Chapter - II

Micropropagation

2.1 INTRODUCTION

Plant tissue culture techniques are essential to many types of academic inquiry, as well as to many applied aspects of plant science. In the past, plant tissue culture techniques have been used in academic investigations of totipotency and the roles of hormones in cyto differentiation and organogenesis. The term "Plant Tissue Culture" is commonly used to describe the *in vitro* and aseptic cultivation of any plant part on a nutrient medium (Dixon, 1987). In simple terms, plant tissue culture can be considered to involve three phases: first, the isolation of the plant (tissue) from its usual environment; second, the use of aseptic techniques to obtain clean materials free of the usual bacterial, fungal, viral and even algal contaminants and third, the culture and maintenance of this material *in vitro* in a strictly controlled physical and chemical environment (Robert, 2002).

The term micropropagation means a technological process consisting of several steps in order to produce numerous propagets from a chosen mother plant under *in vitro* conditions. Large scale technologies have been developed following long experimental procedures worldwide. In the period of 1970 - 1980 research was focused onto the methodology of sterile culturing, and the propagation of economically important plant species. Since, many trials with different nutrient media, investigation on growth regulator effects, experiments concerning interaction of genotype and environmental effects have been studied. Micropropagation is used routinely to generate a large number of high-quality clonal plants, including medicinal, agricultural, ornamental and vegetable species, and in some cases also plantation crops and fruits. Micropropagation has significant advantages over traditional clonal propagation techniques. These include the potential of combining rapid large-scale propagation of new genotypes, the use of small amounts of original germplasm and the generation of pathogen-free propagates (Murashige and Skoog, 1962).

Emergence of an adventitious organ directly from the explant without an intervening callus phase is termed “direct organogenesis”. It is also found out that the plants developed using shoot tip culture often retains the genetic composition of the mother plant (Dixon, 1987). This type of plant development is also called micropropagation. In other words, it means production of genetically identical copies of the organ. In this procedure, the explant is established on a nutrient medium containing moderate level of auxins and cytokinins (to avoid callus production) and subsequently initialize shoot organ development. Moreover, shoot multiplication is achieved through subdivision of the shooting clump and planting out in separate vessels. Plant tissue culture technology has been available to the plant breeders for nearly four decades and has been extensively employed for crop improvement in several oil seed crops. Micropropagation of plant tissues *in vitro* is firmly influenced by culture medium and various plant hormones, such as auxin, cytokinin, and ethylene. The ratio of cytokinin/auxin is also crucial for adventitious shoot formation (Fakhrai and Fakhrai, 1990). The role of ethylene in *in vitro* morphogenesis has been assessed previously (Gonzalez *et al.*, 1997) showing some positive effects on axillary shoot proliferation (Panizza *et al.*, 1993). During the last three decades, micropropagation and other *in vitro* techniques have become more widely used in commercial horticulture and agriculture for the mass propagation of crop, horticultural and medicinal plants (Behera and Sahoo, 2009).

Micropropagation has many advantages over conventional methods of vegetative propagation, which suffers from several limitations (Nehra and Kartha, 1994). Large-scale micropropagation laboratories are providing millions of plants for the commercial ornamental market and the agricultural, clonally-propagated crop market. With selected laboratory material typically taking one or two decades to reach the commercial market through plant breeding, this technology can be expected to have an ever increasing impact on crop improvement as we approach the new millennium (Brown and Thorpe, 1995). With micropropagation, the multiplication rate is greatly increased. It also permits the production of pathogen-free plants. The role of micropropagation in crop improvement has been reviewed in considerable details (Bajaj, 1992).

Due to large scale destruction of plant habitats and unrestricted over exploitation of this natural resource, coupled with limited cultivation and insufficient attempts for its replenishments, the propagation through seed is unreliable due to poor germination and death of young seedlings under natural conditions the wild stock of this species has been markedly depleted. The consequence is possible extinction of the species and this provides justification from conservation and propagation of this valuable germplasm. *In vitro* culture technique is an alternative method for conservation and propagation of this species. The present investigation describes an efficient protocol for micropropagation of *Trichosanthes cucumerina* by using shoot tip and nodal explants.

In this chapter, we have successfully developed a *in vitro* micropropagation protocol for *Tricosanthes cucumerina.* We examined that the effects of different basal media supplemented with cytokinins, carbohydrates, amino acids and different combinations of plant growth regulators for adventitious shoot regeneration from shoot tip and node explants of *Tricosanthes cucumerina*.

2.2 MATERIALS AND METHODS

2.2.1 Collection of seeds

The snake gourd seeds were procured from Tamil Nadu Agriculture University, Coimbatore District, Tamil Nadu, India. The seeds were grown on the field, in the Department of Botany, Bharathidasan University for the collection of explants.

2.2.2 Sterilization of explants

Shoot tip and nodal explants of 10 days old seedling were harvested and washed in tap water to remove loose dust, rinsed with 4 drops of commercial soap solution (Teepol) for 5 mins and kept under running tap water for for 30 mins. The explants then incubated with 0.1 mg/L mercuric chloride $HgCl_2$ for 5 mins aseptically to remove the microbes present in the surface of the explants.

2.2.3 Preparation of media

Throughout the study, the MS medium was tested for the standardization of *in vitro* protocols. The composition of MS basal medium designed by Murashige and

Skoog (1962) was used in this study. Instead of MS, B_5 vitamins (Gomborg *et al.*, 1968) we used modified MS medium in the present study. An aliquot of the stock solution was frozen and thawed at room temperature just before use. To make one litre of medium, about 500 ml of distilled water was taken in a clean Erlenmeyer flask (2 Litre) with appropriate aliquots of the stock solution. Sucrose was added slowly and shake the flask to prevent clumping of sucrose in the bottom of the flask followed by the addition of vitamins and hormones. It was then brought to one litre volume and adjusted to pH 5.8 with 0.1 N HCl or 0.1 N NaOH. Agar (0.8 % w/v) was added slowly while shaking and the medium was distributed to the test tubes (about 10 ml/test tube) or conical flask (about 50 ml/flask) plugged with non-absorbent cotton wrapped in one layer of cheese cloth. After plugging with non absorbent cotton, the containers were autoclaved at 121°C for 15 mins. Likewise MS medium (Murashige and Skoog, 1962); WPM (Loyd and Mc Cown, 1980) and B5 vitamin (Gomborg *et al.*, 1968) were also prepared for micropropagation studies (Table 2.6).

2.2.4 Preparation of hormones

Cytokinins like benzyl adenine (BAP), Kinetin (KIN), 2 isopentanyl adenine (2iP), Zeatin (Ze) were dissolved (10 mg/ml – stock solution) in 0.1 N HCl (2 ml) and diluted with distilled water to make 1 mg/1 concentrations and stored in the refrigerator. Auxins such as Indole acetic acid (IAA-10 mg/L), Indole butyric acid (IBA – 10 mg/L) and Napthalen acetic acid (NAA – 10 mg/L) were dissolved in 0.1 N NaOH (1.0 ml) and diluted with distilled water to make 1.0 mg/1.0 ml each and stored in the refrigerator. These hormones were stable to be added before autoclaving and pH of the media was adjusted to 5.8.

2.2.5 Preparation of glassware

The glass wares like test tubes, conical flasks, measuring cylinders, beakers and bottles in different sizes were washed with liquid detergent (Teepol) and rinsed thoroughly with tap water and then with distilled water. The test tubes, conical flasks, petriplates and other glass wares are taken for the tissue culture study were dried by placing them in oven at 60°C with blowing air. The racks of tubes were covered with clean plastic sheet to prevent deposition dust and flying contaminants.

