at 25 DAI. Because BAP may be involved in cell division, differentiation and also overcome the apical dominance. The MS medium with 3mg per litre BAP was found to be best for multiple shoot induction (Plate 4). Same results were obtained by (Suh and Suh, 1998) reported NAA and BAP were more effective in inducing multiple shoots.

In the present investigation, maximum number of leaves was obtained from medium containing Kinetin 1.5 + NAA 0.1mg per litre at 10, 25 and 40 days after inoculation (6.2, 6.5 and 8.5), respectively. Similar results were noticed by Handley and Chambliss, 1979. They have reported that MS medium with 0.1 mg per litre NAA + Kinetin produced shoots.

In the present study, maximum shoot length (2.3) was obtained from medium supplemented with Kinetin 1.0 + NAA 0.1mg per litre at 10 DAI. At 25 and 40 DAI the maximum shoot length obtained on MS with 3 mg per litre Kinetin + NAA 0.1 mg per litre (4.2 and 9.9), respectively.

5.4 TO STUDY THE EFFECT OF GROWTH REGULATOR ON ROOTING OF *IN VITRO* SHOOTS WITH AUXINS

The root formation of plantlets under *in vitro* from auxillary bud explants of shoot growth was much easier.

In the present study, the maximum number of roots (27.1) was obtained with IBA 1.0 mg per litre (Plate 5). Less number of roots were seen, when medium was supplemented with NAA 2.0 mg per litre. Because IBA has weak auxin activity and is destroyed relatively slowly by auxin destroying

Kinetin 3.0 mg/l + NAA 0.1 mg/l

BAP 3.0 mg/l + NAA 0.1 mg/l

Plate 4: Effect of cytokinin and NAA on shoot proliferation

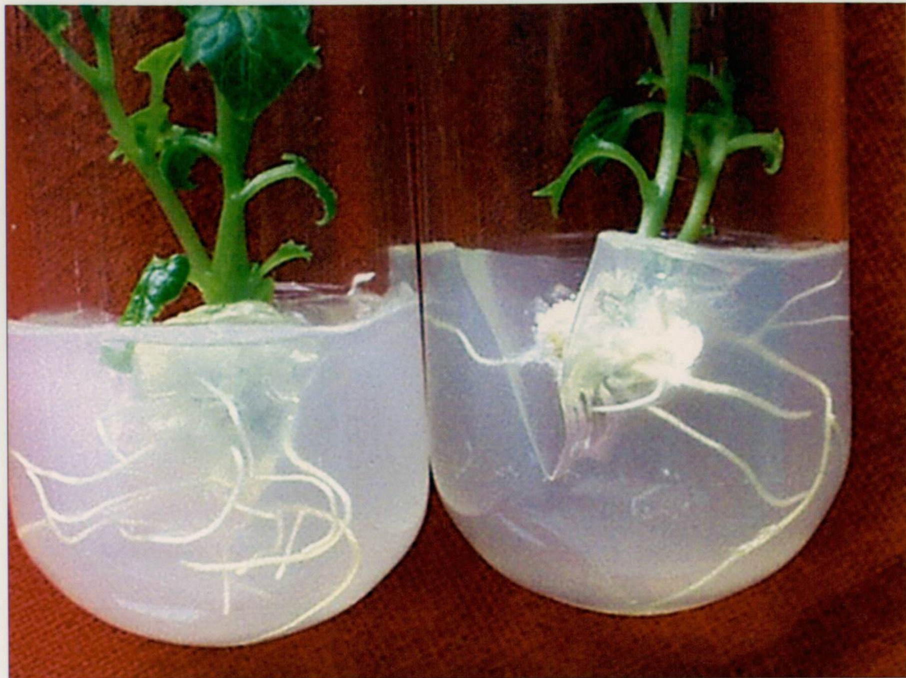


Plate 5: Rooting of *in vitro* shoots in MS + 1 mg / l of IBA

enzymes system, IBA gets poorly translocated, while NAA is phytotoxic at higher concentrations. Similar results were obtained by Sarowar *et al.,*2003. In their experiments shoots were rooted more effectively in 1mg per litre of IBA. Roberto *et al.,* 1982 reported rooting was achieved when the single shoot was transferred to a fresh 1 mg per like IBA medium.

