STANDARDISATION OF MICROPROPAGATION TECHNIQUE IN IVYGOURD (*Coccinia grandis* (L.) Voigt.) VARIETY SULABHA

by

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(2017-12-021)

THESIS

Submitted in partial fulfillment of the requirements for the degree of

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DEPARTMENT OF VEGETABLE SCIENCE
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I, hereby declare that this thesis entitled **"STANDARDISATION OF MICROPROPAGATION TECHNIQUE IN IVYGOURD (*Coccinia grandis* (l.) Voigt.) VARIETY SULABHA"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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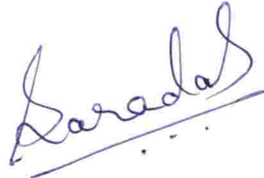

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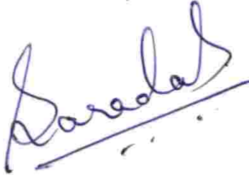

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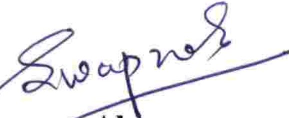

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LIST OF ABBREVIATIONS

Abbreviations	**Full form**
2,4- D	2,4 Dichlorophenoxyacetic acid
et al.	and others
BA	6- benzyl adenine
cm	Centimetre
˚C	Degree Celsius
EDTA	Ethylene diamine tetra acetic acid
Fig.	Figure
g	Gram
$HgCl_2$	Mercuric chloride
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kn	Kinetin
Mg	Microgram
µL	Microlitre
µM	Micromolar
Mg	Milligram
Min	Minute
MS	Murashige and Skoog, 1962
viz.	Namely
NAA	Naphthaleneacetic acid
ppm	Parts per million
%	per cent
PGR	Plant growth regulator
s	Second

NaOH	Sodium hydroxide
Sp. or spp.	Species (Singular and plural)
i.e.	That is

LIST OF SYMBOLS

°C	Degree celsius
%	Per cent
±	Plus or minus

Introduction

1. INTRODUCTION

Ivy gourd (*Coccinia grandis* (L.) Voigt), also known as little gourd, scarlet gourd, is an underexploited vegetable belonging to the family Cucurbitaceae. It is a perennial, dioecious creeper, widely cultivated in India and other tropical countries. The name is derived from the latin word *Coccineus*, meaning scarlet, which refers to the fruit colour. Vernacular names are kundru (hindi), kovakka (malayalam), kovakkai, kovai (tamil) and thondakayi (kannada). In India, ivygourd is grown extensively in West Bengal, Karnataka, Tamil Nadu, Maharashtra, Andhra Pradesh and Gujarat (Peter, 2007). In Kerala, total area coming under ivygourd cultivation is 1643 ha, of which the Kottayam district ranks the first (326 ha) (Farm guide, 2019).

Young and tender green fruits of ivy gourd are eaten raw in salads or cooked. It has been extensively used in Ayurveda and Unani practices in the Indian subcontinent. The leaves, stem, root and whole plant is used in the treatment of jaundice, indigestion, asthma, bronchitis, skin eruption and mainly in diabetes. Medicinally this vegetable is gaining importance among diabetic patients and is described as "Indian substitute for Insulin" (Chopra *et al.*, 1958).

The fruits are rich source of carbohydrates, proteins, vitamin A and C. Chemical composition of *C. grandis* was reported by Khatun *et al.* (2012) *i.e* carbohydrate-12.62%, total protein-15%, water soluble protein-11.25%, lipid-4.0%, total phenol-61.92 mg 100 g^{-1}, vitamin C-25.55 mg 100 g^{-1}, β-carotene-70.05 mg 100 g^{-1}, potassium- mg 100 g^{-1}, phosphorous-1.15 mg 100 g^{-1}, sodium-0.95 mg 100 g^{-1}, iron-2.23 mg 100 g^{-1} and calcium-3.79 mg 100 g^{-1}.

Bio active compounds present in the fruits of ivy gourd are well known for its antimicrobial activity (Shaheen *et al.,* 2009; Satheesh and Murugan, 2011), anticancerous activity (Bhattacharya *et al.,* 2011), antiulcerogenic and antioxidant

effects (Mazumder *et al.,* 2008) and antidiabetic and antioxidant activity (Patel and Ishnava, 2015).

Ivy gourd is propagated through vegetative methods by stem cuttings with 3 or 4 nodes and 30-40 cm length, selected from high yielding female vines, used as planting material and planted at a spacing of 4m x 3m (POP, 2016)

Kerala Agricultural University (KAU) developed a high yielding ivygourd variety Sulabha, which produces long fruits having a length of 9.25 cm and each fruit weighing about 18.48 g (Gopalakrishnan, 2007).

The main problems associated with the cultivation of ivygourd include the shortage of planting material from cuttings of mature stems and this limits the scope of commercial level cultivation of this crop. So it is urgent to establish a rapid and reproducible *in vitro* regeneration system with less cost and high frequency survival success of regenerated plants through nodal explants of *C. grandis,* as an alternative to vegetative propagation through stem cuttings. During the last few years, tissue cultue or micro propagation technique has emerged as a promising technology for rapid and large scale propagation of various plants.