2.2.6 *In vitro* seed germination

The seeds were washed in tap water to remove dust particles and the chemical reagents used for storage of seeds, rinsed with four drops of commercial soap solution (Teepol) for 5 mins and then kept under running tap water for one hour. The seeds were 0.1 % Mercuric chloride ($HgCl_2$) for 3 mins aseptically to remove the microbes adhered on the surface of the seeds followed by five times rinse with sterile distilled water to remove the sterilant and used for inoculation. The sterilized seeds were kept on half strength MS medium containing 0.8 g/L Agar as gelling agent and 15 g/L Sucrose as carbon source in MS medium without hormones for germination. This setup kept to 16 h L/D photoperiod with low light intensity (1,000 Lux). Seeds germinated after two weeks, and the germinated seedlings produced leaves and shoot tips three weeks which served as explant source. The main reason for opting *in vitro* seed germination is to avoid the contamination.

2.2.7 Culture media and conditions for shoot regeneration

MS medium supplemented with appropriate plant growth regulators such as BAP (0.5 to 2.5 mg/L), 2iP (0.5 to 2.5 mg/L) and KIN (0.5 to 2.5 mg/L) Ze (0.5 to 2.5 mg/L) (Table – 2.1) and tightly closed with screw cap. The cultures were kept in culture rooms maintained at 25 ± 2°C for 16 hr L/D photoperiod (90-110µ mol m^{-2} s^{-1}, cool white fluorescent lights Philips F-40 CW). Two weeks after inoculation the explants developed shoots directly from the margins of the nodal segments.

2.2.8 Multiple shoot induction

The explants were placed in vertical position in different media for induction and elongation of multiple shoots. For high frequency adventitious shoot induction, three key steps were followed. In the initial step, individual treatment of different cytokinins like 2iP (1.0 – 2.0 mg/L) and KIN (1.0 – 2.0 mg/L) were evaluated. After the identification of suitable concentration of cytokinin for shoot multiplication, different auxins (IBA, NAA and IAA) in various concentrations (0.5 – 2.5 mg/L) were tested in the second step. During multiple shoot induction, the influence of each plant growth regulator was noticed. In the third step, a different approach was made for high frequency recovery for multiple shoots fro shoot tip, nodal and nodal explants directly by using different hormones, polyamines, amino acids, carbon sources and media

supplements. The amino acids like glutamine and serine (0.5-2.0) mg/L were tested. The carbon sources ranging from 15-40 g/L of sucrose, glucose and maltose tested to trigger the direct regeneration process. The different media formulations like modified MS media (MS+B5 vitamins), MS media (Murashige and Skoog, 1962), White's media (Lawrence and Barker, 1963) and B_5 (Gamborg *et al.*, 1968) were also tested for the more number of multiple shoot induction and proliferation (Table 2.2).

2.2.9 Shoot elongation

The developed shoots were excised from the explant and immediately shifted to elongation medium for shoot elongation, and elongation medium were supplemented with Gibberellic acid (GA_3) (0.5 – 2.5 mg/L) and cytokinins like 2iP and BAP on half strength and full strength MS medium with agar and sucrose. After ten days of subculture, shoots were elongated on elongation medium with significant frequencies.

2.2.10 Root induction

For root induction, regenerated shoots of 4.5cm length were transferred to MS medium supplemented with auxins such as NAA, IBA and IAA. In all these experiments, the culture was kept to 16/8 h L/D photoperiod with low light intensity (1,000 Lux). Rooting evaluated after 20 days in culture. The frequency of rooting the average number and length of roots per shoot were determined and recorded after 45 days. Rooted plantlets were removed from the culture bottles without damaging the roots and then washed in running tap water. Then they were transferred to plastic cups containing sterile sand, soil and vermiculate (1:1:1). Polyethylene bags were inverted over the cups to maintain humidity and kept under 16 h L/D photoperiod (1,500 Lux) at 25 ± 2°C. The plants were irrigated periodically with half strength MS nutrient solution. At the end of 4 weeks, they were transferred to the field, after which survival was assessed.

2.2.11 Hardening and acclimatization

Rooted plantlets are separated aseptically from medium and the developed roots washed with distilled water. Washed plantlets shifted to Plastic cups with sand, soil and vermiculite in the ratio of 1:1:1 and this setup kept under controlled

environment chamber (SANYO, Japan) at 70 % humidity 16:8 hour D/L photoperiod. After about one month, the hardened plants were transferred to polybags containing sand, soil and vermiculite (1:1:1). Then it was transferred in to green house finally to experiment field.

2.2.12 Statistical analysis

Mean and standard error were used throughout the study and the values were assessed by using parametric modes median test (Snedecor and Cochren, 1989). The data were analyzed for variance by Duncan's Multiple Range Test (DMRT) using SAS programme (SAS Institute, Cary, N.C). For multiple shoot induction from the shoot tip and nodal explants, 50 explants tested with 5 replicates and each experiment was repeated three times. During root induction 30 elongated shoots were tested for each experiment was repeated 3 times with 5 replicates.

2.3 RESULTS AND DISCUSSION

2.3.1 Effect of cytokinins on adventitious shoot induction

Adventitious shoots were directly induced from node and shoot tip explants by both the TDZ and BAP. The frequency of shoot formation was influenced by both the type of explants and the choice of cytokinins. Treatment differences for the number of shoots / explant were noticed within 3 – 4 weeks of culture initiation. The number of shoots from node was comparatively high than shoot tip on TDZ (0.3 mg/l) supplemented media and maximal (15.7 shoots and 11.4 shoots / nodal and shoot tip explants) which showed 37.2% and 35.4% of response. Both the explants showed 30.7% and 25.7% with 11.5 and 9.5 shoots / explant on that with 1.5 mg/l of BA from node and shoot tip explants (Table 2.1). Furthermore, continuous culture on both the TDZ and BA supplemented media resulted in the clumping of shoots. Hence, the proliferating shoot clusters from both the cytokinins fortified media were subcultured within 2 weeks of culture initiation to develop into normal shoots. Moreover in explants cultured on BA showed swelling (hyperhydricity) prior to the emergence of shoot buds developing from the pre-existing material after ten days of inoculation. In KN treatment also low number of multiple shoot formation was observed when compared with TDZ. Among the different concentrations tested, 1.5 mg/l KN showed the best response and produced 6.2 and 3.7 shoots / explant from node and shoot tip

explants (Table 2.1). Increasing the cytokinins concentration decreases the formation of multiple shoot in BA and TDZ. Among the individual concentrations of cytokinins tested, TDZ (0.3 mg/l) seems to be best for the formation of multiple shoots from node and shoot tip explants of snake gourd. These results correlate with the earlier studies in snake gourd employed with higher levels of growth regulators and obtained relatively lower frequency of shoots. However, our reports differ from that of Sujatha and Reddy (1998) who speculated that the maximum number of regenerated shoots amply demonstrates the high cytokinins activity of TDZ, as reported for several other woody plants species also (Huetteman and Preece, 1993; Chakravarty *et al.*, 2010). In most of species, the presence of cytokinins alone promoted optimal shoot proliferation, such as 2iP in *Euphorbia lathyris* (Lee *et al.*, 1982) and BA in *E. lathyris* (Tideman and Hawker, 1982; Ripley and Preece, 1986), *E. poplus*, *E. tannensis* (Tideman and Hawker, 1982), Cassava (Nair *et al.*, 1979), *Ipomoea sepiaria* (Cheruvathur *et al.*, 2015) and *T.cucumerina* L. (Devendra *et al.*, 2008 and 2015).