The highest root length (1.4) was obtained on MS medium supplemented with 3mg per litre of NAA. NAA was found to be better for length of root. Which may due to NAA helps in elongation. Early rooting observed with IBA when compared to NAA. Hence it could be concluded that rooting of micro-shoots can be achieved through auxin.

5.5 HARDENING OF *IN VITRO* GROWN PLANTLETS

In the present study the high percent survival of plantlets (5.7) observed in peat medium followed by soilrite medium (5.1) (Plate 6). This may be due to the optimum conditions of aeration, good water holding capacity, and nutrients present in this media. Hence peat media is most suitable for plant growth compared to perlite + vermiculite (1.1) and common potting mixture. Similar results were also observed by Handley and Chambliss, 1979. No survival of plantlets was observed in common potting mixture, This might be observed due to the early hardening of surface, compact nature of the media and due to poor aeration of the media.

The highest plant height, more number of shoots and maximum number of leaves were observed in peat media followed by soilrite media.

It can be concluded that peat is the best medium for the production of *in vitro* plantlets and it can be successfully weaned to greenhouse and

Plate 6: Hardening of plantlets in peat media

finally subjected to field. After one month in field, the plant start producing flowers.

PROTOCOL FOR RAPID MULTIPLICATION OF SHOOT GROWTH *IN VITRO*

Based on the results obtained in the present investigation, protocol for *in vitro* multiplication of shoot growth is as follows.

1. Auxillary buds are the best source of explants which were collected from the main field.

2. The prepared explants are to be treated with detergent teepol for few minutes and rinsing 4-5 times with distilled water.

3. Then the explants are to be dipped in fungicide for 5-10 min. followed by 3-4 times washing with distilled water.

4. The explants should be sterilized with 70% ethanol for one minute followed by soaking in 0.1% HgCl2 for 10 minutes and then rinsed for four to five minutes with sterilized double distilled water under laminar air flow chamber.

5. Then the explants should be cultured on MS medium containing BAP 3mg /litre for culture establishment. The cultures are incubated in culture room where temperature at 25 $\pm$ 2°C and 16 hours light period is maintained.

6. Proliferated shoots should be further sub-cultured on basal MS medium containing Kinetin 0.5mg / litre for better shoot elongation longer shoots are more useful for further sub-culturing and multiplication.

7. After second or third sub-cultures, the shoots are to be placed on MS basal medium containing IBA 1mg per litre for rooting.

8. Rooted plantlets could be transferred to pots containing peat medium and watered regularly and then plantlets are kept under net house for better establishment.

FUTURE LINE OF WORK

1) *In vitro* propagation studies in *Momordica dioica* can be tried with other explants and culture media for high rate of multiplication.

2) Study should be initiated for increasing the survival percentage of *in vitro* plantlets in the main field with different environmental conditions.

Summary

VI. SUMMARY

The present study on "*In vitro* propagation studies in *Momordica dioica*" was conducted at the tissue culture laboratory of Department of Horticulture, University of Agricultural Sciences, Dharwad during the period of 2002-2004. All cucurbits are propagated through seeds except few, spine gourd having hard seed coat dormancy and difficult to germinate in field condition and it is difficult to identify the sex of the crop at initial stage. Moreover it is dioecious in nature and cross pollinated crop. For all these difficulties *in vitro* propagation allows multiplication of shoots. Hence the investigations were carried out to standardize explants, standardization of culture media and growth regulator combination for shoot proliferation, rhizogenesis and hardening of *in vitro* rooted plantlets. Further acclimatization of plantlets was also studied.

The Results are summarized as follows.

1. Five different explants *viz.,* shoot tip, auxillary bud, leaf, tuber and seed were used for micro-propagation and it was observed that auxillary bud gave the quickest response and maximum survival percentage and more number of multiple shoots per explant.