Hence the present study was taken up with the following objective.

- To develop an efficient, large scale multiplication protocol for the regeneration of *C. grandis* from shoot tips/nodal explants.

Review of Literature

2. REVIEW OF LITERATURE

Ivy gourd, *Coccinia grandis* (L.) Voigt., is an underexploited vegetable belonging to the family Cucurbitaceae. Commercially ivy gourd is propagated asexually using cuttings during rainy season. Indira and Peter (1988) reported that though the main marketed vegetables in Kerala constitute only 20, about 60 vegetables are grown in smaller areas for household uses, ivygourd being one among them. Problems associated with its cultivation include the shortage of seedling material from cuttings of mature stems.

In vitro regeneration of *Coccinia* by direct shoot regeneration from hypocotyl segments, shoot tip and nodal segments and indirect regeneration from node and leaf explants had been reported by different workers. Bhatt *et al.* (2009) attempted commercial cultivation of micro propagated *Coccinia indica*. A protocol was standardized for micropropagation using modified MS medium fortified with different concentrations of Kn and IAA. After hardening, field planting was done to observe the flowering, uniformity of fruit size and yield. The plants produced by micropropagation outperformed the conventionally propagated plants by a factor of ten.

The present study was undertaken to establish rapid and reproducible *in vitro* regeneration system with less cost and high frequency of survival through nodal explants of *C. grandis (L.)* voigt variety Sulabha, as an alternative to vegetative propagation through stem cuttings.

In this chapter, literature on micropropagation of some cucurbitaceous vegetables has been reviewed under the following subheads:

2.1 *IN VITRO* REGENERATION

2.2 FACTORS INFLUENCING *IN VITRO* REGENERATION

2.3 *IN VITRO* ROOTING

2.4 HARDENING AND PLANTING OUT

2.1 *IN VITRO* REGENERATION

Plant tissue culture is the technique of growing plant cells, tissues, organs, seeds or other plant parts in a sterile environment on a nutrient medium. With the advent of Haberland' s hypothesis of totipotency of plant cell, there has been an enormous increase in applications of plant tissue culture. Murashige and Huang (1987) opined that rapid clonal production of superior plants or lines for commercial sale is possible through micropropagation, where demand is high and supply is low. Successful *in vitro* propagation has been achieved in several vegetable crops using tissue culture techniques (Kitto, 1997). Nowadays this technique is being commercially exploited for production of larger numbers of disease free and true to type plants in quicker time (Ponnuswami *et al.,* 2014).

According to Murashige (1977), there are different routes of *in vitro* propagation *viz.,* enhanced release of axillary buds, direct organogenesis, indirect organogenesis, direct somatic embryogenesis and indirect somatic embryogenesis.

2.1.1 Enhanced Release of Axillary Buds

The phenomenon involves the stimulation of axillary buds present at the leaf axil to develop into a new shoot by overcoming the apical dominance.

Enhanced shoot formation in cucumber by proliferation of axillary buds on MS medium fortified with cytokinin (BA) and casein hydrolysate (CH) was recorded by Ahmad and Anis (2005).

A study on *in vitro* regeneration of *Coccinia grandis* was conducted by Sarker *et al.* (2008) to develop a regeneration protocol that would enable large scale and commercial cultivation as well as conservation. Juvenile shoot tips and nodes were used as explants. Highest percentage of response (81.25%) was obtained on

MS medium fortified with BAP (1.5 mg L^{-1}). An average of 2-3 multiple shoots were formed when shoot tips and nodal explants were cultured in MS medium containing BAP (1.5 mg L^{-1}) and Kn (1 mg L^{-1}). Shoot elongation was achieved with MS medium containing BAP (1.5 mg L^{-1}) + NAA (0.5 mg L^{-1}).

Micro propagation by axillary bud proliferation is the most simple and reliable route for the large scale production of many crops, including vegetables. The plants regenerated *via* axillary shoot proliferation would be true to type as they develop from the pre-existing meristem (Nair and Reghunath, 2009).

Rapid micropropagation of *Coccinia grandis* through axillary bud proliferation from nodal segments was reported by Sundari *et al.* (2011) on MS medium supplemented with Kn, BA and TDZ in various concentrations (0.1 - 2.0 mg L^{-1}), in combination with IBA (0.1 - 2.0 mg L^{-1}) and IAA (0.1 - 2.0 mg L^{-1}). The media composition, MS + Kn 0.5 mg L^{-1} + BA 1.0 mg L^{-1} + IBA 0.3 mg L^{-1} produced higher response (80 %) with an average number of shoots of 8.3 ± 0.9.

Saha and Gosh (2014) developed a protocol for axillary shoot multiplication and *in vitro* flowering in ridge gourd (*Luffa acutangula*). Maximum number of shoots were obtained, when nodal explants were cultured on MS medium supplemented with BA 4.44 μM. Different concentrations of IAA (0.57, 1.14 μM) and NAA (0.54, 1.08 μM) in combination with the optimal concentration of BA did not play any significant role in multiple shoot induction within 30 days of culture.