Conditions facilitating optimal shoot bud proliferation from shoot tip and node explants of snake gourd were studied. Similar to our results, Sujatha and Reddy (1998); Ahn *et al.*, (2007); Sujatha and Reddy (2007) reported that TDZ favours multiple shoot proliferation from shoot apex and embryo axes explants of snake gourd. In contrast, Athma and Reddy (1983) reported the development of a single shoot from shoot apices on medium supplemented with 0.5 to 2.0 mg/l BA. Sanguduen *et al.* (1987) reported strong proliferation of multiple shoots from the shoot apex on medium supplemented with 4.0 mg/l BA. The same medium was found to give the optimal caulogenic response from embryo explants (Khumsub, 1988). Molina and Schobert (1995) obtained a maximum of 4.4 shoots on the medium supplemented with 1.0 µM of BA and 30 g/l sucrose. Molina and Schobert (1995); Khumsub (1988), Sanguduen *et al.* (1987) and (Cheruvathur *et al.*, 2015) reported BA as the best cytokinins for multiple shoot proliferation from snake gourd shoot apex. These reports are contradictory to our results where lower concentrations of TDZ (0.3 mg/l) treated cultures showed the highest percentage of response and produced more number of multiple shoots (15.7) with nodal explants compared with 11.4 shoots / Shoot tip.

2.3.2 Influence of auxins on multiple shoot induction

Supplementation of 0.3 mg/l TDZ was found as suitable cytokinin and showed the best response for multiple shoot induction from both the explants. The cytokinins combined with auxins were used for the induction of multiple shoots in several plant species (Khan *et al.*, 1999). Hence, in this experiment, the impact of auxins like IAA, IBA and NAA were also evaluated along with TDZ (0.3 mg/l). The influence of auxins decreases the formation of multiple shoots drastically. Among the various concentrations of auxins tested, MS media fortified with 30 g/l sucrose, 0.3 mg/l TDZ with 0.75 mg/l NAA produced multiple shoots from node explants. Maximum of 6.2 shoots were regenerated from 0.75 mg/l of NAA with 35.2% of response. In the shoot tip explants also the above said media did not favour the formation of multiple shoots as expected and produced 5.2 shoots with 30.3% of response (Table 2.2). Combination of auxin and cytokinins decreased the multiple shoot formation in snake gourd. Auxins only induce basal callus from both the explants. Combination of auxin with cytokinins reduces the production of multiple shoots due to the formation of basal callus and bulging of explants. Similar to our results Reddy and Bahadur (1989b) obtained the highest mean of 5.2 shoots on medium supplemented with 2.0 mg/l KN plus 1.0 mg/l IBA. Bin *et al.* (2008) in *Euphorbia esula* obtained multiple shoots from axillary buds in the combination of BA (1.11 µM) with IBA (1.97 µM). In other dicot plants such as in *Dioscorea nipponica* microtubers were produced on all the auxin IAA, IBA and NAA (Chen *et al.*, 2007) and also by Junli *et al.*, (2009) in *Pinellia ternate*. Similar to our results, Reddy *et al.*, (1987b); Reddy and Bahadur (1989a; 1989b) experiments proved that KIN 2.0 mg/l with IAA 1.0 mg/l produces low number of multiple shoots and failed to obtain shoots directly from shoot apex and nodal explants of snake gourd. Since the young shoot apex is an active site for auxin biosynthesis, exogenous auxin is not always needed when relatively large shoot tip explants from actively growing plants are used. Moreover, tissue culture studies using several explants have indicated the presence of high levels of exogenous auxins in snake gourd tissues (Sujatha, 1996).

These results proved that combination of cytokinin with any auxin showed inferior results for micropropagation in snake gourd. Hence the process of

multiplication of shoots depends on types of auxins and cytokinins. In our study also variations in the use of cytokinins and auxin for multiplication of shoot tip and nodal explants were observed.

2.3.3 Effect of aminoacids on multiple shoot induction

Both the Shoot tip and nodal explants have been used to see the effect of aminoacids on shoot formation. There is evidence to prove that aminoacids act as an efficient morphogenic regulators in a number of species (Tupy *et al.*, 1983; John and Guha – Mukherjee, 1997). Media enriched with aminoacids or hydrolyzed proteins enhanced regeneration of explants *in vitro* (Sen *et al.*, 2002). Therefore, different aminoacids namely Alanine, Serine, Proline and Glutamine were employed in the MS medium independently to study their effect on multiple shoot induction from both the explants of snake gourd along with TDZ at 0.3 mg/l (Table 2.3). At 20 mg/l Glutamine with 0.3 mg/l TDZ, 55.2% and 41.0% of Node and shoot tip explants responded in producing multiple shoots with an average of 25.8 shoots and 20.4 shoots per explant. At the same time alanine at 30 mg/l evoked 46.3% and 34.3% of culture response and produced 20.7 and 18.4 shoots per nodal and shoot tip explants. The other aminoacids viz., Proline at 20 mg/l produced 19.5 and 16.8 shoots / nodal and shoot tip explants whereas, serine 30 mg/l produced 18.9 and 15.9 shoots / nodal and shoot tip explants respectively (Table 2.3). Though their response was less than those to Glutamine and Alanine treatments, their effects on shoot induction were significantly higher than with TDZ alone (Table 2.1). The effects of alanine, proline, Glutamine and serine were studied here for the first time in shoot differentiation from both the explants of snake gourd. Mariyana and Johannes (2009) speculated that good quality new shoots were obtained on media with glutamine as well as from shoot tip cultures of cucumber (Vasudevan *et al.*, 2004, 2007). Supportive results were also given in other dicot plants by Rastogi *et al.* (2008); Sanjaya *et al.* (2006); Muhammad Akram and Faheem Aftab (2009).

2.3.4 Effect of carbon sources on multiple shoot induction

The responses of *in vitro* culture to different carbon sources added to the medium were frequently tested. Although carbohydrates are of prime importance for *in vitro* organogenesis, carbon metabolism *in vitro* is still not clearly understood

(Kozai, 1991). It is well established that carbohydrate requirements depend upon the stage of culture and may show differences according to the species (Thomson and Thrope, 1987). Both the explants were cultured in MS medium containing 10 – 50 g/l of Sucrose, Maltose, Fructose and Maltose along with the TDZ (0.3 mg/l) and Glutamine (20 mg/l). Among the four carbon sources tested, Sucrose 30 g/l proved to be the best for shoot regeneration than the other three carbon sources (Table 2.4). Sucrose to be the most effective in terms of shoot number and produced 25.8 and 20.4 shoots / explant using nodal and shoot tip cultures. The number of nodes per regenerated shoots was also higher in Sucrose 30 g/l treated cultures. Similar to our observation, results were obtained in micropropagation of snake gourd (Molina and Schobert, 1995; Sujatha and Reddy, 1998; Sujatha and Reddy, 2007; Ahn *et al.*, 2007). Other supportive results are by Romano *et al.* (1995) in Cork Oak; Ghimire *et al.* (2010) in Drymaria; Madhulatha (2006) in Banana. Contradictory results were published by the experiments on beech cultivars by Cuena and Vieitez (2000b) that glucose was the best carbon source for both axillary branching and adventitious shoot regeneration. However, Sucrose and Glucose induced the highest frequency of shoot formation in *Bixa orellana* (DePaiva Neto *et al.*, 2003).