2. Among the different explants, auxillary bud gave the highest number of shoots (4.66), maximum survival percentage (5.74%) and early days for initiation of growth (4.0).

3. Growth regulator like BAP and Kinetin at different concentrations were used for the study. The highest number of shoots (14.7) was observed on medium with 1mg/ litre BAP., maximum shoot length (7.9) on MS medium with Kinetin 0.5 mg per litre, more number of leaves (5.2) on 3 mg per litre Kinetin and maximum shoot dry weight (125) on Kinetin 1.0 mg per litre .

4. Among the growth regulators like BAP, Kinetin and NAA at different concentration and combination. The maximum shoots was obtained with the combination of growth regulator 3 mg /litre BAP + 0.1 mg /litre NAA.

5. The more number of leaves was observed by the addition of 1 mg /litre Kinetin + 0.1 mg /litre NAA to the medium.

6. The highest shoot length (9.9) was obtained with the combination of growth regulator 3 mg / litre Kinetin + 0.1 mg /litre NAA.

7. The more number of roots (27.16) was observed by the addition of 1 mg / litre of IBA to the medium.

8. The highest root length (1.46) was obtained on the medium with 3 mg / litre NAA.

9. The *in vitro* rooted plantlets are separated from the agar, washed with water and the plantlets are dipped in fungicide. Then plantlets were transferred to peat medium.

10. The peat medium is best for *in vitro* rooted plantlets and highest percent survival (5.70) was also noticed on this media .

11. Finally the plantlets are transferred to field.

Multiplication of propagules under *in vitro* is the best method compared with other method of multiplication. The *in vitro* method is not only a suitable method to increase the rate of multiplication, also breaks the dormancy, but also helps in obtaining the virus free plantlets and to obtain the true to type plants.

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ABBREVIATIONS

BA	:	6-benzyladenine
BAP	:	6-benzyl aminoplurine
KIN	:	Kinetin
Zip	:	2 isopentyl adenine
GA_3	:	Gibberellic acid
2, 4-D	:	2,4-dichlorophyenoxyacetic acid
NAA	:	1 napthalene acetic acid
IAA	:	Indole-3-acetic acid
IBA	:	Indole-3-butyric acid
EDTA	:	Ethylene diamine tetra acetic acid
MS	:	Murashige and Skoog
$HgCl_2$	:	Mercuric chloride
μM	:	Micromole
Psi	:	Per square inch
v/v	:	Volume by volume

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IN VITRO PROPAGATION STUDIES IN *Momordica dioica* Roxb.

REKHA B. 2004 Dr.P. R. DHARMATTI
MAJOR ADVISOR

ABSTRACT

In vitro propagation studies in *Momordica dioica* was carried out at Department of Horticulture, University of Agricultural Sciences, Dharwad. *Momordica dioica* is an under exploited vegetable crop whose seeds are having hard seed coat dormancy and difficult to germinate in field condition. Hence, the investigation were carried out to standardize the explants, growth regulator combination for shoot proliferation, rhizogenesis and hardening. In this experiment five different explants *viz.*, shoot tip, auxillary bud, leaf, tuber and seed were used for micropropagation and observed that auxillary bud gave the quickest response and more number of multiple shoots per explant. Among the growth regulators like BAP and kinetin at different concentrations, the maximum number of shoots was observed with 1 mg per litre BAP and maximum shoot length on MS medium with 0.5 mg per litre kinetin. Among the growth regulator combinations, maximum number of shoots was obtained on MS medium containing 3 mg per litre BAP + 0.1 mg per litre NAA.

The more number of roots was observed by the addition of 1 mg / litre of IBA to the medium. The highest root length was obtained on the medium with 3 mg / litre NAA. The *in vitro* rooted plantlets are separated from the agar, washed with water and the plantlets were dipped in fungicide. Then plantlets were transferred to peat medium which is best for *in vitro* rooted plantlets and highest percent survival was noticed. Finally, the plantlets were transferred to field.

Multiplication of propagules under *in vitro* is the best method compared with other method of multiplication which breaks the dormancy, helps in obtaining the virus free plantlets and to obtain the true to type plants.