Successful axillary bud proliferation in gynoecious lines of bittergourd cultured on MS medium fortified with 1 mg L^{-1} BAP (6- Benzyl amino purine) was recorded by Verma *et al.* (2014). Maximum shoot multiplication was obtained in half MS medium supplemented with 0.5 mg L^{-1} BAP.

2.1.2 Direct Organogenesis

Emergence of adventitious organ directly from the explant without an intervening callus phase is termed direct organogenesis.

Direct organogenesis from leaf and nodal explants of *Coccinia grandis* was reported by Josekutty *et al.* (1993) on MS medium supplemented with BAP 2.5 mg L^{-1}, Kn 0.5 mg L^{-1} and IBA 0.1 mg L^{-1}.

Transformation in cucumber (*Cucumis sativus*) was attempted by Ganapathi and Treves (2000) using *Agrobacterium tumefaciens via* direct organogenesis from the proximal ends of cotyledons cultured on low concentrations of BAP.

Kausar *et al.* (2013) developed an efficient direct organogenesis protocol in ash gourd (*Benincasa hispida*) from shoot tips and nodal segments cultured on MS medium supplemented with different concentrations and combinations of cytokinins for multiple shoot induction and root formation. The highest number of multiple shoots were obtained from the shoot tip explants cultured on MS medium supplemented with 1.5 mg L^{-1} BAP + 0.2 mg L^{-1} GA_3. The concentration of 1.0 mg L^{-1} IBA was found to be most effective for root initiation.

A study on *in vitro* regeneration of *Momordica cymbalaria* Fenzl., a perennial herbaceous climber belonging to the family Cucurbitaceae, revealed that shoot regeneration was obtained in 2.0 mg L^{-1} BAP through direct organogenesis using axillary buds (Devi *et al.*, 2017).

2.1.3 Indirect Organogenesis

In indirect organogenesis, callus is first produced from the explant and then organs are produced from the callus tissue or from a cell suspension produced from that callus.

Josekutty *et al.* (1993) obtained callus cultures through indirect organogenesis from leaf explants of *Coccinia indica* on MS medium supplemented with 1.5 mg L^{-1} BAP, 0.5 mg L^{-1} Kn and 0.5 mg L^{-1} naphthalene acetic acid (NAA) and maximal proliferation of callus was obtained in MS medium containing 2 mg L^{-1} BAP, 0.5 mg L^{-1} Kn and 1 mg L^{-1} NAA.

Micropropagation in *Coccinia grandis* (L.) was attempted by Chandra (1999) in order to establish callus culture and to standardise micropropagation

strategies and recorded that the highest percentage of callus induction in *Coccinia grandis* was obtained when explants were cultured on MS medium with 2,4-D (1.0 mg L^{-1}) and BAP (1.0 mg L^{-1}).

Callus induction and plant regeneration from leaf segments of watermelon was studied by Sultana *et al.* (2004) by using different concentrations and combinations of growth regulators. Highest percentage of callus induction was obtained in MS medium supplemented with 2.5 mg L^{-1} 2,4 D. Roots are developed after the transfer of this callus to MS medium containing BA 1 mg L^{-1} and NAA 0.1 mg L^{-1}.

Pal *et al.* (2006) developed an efficient plant regeneration protocol in summer squash through indirect organogenesis using hypocotyl and cotyledon derived calli. Hypocotyl explants were more responsive than cotyledon explants with respect to callus induction and subsequent plant regeneration. Callus induction rate of 86 % was obtained from hypocotyl explants cultured on MS medium containing 2.5 mg L^{-1} 2,4-D.

The maximum frequency of organogenic callus induction was observed in ivy gourd from nodal explants cultured on MS medium supplemented with 0.1 mg L^{-1} NAA and 1 mg L^{-1} BA (Thiripurasundari and Rao, 2012).

Resmi and Sreelathakumary (2015) reported the induction of organogenic callus from shoot tip explants of bittergourd in the medium supplemented with IBA 4 mg L^{-1} or NAA 2 mg L^{-1} + BA 0.5 mg L^{-1} +2,4- D 2 mg L^{-1}.

Nodal and root segments as explants was attempted for *in vitro* propagation in bittergourd (*Momordica charantia)* by Kumar *et al.* (2019). Highest percentage of callus (95 %) was produced from nodal segments in MS medium supplemented with 1 mg L^{-1} BAP and 1 mg L^{-1} BA and from root segments (85 %) in the combination of 2.5 mg L^{-1} and 0.6 mg L^{-1} NAA.

2.1.4 Somatic Embryogenesis

The process of a single cell or a group of cells initiating the developmental pathway that leads to reproducible regeneration of non-zygotic embryos capable of germinating to form complete plants is known as somatic embryogenesis.