2.3.5 Effect of PF - 68 on multiple shoot induction

The regeneration protocol described above produced multiple shoots supplemented with 0.3 mg/l TDZ, 20 mg/l Glutamine. Although the number of explants producing microshoots reached up to 55.2%, further development of these shoots was very slow. It has been shown that surfactants can induce and / or enhance organogenesis (Khatun *et al.*, 1993 a, b; Khehra *et al.*, 1995). Addition of Pluronic F – 68 (0.6 mg/l) to the regeneration medium significantly enhanced the percentage of multiple shoot formation to 85.5% and 75.4% and produced 52.7 shoots and 39.4 shoots / nodal and shoot tip explants (Table 2.5) (Plate - 1, 2). Increasing concentration of PF 68 decreases the shoot formation. Similar to our results Konan *et al.*, (1997) speculated that Cassava cultivars when supplemented with PF 68 produce more number of multiple shoots. Supporting results were also published by Khehra *et al.* (1995) in Chrysanthemum, Konan *et al.* (1994 b) in cassava, Lloyd and McCown (1980) in Kalmia. The above scientists in their publication mentioned that PF - 68 seems to be highly efficient in micropropagation technique. Among the

different PGRs tested, TDZ (0.3 mg/l) + PF - 68 (0.6 mg/l) + Glutamine (20 mg/l) + Sucrose (30 g/l) was proved to be the best for the production of multiple shoots in snake gourd (Plate – 1 & 2). Higher concentration of all these hormones produces hyperhydricity of explants, formation of basal callus and low rooting was also noticed so lower concentrations proved to be best for micropropagation of snake gourd. This hyperhydricity of explants was also observed by Witzrens *et al.* (1988); Nestares *et al.* (1996); Baker *et al.* (1999) in Sunflower.

2.3.6 Effect of different Media on multiple shoot induction

In the present investigation, the MS media containing MS salts with B5 vitamins showed superior response and produced 52.7 shoots per nodal explants (Plate 2) and 39.4 shoots with shoot tip explants (Plate 1) when compared with MS, WPM and B5 medium. In the above Medias, the multiple shoots were decreased from 52.7, 32.5, 17.5 and 5.5 shoots / nodal explant respectively. In the case of shoot tip explants also the shoots were decreased from 39.4, 18.5, 7.6 and 2.4 shoots / shoot tip explants respectively (Table 2.6). In most of the snake gourd micropropagation, MS medium proves to be the best (Sujatha and Reddy, 1998; Sujatha and Reddy, 2007; Ahn *et al.*, 2007). But we proved that MS seems to be the best compared to MS in shoot formation. Condradictory to our results, Reddy *et al.* (1987b) and Reddy and Bahadur (1989b) failed to obtain shoots directly from shoot tip explants cultured on MS and B5 medium with various cytokinins. To our knowledge, no results were published for the micropropagation of snake gourd by using WPM and MS medium.

2.3.7 Elongation of shoots

Individual treatment of GA_3 produced elongated shoots but the leaves fall after ten days of culture and led to loss of their multiplication ability. Hence (0.1 – 0.5 mg/l) GA_3 was used in conjunction with all the cytokinins for shoot elongation. Shoot elongation (>5cm in length) of adventitious shoot buds was successfully achieved on an MS medium supplemented with PF - 68 (0.6 mg/l) with GA_3 (0.3 mg/l) within a period of four weeks and showed 67.4% of response with 5.5 cm in shoot length. Kinetin 1.5 mg/l + 0.3 mg/l GA_3 produced 5.0 cm in shoot length and showed 60.3% of response. The other cytokinins TDZ and BA produced 50.5% and 45.7% of response with 4.8 cm and 4.4 cm of shoot length. GA_3 (0.3 mg/l) + PF 68 (0.6 mg/l)

proved to be the best for the elongation of shoots (Table 2.7) (Plate 1 & 2). Similar to our results, elongation of shoots was obtained when 0.1 – 1.0 mg/l of GA_3 was used in conjunction with 0.2 mg/l of BA (Sujatha and Reddy, 1998; Alam *et al.*, 2010). Low concentration of GA_3 was found useful in shoot elongation in snake gourd which also correlates with Alam *et al.,* (2010). But, Purkayastha *et al.,* (2008) proved that elongation was also achieved on MS basal medium supplemented with GA_3 in varying concentrations. Combined treatment of GA_3 with PF - 68 showed best results for elongation in snake gourd. In some dicot plants, elongation was achieved on multiple shoot induction medium itself without the addition of GA_3 (Meena *et al.*, 2010; Maria *et al.,* 2009).

2.3.8 Rooting and Acclimatization

Excised shoots were rooted on half – strength and full - strength MS medium with different types of auxin. The promoting effect of reducing the salt concentration of MS on *in vitro* rooting of shoots has been described in snake gourd reports (Sujatha and Reddy, 1998) and other dicot plants (Constantine, 1978; Skirvin *et al.*, 1980). Plants rooted in MS medium significantly developed stunted roots and the root induction was strengthened after 30 days of culture. MS medium fortified with IBA (1.5 mg/l) showed 50.5% of response was found to be more effective for root induction (Table 2.8) than IAA and produced 4.0 roots with 3.5 cm root length. This results correlate with Ahn *et al.* (2007) where IBA at 5 µM produces two fold increases in the formation of roots and the survival percentage. Sujatha and Reddy (1998) evoked that rooting was maximum on the medium supplemented with 1.0 mg/l IBA followed by NAA. Along with the identified concentration of auxin IBA (1.5 mg/l) $AgNO_3$ was added to limit the time of rooting. $AgNO_3$ at 0.2 – 1.0 mg/l significantly increased the root number per shoot (Table 2.8). $AgNO_3$ (0.6 mg/l) with IBA (1.5 mg/l) significantly increased the root number 6.2 roots/explants with 75.6% of response and produced 5.8 cm of root length (Table 2.8) (Plate 1,2). Our result is opposite to the results of Wan Jin Xiang and Pan Rui – Chi (2004) who proved that $AgNO_3$ inhibited rooting in mung bean. Our result coincides with Mutasim and Kazumi (2000) in faba bean where $AgNO_3$ proved to be the best in root formation. Bais et al. (2000a) reported that $AgNO_3$ can improve and enhance *in vitro* rooting in

shoot cultures of *D. hamiltonii*. Yonghua *et al.* (2005) showed that half - strength MS containing 1.0 mg/l $AgNO_3$ was an optimum medium for rooting in strawberry.

This result clearly demonstrates that $AgNO_3$ can enhance root emergence, root growth rate, root number per shoot, root length and improve rooting efficiency. For acclimatization, the rooted shoots were washed with sterile distilled water to remove agar sticking then and transferred to pots containing sterile sand, soil and vermiculite in the ratio 1:1:1 for proper acclimatization. These plantlets showed 73% of survival (Plate 1,2).

2.4 CONCLUSION

Genetic improvement of snake gourd cultivars for insect pest and disease resistance, among other characters, can be efficiently achieved through genetic engineering. To date, difficulties in regeneration and non – reproducibility of results in tissue cultures of snake gourd have restricted work on genetic transformation. Direct DNA transfer methods using shoot apical meristems have revolutionized plant genetic engineering of key agronomic crops previously considered recalcitrant to gene transfer, by passing tissue culture related regeneration difficulties (Potrykus, 1991; Sautter, 1993). The importance of having access to a prolific regeneration system for transformation experiments using particle bombardment technology is well documented (Christou, 1994). The standardized protocols are of cardinal importance to snake gourd improvement, their high proliferation rates enabling mass multiplication of elite stocks, such as pistillate lines, and the development of genetic transformation.