Two ways of somatic embryogenesis exists i.e Direct somatic embryogenesis and Indirect somatic embryogenesis. In direct somatic embryogenesis, the embryo is formed directly from a cell or small group of cells, without the production of an intervening callus. In indirect somatic embryogenesis, callus is first produced from the explant and embryos are then produced from the callus tissue or from a cell suspension produced from that callus (Bhojwani and Razdan, 2004).

Jelaska (1974) observed that embryogenic callus was induced in pumpkin by culturing hypocotyl explants on MS medium containing growth factors. Hence formation of embyogenic callus in pumpkin was attributed more to the physiological capacities of the tissue than to a specific chemical substance.

The interaction between the sucrose concentration in the initiation medium and the embryo induction medium for embryogenic callus formation and somatic embryogenesis in seven cultivars of cucumber was studied by Lou and Kako (1994). Initiation medium containing high levels (6% to 12%) of sucrose concentrations significantly increased the percentage of somatic embryo formation compared to 3% sucrose. With increasing sucrose concentration in the initiation medium, the number of somatic embryos formed on the embryo induction medium also increased quadratically.

The regeneration of cucumber plants through direct somatic embryogenesis was studied by Burza and Malepszy (1995) and elaborated a procedure for the isolation and culture of protoplasts from embryogenic callus (gel-like callus -GLC) and embryogenic suspension cultures (ESC) of *Cucumis sativus* c.v. Borszczagowski. The highest percentage of direct somatic embryogenesis (80%) was observed with embryogenic suspension cultures (ESC).

Kintzios *et al.* (2002) studied the effect of pretreatment with different growth regulators 2,4 D (113.1, 226.2 and 452.4 µM) and Kinetin (46.5, 93 and 186 Mm) or a combination of both for different duration (6, 24 and 48 h) on somatic embryogenesis from leaves of squash (*Cucurbita pepo*) and melon (*Cucumis melo*). The pretreatment of squash explants with 186 µM kinetin and melon explants with 226.2 µM 2,4-D for 48 h promoted the formation of somatic embryos which developed further to the torpedo-shape stage and germinated.

2.2 FACTORS INFLUENCING *IN VITRO* REGENERATION

In vitro regeneration of plants mainly depends on different factors such as explant type, media composition, plant growth regulators and the culture conditions. A consistent and reproducible protocol looks into the appropriate use of basal media and various plant growth regulators.

2.2.1 Selection of explant

The choice of explant is very important for the success of any *in vitro* propagation system. Maintaining the mother plants in clean and controlled environmental conditions will provide healthy explants.

In vitro plant regeneration has been reported in different cucurbits from different explants *i.e.* from axillary buds in cucumber (Ahmad and Anis, 2005), *Coccinia grandis* [(Sarker *et al.,* 2008), (Sundari *et al.,* 2011)], ridge gourd (Saha and Gosh, 2014) and bittergourd (Verma *et al.,* 2014), through enhanced release of axillary buds.

Direct organogenesis from leaf and nodal explants of *Coccinia grandis* (Josekutty *et al.,* 1993), proximal ends of cotyledons of cucumber (Ganapathi and Treves, 2000), shoot tips and nodal segments of ash gourd (Kausar *et al.,* 2013) and nodal segments in bittergourd (Saha *et al.,* 2016).

Indirect organogenesis has been reported from leaf explants in ivygourd (Josekutty *et al.,* 1993) and watermelon (Sultana *et al.,* 2004), hypocotyl and

cotyledon explants of summer squash (Pal *et al.,* 2006) and nodal explants in ivygourd (Thiripurasundari and Rao, 2012) .

There are several reports of somatic embryogenesis in cucurbits *i.e* from hypocotyl explants in pumpkin (Jelaska, 1973) and from leaves of squash (*Cucurbita pepo*) and melon (*Cucumis melo*) (Kintzios *et al.,* 2002).

2.2.2 Surface Sterilization of Explant

Explants collected from the field may contain various microorganisms. To get rid off these micro organisms, surface sterilization should be done with common sterilizing agents like Sodium hypochlorite (1-4%; 15 – 30 mins), Calcium hypochlorite (0.5-1%; 20 – 30 mins), Mercuric chloride (0.05 – 0.5%; 3 - 20 mins) *etc.* followed by several rinses in sterile water.

Detergent washed explants of ivy gourd were treated with 0.2% mercuric chloride solution for 1.5 to 2 min and rinsed many times with sterile distilled water for surface sterilization (Josekutty *et al.,* 1993).

Sundari *et al.* (2011) reported that nodal segments of *Coccinia grandis* were washed under continuous tap water for 30 min and then treated with few drops of Teepol (detergent solution) for 5 min. After that the explants were disinfected with freshly prepared 70% alcohol for 30 sec followed by treating with surface sterilants such as sodium hypochlorite (3%) for 3 min and with 0.1% mercuric chloride for 3 min in Laminar air flow chamber and the explants were washed thrice with sterile distilled water after every treatment.

In node culture technique of *Cucumis sativus* by Mahmoud and Arash (2014), sterile distilled water washed seeds were soaked in fungicide solution of 0.5% Captan for about one hour. The treated seeds were disinfected with 0.1% mercuric chloride solution for 40 minutes, immersed in 70% ethanol for 1 minute and then soaked in 5% sodium hypochlorite with 1 drop tween-20 for 20 minutes.

Saha *et al.* (2016) gave pre-treatments before *in vitro* inoculation of nodal explants of bitter gourd with carbendazim (0.2%) + 8 HQC (200 mg/l + mancozeb-

45 (0.2%) for 1 h followed by three rinses with sterile double-distilled water. The pretreated explants were surface-sterilized with mercuric chloride (0.1%) for 3.0 and 3.5 min. and were rinsed 4 to 5 times with double-distilled sterile water.

2.2.3 Culture Media and Plant Growth Regulators

TC media provides nutrients for growth of the plant and hormones in the media directs development and morphogenesis. Common media used for plant tissue culture includes, White's medium (White, 1943), MS medium (Murashige and Skoog, 1962), B5 medium (Gamborg *et al.,* 1968), Woody plant basal medium (WPM) (Lloyd and Mc Cown, 1980) *etc*. MS medium is the most commonly used plant tissue culture media.

A plant growth regulator is an organic compound, either natural or synthetic, that modifies or control one or more specific physiological processes within a plant. It will function as chemical messengers for intercellular communication. The success of any tissue culture system is mainly dependent on type and concentration of plant growth regulators added in culture medium (Bhojwani and Razdan, 2004).

The type and concentration of mineral nutrients supplied in various media have a profound influence on tissue proliferation and morphogenetic responses (Gantait *et al.,* 2016).

Gulati (1988) attempted tissue culture of *Coccinia grandis* by placing the shoot tip, hypocotyl, internode, leaf and nodal explants on MS medium supplemented with various concentrations of IAA, IBA, 2,4 D and kinetin (10^{-7} M to 10^{-5} M).

Shoot tip explants from aseptically germinated watermelon seedlings were incubated on MS medium containing different concentrations of BA (0, 1, 5 and 10 μM), Kinetin (0, 1, 5 and 10 μM) and thidiazuron (0, 0.1, 1 and 5 μM) by Compton *et al.* (1992) and obtained 60-100 % rooted shoots and the percentage of acclimatized plants ranged from 21-96 % depending upon the genotype and length of time in culture.

Josekutty *et al.* (1993) obtained direct and in direct organogenesis from leaf and nodal explants of coccinia by culturing them on MS medium supplemented with BAP, Kinetin and IBA. Callus was obtained from leaf explants on MS medium supplemented with 1.5 mg L^{-1} BAP, 0.5 mg L^{-1} Kn and 0.5 mg L^{-1} naphthalene acetic acid (NAA), while 2 mg L^{-1} BAP, 0.5 mg L^{-1} Kn and 1 mg L^{-1} NAA was required to obtain maximal proliferation of callus when cultured on MS medium.

In a study on *in vitro* mass propagation of *Cucumis sativus L.* from nodal segments, Ahmad and Anis (2005) reported that MS medium containing 1.0 μM 6-benzyladenine (BA) and 200 mg L^{-1}casein hydrolysate induced higher shoot regeneration.

In vitro plant production in bittergourd (*Momordica charantia* L.) was attempted by Huda and Sikdar (2006) by culturing the apical meristem on MS medium supplemented with 0.05 mg L^{-1} Kn + 0.1 mg L^{-1} GA_3. After three weeks, the meristems were transferred to MS medium supplemented with BA, Kn, IBA, NAA and IAA individually or in combination for shoot elongation and root initiation. Shoot initiation and elongation was best in the combination of 1.0 mg L^{-1} BA + 0.1 mg L^{-1} IBA + 0.3 mg L^{-1} GA_3 and good rooting was observed in 0.5 mg L^{-1} IBA and 0.1 mg L^{-1} NAA.

Saha and Kazumi (2007) developed an efficient and novel method of micropropagation of bottle Gourd (*Lagenaria siceraria*). The seven days old cotyledonary explants were cultured on MS basal medium containing 2 mg L^{-1} BA and obtained highest number of shoots (7 shoots/explant). Elongated shoots were successfully rooted on half strength MS medium supplemented with 0.1 mg L^{-1} IAA for 2-3 weeks.

Mahzabin *et al.* (2008) attempted micropropagation of *Cucurbita maxima* duch. through shoot tip culture and reported that the best media composition for shoot induction was MS + BA 3.0 mg L^{-1}. Half MS medium supplemented with 1.0 mg L^{-1} IBA was found to be the best for rooting.

Nodal segments of *Coccinia grandis* were cultured on MS medium supplemented with Kn, BA, TDZ individually and in combination for shoot bud proliferation and multiplication. The proliferated shoots were transferred to the

shoot multiplication medium containing different concentrations of IAA, IBA (0.1 - 2.0 mg L^{-1} and BA (1.0 mg L^{-1}) + Kn (0.5 mg L^{-1}). Among various combinations higher percentage of response (80%) was obtained with an average number of 8.3 ± 0.9 shoots on MS + 0.5 mg L^{-1} Kn + 1.0 mg L^{-1} BA + 0.3 mg L^{-1} IBA (Sundari *et al.,* 2011).