Table 2.1 Effect of cytokinins on multiple shoot proliferation from Shoot tip and Node explant cultured on MS medium.

Concentrations Cytokinins (mg/l)	Shoot tip explant		Node explant	
	Percentage of response	Mean No. of shoots / explant	Percentage of response	Mean No. of shoots / explant
BAP				
0.5	21.5 ± 0.1^{g}	6.6 ± 0.1^{h}	26.5 ± 0.2^{f}	9.4 ± 1.3^{g}
1.0	22.5 ± 0.2^{fg}	8.5 ± 0.4^{f}	28.4 ± 0.4^{de}	10.4 ± 1.3^{f}
1.5	25.7 ± 0.2^{e}	9.5 ± 1.5^{d}	30.7 ± 0.1^{bc}	11.5 ± 1.1^{de}
2.0	22.8 ± 1.0^{f}	7.5 ± 0.3^{g}	29.4 ± 1.1^{d}	9.7 ± 1.4^{fg}
2.5	20.4 ± 0.5^{gh}	6.3 ± 0.4^{hi}	28.1 ± 1.2^{e}	7.5 ± 0.6^{h}
3.0	09.5 ± 1.2^{h}	6.0 ± 0.6^{hi}	27.1 ± 1.7^{ef}	5.3 ± 0.3^{j}
KIN				
0.5	12.1 ± 1.1^{mn}	3.0 ± 1.1^{l}	17.2 ± 0.1^{k}	1.4 ± 0.4^{n}
1.0	16.5 ± 1.2^{j}	3.4 ± 1.3^{k}	18.7 ± 0.5^{j}	2.8 ± 0.3^{m}
1.5	18.7 ± 0.4^{hi}	3.7 ± 0.5^{j}	20.4 ± 1.1^{i}	6.2 ± 1.1^{i}
2.0	15.3 ± 0.3^{jk}	3.5 ± 0.3^{jk}	18.1 ± 1.3^{jk}	5.2 ± 1.7^{jk}
2.5	13.7 ± 0.3^{l}	3.3 ± 0.2^{k}	17.5 ± 0.2^{jk}	4.4 ± 1.1^{k}
3.0	12.5 ± 1.2^{lm}	3.1 ± 0.5^{kl}	16.4 ± 0.1^{kl}	4.3 ± 0.3^{kl}
TTDZ				
0.1	31.7 ± 0.3^{cd}	9.4 ± 0.1^{de}	31.0 ± 0.2^{b}	14.2 ± 0.5^{bc}
0.2	33.7 ± 1.3^{b}	10.2 ± 0.3^{bc}	34.1 ± 0.4^{b}	14.6 ± 0.4^{b}
0.3	35.4 ± 1.0^{a}	11.4 ± 0.4^{a}	37.2 ± 0.5^{a}	15.7 ± 0.1^{a}
0.4	33.2 ± 0.5^{bc}	10.4 ± 0.2^{b}	26.3 ± 1.4^{fg}	13.5 ± 1.3^{c}
0.5	32.8 ± 0.2^{c}	9.3 ± 0.3^{de}	22.4 ± 1.5^{h}	12.1 ± 1.5^{d}

Number of explants tested = 30. Values are means □ SE of 5 replication of 3 repeated experiments. Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table 2.2 Influence of different auxins on multiple shoot proliferation from Shoot tip and Node explant cultured on MS medium supplemented with TDZ (0.3 mg/l)

Concentrations Cytokinins (mg/l)	Shoot tip explant		Node explant	
	Percentage of response	Mean No. of shoots / explant	Percentage of response	Mean No. of shoots / explant
NAA				
0.25	20.1 ± 0.5^{a}	3.4 ± 1.1^{i}	26.5 ± 1.3^{h}	5.2 ± 0.4^{e}
0.50	22.4 ± 0.3^{g}	4.2 ± 1.3^{e}	27.9 ± 1.2^{fg}	5.3 ± 0.2^{e}
0.75	24.5 ± 0.1^{de}	4.8 ± 1.7^{c}	28.4 ± 0.1^{f}	5.5 ± 1.2^{de}
1.00	26.4 ± 1.5^{bc}	5.0 ± 0.4 ^{b}	29.7 ± 0.3^{e}	5.6 ± 0.3^{d}
1.00	26.4 ± 1.5^{bc}	5.0 ± 0.4 ^{b}	29.7 ± 0.3^{e}	5.6 ± 0.3^{d}
1.50	19.7 ± 1.5^{j}k	4.1 ± 0.2 ^{f}	21.5 ± 0.3 ^{l}	5.1 ± 0.2^{ef}
NIAA				
0.25	22.2 ± 1.6^{gh}	4.8 ± 0.1^{c}	27.8 ± 0.2^{g}	5.8 ± 0.2^{c}
0.50	25.1 ± 0.7^{d}	5.0 ± 0.4^{b}	30,2 ± 0.5^{de}	6.1 ± 0.5^{ab}
0.75	30.3 ± 0.4^{a}	5.2 ± 0.5^{a}	35.2 ± 0.4^{d}	6.2 ± 0.6^{a}
1.00	26.1 ± 1.4^{bc}	4.6 ± 0.5^{d}	28.4 ± 1.6^{f}	5.5 ± 1.7^{de}
1.25	22.3 ± 0.4^{gh}	3.9 ± 0.4^{g}	26.4 ± 1.3^{hi}	5.1 ± 1.3^{ef}
1.50	20.4 ± 1.2^{ij}	3.7 ± 0.2^{g}	24.5 ± 1.2^{j}	5.0 ± 1.5^{ef}
IBA				
0.25	18.7 ± 1.3k^{l}	3.3 ± 1.3^{i}	32.5 ±0.4^{de}	4.4 ± 1.1^{g}
0.50	19.1 ± 1.4k	3.7 ± 1.2^{g}	34.5 ± 0.3^{c}	4.6 ± 1.6^{g}
0.75	19.3 ± 0.3^{j}k	4.4 ± 0.4^{e}	35.2 ± 1.4^{bc}	4.8 ± 0.5^{g}
1.00	20.6 ± 0.5^{i}	4.6 ± 0.5^{d}	35.9 ± 1.5^{ab}	5.2 ± 0.3^{e}
1.25	17.3 ± 1.2m	4.2 ± 0.5^{e}	36.5 ± 1.7^{a}	4.0 ± 1.8^{h}
1.50	16.5 ± 1.1n	3.5 ± 1.6^{h}	35.4 ± 1.4^{b}	3.7 ± 1.4^{i}

Number of explants tested = 30. Values are means ± SE of 5 replication of 3 repeated experiments. Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table 2.3 Influence of different amino acids in the multiplication of Shoot tip and Node explant cultured on MS medium supplemented with TDZ (0.3 mg/l)