Direct multiple shoot proliferation of muskmelon (*Cucumis melo* (L.) from shoot tip explant was studied by Venkateshwarlu (2012). MS + IAA (0.5 mg L^{-1})+BAP (2.0 mg L^{-1}) was found to be the best medium for multiple shoot induction. All regenerated plantlets were rooted on MS +1.0 mg L^{-1} IAA and the regenerated plants grew normally in the green house conditions.

Thiripurasundari and Rao (2012) reported an efficient protocol for indirect shoot organogenesis and plantlet regeneration of *Coccinia grandis* by culturing the nodal explants on MS medium supplemented with different concentrations and combinations of IAA, IBA, NAA (0.1 – 2.0 mg L^{-1}) and BA (1 mg L^{-1}). The maximum frequency of organogenic callus induction was observed in MS medium supplemented with NAA (0.1 mg L^{-1}) and BA (1 mg L^{-1}), while the highest shoot multiplication with increased number of shoots and shoot mean length was achieved on MS medium with NAA (0.1 mg L^{-1}) BA(1 mg L^{-1}) and Kn (0.5 mg L^{-1}).

MS medium supplemented with 2 mg L^{-1} 6-Benzylaminopurine (BAP) + 2 mg L^{-1} L- Glutamic acid was the effective medium for multiple shoot induction of spine gourd (Mustafa *et al.,* 2013).

Regeneration of plantlets from hypocotyl and cotyledonary explants of tetraploid watermelon was attempted by Rasheed *et al.* (2013). Maximum callus induction was achieved on MS basal media supplemented with BAP 5 mg L^{-1} from cotyledon (76.66%) and from hypocotyls explants (73.33%). Highest shoot induction was obtained from cotyledon explant *i.e* 96.66% and 76.66% from hypocotyl explants on shoot regeneration medium MS + 1.0 mg L^{-1} BAP + 0.2 mg L^{-1} NAA.

Rapid micropropagation of *Cucumis sativus* var. Dastgerdi (Iranian cultivar) by node culture technique was established by Mahmoud and Arash (2014)

in MS medium containing various concentrations of Kn (0, 0.5, 1 and 1.5 mg L^{-1}) in combination with IBA (0, 0.025 and 0.5 mg L^{-1}).

Verma *et al.* (2014) developed a protocol for micro propagation of gynoecious bitter gourd (*Momordica charantia*). The axillary bud and apical bud of identified gynoecious lines of bitter gourd were cultured on MS medium for initiation. Maximum shoot length was obtained in half MS medium supplemented with 0.5 mg ml^{-1} BAP, while rooting was best observed in half MS medium supplemented with 1.0 mg ml^{-1} IBA.

A study was conducted by Patel and Ishnava (2015) to develop an efficient protocol for *in vitro* shoot multiplication from nodal explants of *Coccinia grandis* (L.) Voigt. The nodal segments were cultured in MS medium with different concentrations of BAP, Kn, NAA and NB_6 for shoot multiplication and callus formation. Maximum number of multiple shoots (16±1.5) were formed on MS medium containing 0.5 mg L^{-1} BAP in combination with 0.2 mg L^{-1} BA after 15 days of inoculation.

Resmi and Sreelathakumary (2015) obtained indirect organogenesis in bittergourd (*Momordica charantia* L.) using shoot tip explants by culturing them on MS medium supplemented with IBA 4 mg L^{-1} or NAA 2 mg L^{-1} +BA 0.5 mg L^{-1} + 2,4- D 2 mg L^{-1}.

Saha *et al.* (2016) introduced a novel strategy for maintenance and mass multiplication of gynoecious line in bitter gourd through micropropagation. Murashige and Skoog (MS) basal medium supplemented with 6 benzyl-aminopurine (2 mg L^{-1}) + α-Napthalene acetic acid (0.2 mg L^{-1}) was found best for *in vitro* survival of plantlets.

Bhardwaj et al. (2017) reported *in vitro* regeneration of parthenocarpic cucumber (*Cucumis sativus* L.) by growing the seeds initially in half strength MS basal medium, cotyledonary leaf explants were then transferred to eight media compositions of half strength MS medium with varying IAA and BAP concentrations in the culture test tubes.

2.2.4 Culture Conditions

Aseptic conditions should be maintained in culture rooms. Temperature, humidity and light conditions are regulated properly. For most of the crops, temperature is maintained at 25 ± 2°C. The optimum light intensity for shoot formation in a large number of herbaceous species is around 1000 lux (Murashige, 1977).

Ahmad and Anis (2005) placed the cultures of cucumber at 25 ± 2°C under 16 hrs. of photoperiod provided by cool, white fluorescent tubes with light intensity of 2000 lux.

Sundari *et al.* (2011) maintained cultures of *Coccinia grandis* at 25 ± 2ºC, under 16/8 hrs light regime provided by cool, white fluorescent lamp (60 μmol m2/s) with 55 - 60% relative humidity.