Concentrations of amino acids (mg/l)	Shoot tip explant		Node explant	
	Percentage of response	Number of shoots / explant	Percentage of response	Number of shoots / explant
Alanine				
10	28.3 ± 0.2^{f}	17.5 ± 0.4^{h}	43.5 ± 0.3^{g}	17.6 ± 1.2^{l}
20	33.7 ± 1.3^{e}	17.8 ± 1.2^{fg}	45.4 ± 0.2^{ef}	17.8 ± 0.5^{kl}
30	34.3 ± 1.4^{d}	18.4 ± 1.5^{de}	46.3 ± 1.2^{e}	20.7 ± 1.4^{e}
40	21.5 ± 1.7^{k}	16.7 ± 1.2^{ij}	42.4 ± 1.5^{gh}	18.5 ± 1.6^{j}
50	19.7 ± 1.2^{kl}	16.3 ± 0.9^{j}	41.5 ± 1.6^{h}	18.1 ± 0.5^{k}
Glutamine				
5	34.1 ± 1.6^{de}	18.0 ± 0.5^{f}	50.6 ± 1.6^{d}	21.7 ± 0.4^{d}
10	36.3 ± 1.2^{bc}	18.6 ± 0.3^{d}	51.2 ± 1.3^{cd}	22.7 ± 0.3^{d}
15	38.2 ± 0.7^{b}	19.4 ± 0.4^{bc}	53.6 ± 1.6^{bc}	24.8 ± 0.5^{b}
20	41.0 ± 1.6^{a}	20.4 ± 0.5^{a}	55.2 ± 1.6^{a}	25.8 ± 0.3^{a}
25	37.5 ± 1.3^{bc}	19.6 ± 1.4^{b}	53.7 ± 1.3^{b}	23.4 ± 1.4^{c}
30	35.2 ± 1.5^{cd}	19.4 ± 0.5^{bc}	52.5 ± 1.6^{c}	20.4 ± 1.5^{e}
Serine				
10	10.5 ± 0.5^{n}	15.0 ± 0.3^{n}	26.4 ± 1.6^{n}	18.1 ± 0.4^{k}
20	12.9 ± 0.4^{m}	15.6 ± 0.4^{lm}	28.4 ± 0.4^{m}	18.5 ± 1.3^{j}
30	21.7 ± 0.6^{j}	15.9 ± 1.2^{kl}	30.3 ± 0.5^{l}	18.9 ± 1.5^{h}
40	10.3 ± 0.3^{no}	14.6 ± 1.3^{n}	25.3 ± 0.5^{op}	17.5 ± 1.5^{lm}
50	8.5 ± 0.6^{p}	14.0 ± 0.8^{n}	24.5 ± 0.2^{p}	16.1 ± 1.3^{n}
Proline				
10	25.3 ± 1.1^{hi}	16.0 ± 1.4^{k}	30.1 ± 0.5^{lm}	19.2 ± 0.5^{gh}
20	27.4 ± 2.5^{fg}	16.8 ± 1.2^{i}	38.3 ± 0.6^{i}	19.5 ± 0.3^{ef}
30	25.4 ± 2.6^{h}	16.5 ± 0.5^{j}	32.3 ± 0.5^{k}	19.4 ± 0.1^{g}
40	21.2 ± 2.5^{j}	15.8 ± 0.7^{l}	36.3 ± 1.2^{ij}	18.5 ± 0.4^{j}
50	19.0 ± 2.4^{jk}	15.4 ± 0.2^{lm}	33.5 ± 1.4^{j}	18.2 ± 0.1^{jk}

Number of explants tested = 30. Values are means ± SE of 5 replication of 3 repeated experiments. Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table 2.4 Effect of different concentrations of carbohydrates on multiplication of Shoot tip and Node explant cultured on MS medium supplemented with TDZ (0.3 mg/l) and glutamine (20 mg/l)

Concentrations Cytokinins (mg/l)	Shoot tip explant		Node explant	
	Percentage of response	Mean No. of shoots / explant	Percentage of response	Mean No. of shoots / explant
Sucrose				
10	82.4 ± 0.3^{c}	16.5 ± 0.4^{d}	80.3 ± 0.9^{d}	24.4 ± 0.5^{bc}
20	85.4 ± 0.5^{b}	18.2 ± 1.2^{b}	85.8 ± 0.5^{b}	25.1 ± 0.6^{b}
30	90.2 ± 0.3^{a}	20.4 ± 0.5^{a}	95.2 ± 1.1^{a}	25.8 ± 0.3^{a}
40	81.3 ± 1.3^{cd}	16.2 ± 1.0^{de}	79.5 ± 0.4^{de}	22.4 ± 1.0^{e}
50	77.4 ± 1.5^{e}	14.2 ± 1.4^{g}	75.5 ± 0.2^{fg}	21.1 ± 1.3^{g}
Maltose				
10	65.7 ± 1.6^{i}	14.2 ± 1.4^{g}	55.5 ± 0.4^{kl}	16.3 ± 1.7^{ij}
20	69.6 ± 1.5^{h}	15.2 ± 1.6^{fg}	69.5 ± 0.5^{h}	17.4 ± 1.5^{hi}
30	70.4 ± 1.5^{gh}	15.4 ± 0.4^{f}	76.5 ± 0.5^{f}	18.0 ± 1.2^{h}
40	65.7 ± 0.4^{i}	12.5 ± 0.5^{j}	52.5 ± 1.2^{lm}	16.7 ± 0.3^{i}
50	62.6 ± 0.4^{j}	11.0 ± 0.3^{k}	48.5 ± 1.1^{n}	15.3 ± 0.6^{j}
Fructose				
10	40.7 ± 1.2^{lm}	6.9 ± 1.4o	45.5 ± 0.4o	12.2 ± 0.5^{lm}
20	42.1 ± 1.4^{l}	9.5 ± 1.2^{m}	52.5 ± 0.3^{lm}	14.8 ± 0.4^{jk}
30	45.4 ± 0.4^{k}	10.5 ± 1.7^{kl}	54.5 ± 0.5^{l}	15.9 ± 0.9^{ij}
40	35.3 ± 1.5^{n}	8.0 ± 1.5^{m}	35.2 ± 0.6op	12.4 ± 0.5^{l}
50	32.6 ± 1.4^{nm}	7.5 ± 0.5^{mn}	24.5 ± 0.6p	10.1 ± 0.6^{n}
Glucose				
10	73.1 ± 0.5^{f}	13.5 ± 1.3^{h}	57.5 ± 0.6^{k}	22.2 ± 0.5^{ef}
20	77.4 ± 0.3^{e}	16.5 ± 1.6^{d}	65.5 ± 0.2^{i}	22.1 ± 1.2^{f}
30	80.6 ± 0.5^{d}	17.2 ± 0.5^{c}	85.5 ± 1.2^{bc}	23.2 ± 1.4^{d}
40	72.5 ± 0.7^{fg}	16.0 ± 0.3^{e}	60.5 ± 1.3^{j}	21.5 ± 1.6^{fg}
50	71.3 ± 1.0^{g}	13.2 ± 1.1^{hi}	51.2 ± 1.6^{m}	21.1 ± 0.5^{g}

Number of explants tested = 30. Values are means ± SE of 5 replication of 3 repeated experiments. Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table 2.5 Effect of different concentrations of Pluronic F-68 on multiplication of Shoot tip and Node explant cultured on MS medium supplemented TDZ (0.3 mg/l), glutamine (20 mg/l) and Sucrose (30 g/l)