Venkateshwarlu (2012) incubated shoot tip cultures of muskmelon at 25 + 2°C under white fluorescent light of 40-60 μ mol m^{-2} s^{-1} intensity for 16 hrs light /8 hrs dark period.

Maintanence of cultures of spine gourd at 25 ± 2˚C, 2000 lux light intensity with a photo period of 16 hours was reported by Mustafa *et al.* (2013).

Rasheed *et al.* (2013) incubated cultures of watermelon at 25°C with 16 h photoperiod.

Patel and Ishnava (2015) incubated cultures of Ivy gourd in controlled conditions of diffused light (2000 lux) for 10 h daily at 28±2°C temperatures and 50-60% relative humidity.

Saha *et al.* (2016) reported that bittergourd cultures could be kept under 16/8h photoperiod, having light intensity of 47 μmol m^{-2} s^{-1} using white fluorescent lights at regulated temperature of 26 ± 1°C.

2.3 *IN VITRO* ROOTING

In vitro rooting is an important step in the processs of micro propagation. The plantlets produced *in vitro* should have a developed, strong and functional root system. Roots are generally induced in basal medium containing a suitable auxin. IAA, IBA and NAA are the commonly used auxins.

Normally in most of the cucurbits, root induction could be achieved on either basal medium alone or with a very low level of an auxin (Mythili and Thomas 1999).

Josekutty *et al.* (1993) achieved good root formation in ivygourd in hormone free half MS basal medium.

Sundari *et al.* (2011) reported 100% rooting in *Coccinia grandis*, when nodal explants were cultured on MS basal medium supplemented with 0.1 mg L^{-1} IBA.

According to Thiripurasundari and Rao (2012), in a study on indirect organogenesis of *Coccinia grandis* from nodal explants, regenerated micro shoots produced maximum number of roots (8.2 ± 0.8) with a mean length of 4.7 ± 0.3 in MS medium supplemented with IBA (0.1 mg L^{-1}).

The micro-shoots of bittergourd were rooted successfully on MS medium supplemented with GA_3 (1 mg L^{-1}) + activated charcoal (100 mg L^{-1}), when apical bud and nodal segments were taken as explants. Minimum days for rooting (10.81) were recorded for apical bud and 11.49 days for nodal segment (Saha *et al.,* 2016).

Bhardwaj *et al.* (2017) observed maximum rooting in half MS medium containing 0.25 mg L^{-1} IAA by taking minimum days for root initiation (5.17±0.90) during *in vitro* regeneration of parthenocarpic cucumber.

2.4 HARDENING AND PLANTING OUT

Plants grown in *in vitro* conditions are different from field grown plants. The success of micropropagation on a commercial scale mainly depends on the ability to transfer plants out of culture on a large scale, at low cost and with high survival rates. During field transfer, the *in vitro* grown plantlets are unable to compete with soil microbes and to adapt with the external environmental conditions. Hardening and acclimatization are important steps in micro propagation (Chandra *et al.*, 2010).

Huda and Sikdar (2006) reported that plantlets of bittergourd with well developed roots were successfully planted out in growth chamber in plastic pots, covered by polythene cover for maintaining high humidity for a week and transferred to greenhouse

Sundari *et al.* (2011) transferred the micro propagated well-rooted ivygourd plantlets to paper cups containing autoclaved red soil, sand and coconut coir (1 : 1 : 1), under controlled growth chamber conditions (25± 2°C, 16 hrs photoperiod, 75 - 80% relative humidity and 35 μmol m^2/s light intensity). The potted plants were fed with MS basal salt solutions at every 4 days intervals for two weeks and the plantlets were covered with porous polythene to maintain high humidity and after 15 days, the plants were transferred to green house conditions.

Rasheed *et al.* (2013) reported that *in vitro* rooted plantlets of watermelon were successfully transferred to small pots containing 2:1:1:1 mixture of soil, sand, leaf manure and compost.

Saha *et al.* (2016) tried two types of acclimatization strategies for micro propagated bittergourd plantlets using glass jar and plastic pot. Among the two glass jar with PP cap was found to be comparatively better than plastic pot with polythene covering for hardening with respect to survival percentage, number of leaves per plantlet, plant height etc. The *ex vitro* survival (81.25%) was maximum in glass jar with PP cap in shoot tip derived plantlets.

Materials and Methods

3. MATERIALS AND METHODS

The present study on "Standardisation of micropropagation technique in ivygourd (*Coccinia grandis* (L.) Voigt.) variety Sulabha" was carried out at the Department of Vegetable Science, College of Agriculture, Vellayani during 2017-2019. The objective of the study was to develop an efficient, large scale multiplication protocol for the regeneration of C. *grandis* from shoot tips/ nodal explants. The details of experimental materials and methodology followed in this work are presented in this chapter.

3.1 COLLECTION OF EXPLANT

Cuttings of ivy gourd variety Sulabha were collected from the Department of Vegetable Science, College of Horticulture, Vellanikkara. The cuttings were planted in the experimental field of Department of Vegetable science, College of Agriculture, Vellayani. Shoot tips and nodal segments containing an axillary bud from tender shoots were taken as explant.