Concentrations of Pluronic F-68 (mg/l)	Shoot tip explant		Node explant	
	Percentage of response	Number of shoots / explant	Percentage of response	Number of shoots / explant
0.1	65.8 ± 0.9^{f}	21.1 ± 0.6^{g}	74.5 ± 0.3^{hi}	23.9 ± 1.5^{h}
0.2	67.6 ± 0.5^{f}	22.5 ± 0.4^{fg}	75.4 ± 0.5^{h}	24.7 ± 1.5^{gh}
0.3	69.4 ± 0.4^{e}	23.5 ± 0.6^{f}	77.5 ± 1.3^{ef}	25.4 ± 1.4^{g}
0.4	71.5 ± 0.7^{cd}	25.4 ± 0.5^{de}	80.3 ± 0.5^{c}	39.7 ± 0.6^{d}
0.5	72.4 ± 1.6^{bc}	32.5 ± 0.3^{b}	81.5 ± 1.3^{b}	48.2 ± 1.5^{b}
0.6	75.4 ± 1.2^{a}	39.4 ± 1.0^{a}	85.5 ± 1.6^{a}	52.7 ± 0.6^{a}
0.7	73.3 ± 0.5^{b}	30.0 ± 1.3^{c}	80.0 ± 1.6^{cd}	42.5 ± 1.2^{c}
0.8	72.4 ± 0.6^{bc}	27.3 ± 1.2^{c}	78.4 ± 0.5^{e}	31.4 ± 1.5^{e}
0.9	72.0 ± 0.6^{c}	27.0 ± 0.6^{cd}	77.3 ± 1.5^{f}	31.0 ± 0.5^{ef}
1.0	71.4 ± 0.4^{d}	26.5 ± 1.3^{d}	76.8 ± 1.7^{fg}	30.6 ± 1.5^{ef}

Number of explants tested = 30. Values are means ± SE of 5 replication of 3 repeated experiments. Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table 2.6 Effect of different types of media on induction of multiple shoots from Shoot tip and Node explant cultured on MS medium supplemented with TDZ (0.3 mg/l), glutamine (20 mg/l), Sucrose (30 g/l) and PF - 68 (0.6 mg/l)

Types of media	Shoot tip explant		Node explant	
	Percentage of response	Number of Shoots/explant	Percentage of response	Number of shoots/explant
MS	75.4 ± 0.4^{a}	39.4 ± 1.5^{a}	85.5 ± 1.5^{a}	52.7 ± 0.5^{a}
MS	50.4 ± 1.2^{b}	18.5 ± 0.5^{b}	65.5 ± 0.6^{b}	32.5 ± 0.6^{b}
WPM	21.5 ± 0.4^{c}	7.6 ± 1.5^{c}	36.5 ± 1.4^{c}	17.5 ± 1.4^{c}
B5	9.5 ± 1.4^{d}	2.4 ± 1.6^{d}	12.5 ± 0.5^{d}	5.5 ± 1.5^{d}

Number of explants tested = 30. Values are means ± SE of 5 replication of 3 repeated experiments. Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

Table 2.7 Effect of GA_3 in combination with BAP, KIN, TDZ and Pluronic F-68 on elongation of shoots in snake gourd (*Tricosanthes cucumerina* L.)

Concentrations of Plant growth regulators (mg/l)	Percentage of response	Shoot length explants (cm)
BA + GA_3		
1.5 + 0.1	43.6 ± 0.5^{no}	4.3 ± 0.5^{gh}
1.5 + 0.2	45.7 ± 0.4^{m}	4.4 ± 1.2^{gh}
1.5 + 0.3	44.8 ± 1.2^{mn}	4.1 ± 1.5^{i}
1.5 + 0.4	43.7 ± 1.5^{n}	3.8 ± 1.6^{j}
1.5 + 0.5	42.8 ± 0.5^{no}	3.6 ± 0.6^{k}
KN + GA_3		
1.5 + 0.1	57.3 ± 0.5^{gh}	4.6 ± 0.4^{e}
1.5 + 0.2	58.3 ± 0.4^{g}	4.8 ± 0.3^{cd}
1.5 + 0.3	60.3 ± 1.2^{ef}	5.0 ± 1.2^{c}
1.5 + 0.4	57.3 ± 1.1^{gh}	4.8 ± 1.7^{cd}
1.5 + 0.5	55.3 ± 1.6^{h}	4.4 ± 1.5^{e}
TDZ + GA_3		
0.3 + 0.1	51.6 ± 1.5^{ij}	4.1 ± 0.5^{ef}
0.3 + 0.2	52.4 ± 0.9^{j}	4.3 ± 0.3^{e}
0.3 + 0.3	50.5 ± 0.5^{j}	4.8 ± 0.2^{g}
0.3 + 0.4	47.5 ± 0.3^{k}	4.7 ± 0.3^{g}
0.3 + 0.5	47.2 ± 0.2^{kl}	3.6 ± 1.1^{g}
PF - 68 + GA_3		
0.6 + 0.1	61.5 ± 0.5^{e}	5.0 ± 1.7^{c}
0.6 + 0.2	63.2 ± 0.2^{d}	5.3 ± 0.5^{b}
0.6 + 0.3	67.4 ± 1.4^{a}	5.5 ± 0.3^{a}
0.6 + 0.4	65.4 ± 0.4^{b}	4.9 ± 0.1^{cd}
0.6 + 0.5	65.1 ± 0.5^{bc}	4.8 ± 0.3^{cd}

Number of explants tested = 30. Values are means ± SE of 5 replication of 3 repeated experiments. Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

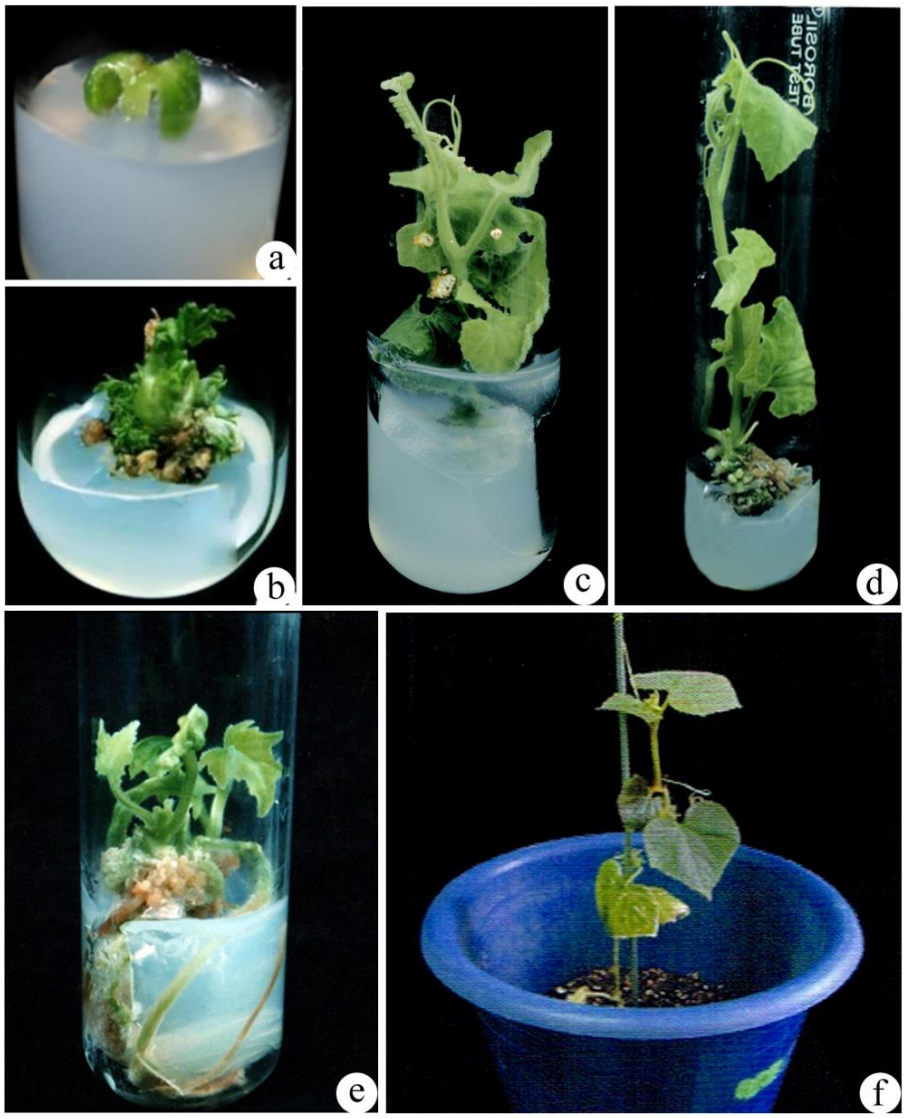
Table 2.8 Effect of different concentrations of auxins on root induction of elongated shoots obtained from shoot tip and Node explants cultured on the MS medium

Concentrations of auxins (mg/l)	Number of roots / shoot	Percentage of response	Root length (cm)
IBA			
0.5	2.6 ± 0.4^{i}	31.2 ± 0.4^{j}	3.0 ± 0.4^{g}
1.0	3.2 ± 1.5^{i}	42.4 ± 1.2^{h}	3.2 ± 0.5^{f}
1.5	4.0 ± 1.6^{g}	50.5 ± 0.3^{f}	3.5 ± 0.3^{e}
2.0	3.5 ± 0.5^{i}	44.5 ± 0.3^{g}	3.2 ± 1.2^{f}
2.5	2.9 ± 0.3^{i}	44.0 ± 0.5^{gh}	2.8 ± 0.5^{g}
3.0	2.0 ± 0.2^{i}	41.0 ± 1.2^{hi}	2.4 ± 0.3^{g}
IAA			
0.5	-	-	-
1.0	-	-	-
1.5	-	-	-
2.0	2.5 ± 1.6^{ij}	26.4 ± 0.3^{k}	1.8 ± 0.4^{i}
2.5	1.8 ± 1.5^{kl}	19.5 ± 0.5^{l}	1.0 ± 1.2^{j}
3.0	-	-	-
$AgNO_3$			
0.2	4.4 ± 1.7^{f}	57.6 ± 0.5^{e}	5.3 ± 0.5^{b}
0.4	4.7 ± 1.4^{de}	59.4 ± 1.7^{cd}	5.4 ± 0.1^{ab}
0.6	4.9 ± 0.5^{d}	61.2 ± 1.0^{c}	5.5 ± 0.5^{ab}
0.8	4.0 ± 0.3^{g}	58.4 ± 1.3^{d}	5.1 ± 1.2^{c}
1.0	3.8 ± 1.6^{gh}	57.7 ± 1.2^{de}	5.0 ± 0.5^{cd}
IBA + $AgNO_3$			
1.5 + 0.2	5.8 ± 0.5^{ab}	74.1 ± 0.5^{b}	5.6 ± 1.2^{ab}
1.5 + 0.4	6.0 ± 0.2^{ab}	74.3 ± 1.2^{ab}	5.7 ± 1.3^{ab}
1.5 + 0.6	6.2 ± 1.5^{a}	75.6 ± 0.5^{a}	5.8 ± 1.5^{a}
1.5 + 0.8	5.4 ± 1.1^{b}	73.2 ± 1.2^{bc}	5.4 ± 0.4^{ab}
1.5 + 1.0	5.2 ± 0.5^{bc}	72.2 ± 0.5^{c}	5.0 ± 1.1^{cd}

Number of explants tested = 30. Values are means ± SE of 5 replication of 3 repeated experiments. Means within a column followed by the same letters are not significantly different according to Duncan's Multiple Range Test (DMRT) at 5 % level.

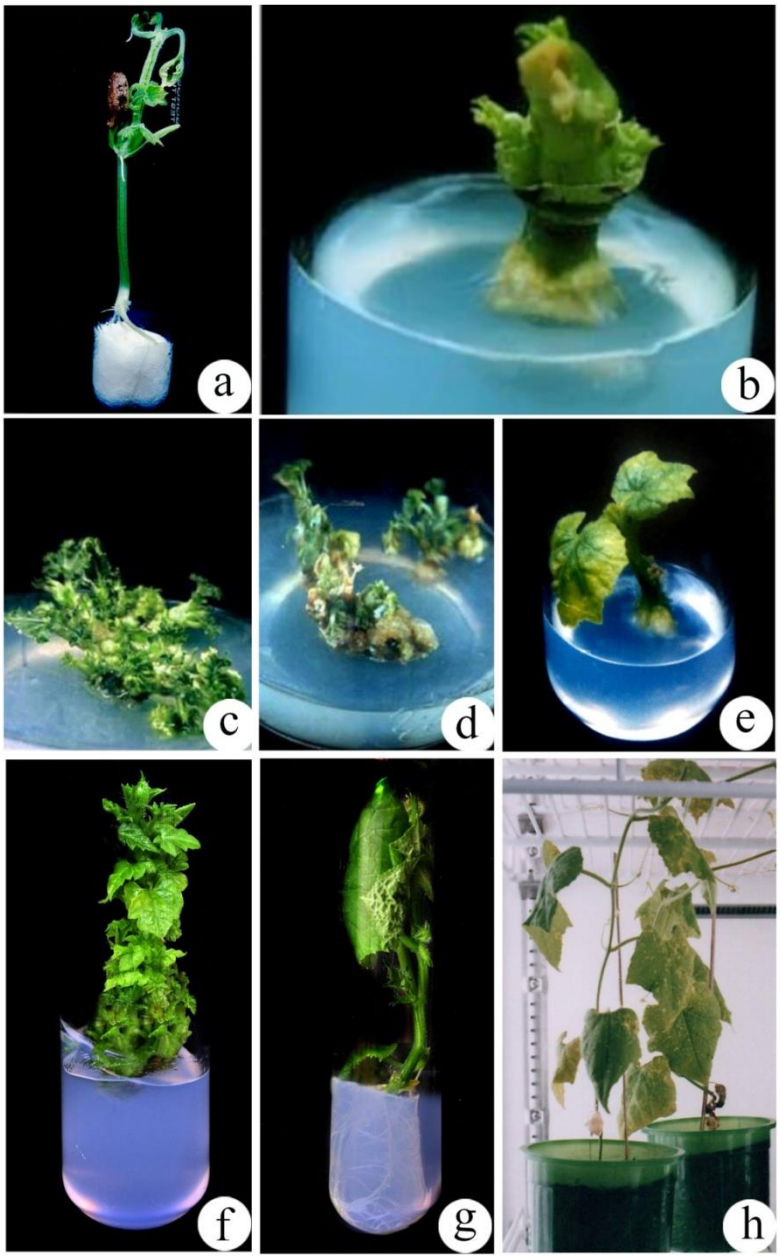
Plate - 2

Micropropagation of *Trichosanthes cucumerina* L. using shoot tip explants



a. Shoot tip
b. Shoot bud regeneration
c. Multiple shoot initiation
d. Shoot elongation
e. Shoot elongation and root initiation
f. Hardening of in vitro derived plants

Plate 3
Micropropagation of *Trichosanthes cucumerina L.* using nodal explant



a). *In vitro* plant
b). Nodal explant
c & d). Multiple shoot formation
e & f). Shoot elongation
g). Rooting
h). Hardening plant in growth chamber