3.2 CLEANING AND STERILIZATION OF GLASSWARES AND EQUIPMENTS

Glasswares (Jam bottles, beakers, petriplates etc.) were soaked in detergent solution for one day and then washed with tap water followed by distilled water. The washed glassware's were kept in hot air oven for 2 hours for drying in a controlled temperature of 100°C (Dry heat sterilisation). The dried glasswares, petriplates, forceps, blade holder etc were sterilized by autoclaving (Moist heat sterilization) at 121° C, 100 Kpa for 45 minutes in pressure cooker. Before autoclaving the petriplates, forceps and blade holder were wrapped in polypropylene bags. A horizontal laminar air flow (LAF) cabinet equipped with high efficiency particulate air (HEPA) filter of 0.2 μ was used for the *in vitro*

culture. Air coming through HEPA filter is free of bacterial and fungal contamination. The hood surface was cleaned by using 70% ethanol and sterilized by germicidal UV light for 20-30 minutes before work. After UV sterilization, LAF should be kept open for 10 min with blower on condition. Scalpel and forceps were sterilized by dipping in 100% ethyl alcohol and flaming prior to use.

3.3 PREPARATION OF STOCK SOLUTION

Stock solutions (Stock A, B, C, D, E & F) of macronutrients, micronutrients, vitamins and plant growth regulators (BA, Kinetin, IAA and IBA) were prepared and stored in refrigerated conditions. Details of stock solutions of MS is given in Appendix I. Each compound was dissolved separately in double distilled water. Components are added one at a time and dissolved and the volume made upto 100 ml in a volumetric flask.

Cytokinins and auxins were the two important plant growth regulators used in different concentrations and combinations for bud proliferation, shoot multiplication and *in vitro* rooting etc.

Stock solution of cytokinins were prepared by weighing the required quantity of BA or Kinetin and dissolving in 1 N NaOH or 1 N HCl and the volume made upto 100 ml with double distilled water.

Stock solution of auxins were prepared by weighing the required quantity of IAA or IBA and dissolving in 95% ethyl alcohol and the volume made upto 100 ml with double distilled water.

3.4 PREPARATION OF MEDIA

MS (Murashige and Skoog, 1962) was used as the basal media for the experiment. The composition of MS media is given in appendix I. For the preparation of 1 L MS media, required quantity of stock solutions were pipetted out one by one to a beaker containing 600-800 ml distilled water. Then sucrose (30 g L^{-1}), Myoinositol (100 mg L^{-1}), and MS supplement (3.55 g L^{-1}) were weighed and dissolved in it. The pH of the medium was adjusted to 5.7-5.8 by using 1 N NaOH or 1 N HCl. After adjusting the p^H, volume was made upto 1 L. Agar (6.5 - 7 g L^{-1})

was added and melted in microwave oven. Then the prepared medium was poured into clean, autoclaved culture bottles and autoclaved for 20 minutes at 15 lb pressure at 121° C. The media were allowed to cool and kept at room temperature for one week and examined for any type of contamination on it.

3.5 SURFACE STERILIZATION OF EXPLANT

Nodal segments and shoot tips from tender shoots of ivy gourd were collected in a polythene bag with a moist cotton. The explants were defoliated and washed under tap water for some time and then treated with detergent solution for 10-15 min. Later the explants were disinfected with 1% bavistin for 30 minutes for preventing fungal contamination. After every treatment, the explants were washed several times with double distilled water.

The explants were treated with 70% alcohol for 30 sec followed by surface sterilization using mercuric chloride at different concentrations for different duration. Shoot tips were treated with $HgCl_2$ 0.1 % for 3 or 2 minutes and 0.08 % for 3 minutes. Nodal explants were treated with $HgCl_2$ 0.1 % for 5 or 3 minutes. After every treatment, explants were washed 3-4 times with sterile distilled water. The exposed ends of nodal segments were again cut to remove the dead tissues due to the exposure of $HgCl_2$.

3.6 INOCULATION

3.6.1 Bud proliferation and multiple shoot induction

Sterilized nodal explants of 1-2 cm length were inoculated in basal MS medium supplemented with Kn and BA, individually and in combination for bud proliferation and multiple shoot induction (Table 1).

Each treatment was replicated thrice and number of bottles per replication was four. Observations were recorded, days for shoot bud initiation, number of shoots/ explant, shoot length (cm) and percentage of response.

Table 1. Growth regulators used for bud proliferation and multiple shoot induction

Treatments	Plant growth regulator con. (mg L^{-1})	
	BA	Kinetin
BP_1	-	0.1
BP_2	-	0.2
BP_3	-	0.3
BP_4	-	0.5
BP_5	-	1
BP_6	0.1	-
BP_7	0.2	-
BP_8	0.3	-
BP_9	0.4	-
BP_{10}	0.5	-
BP_{11}	Best of BA	Best of Kinetin

3.6.2 Shoot multiplication

Proliferated shoots from the best treatment of bud proliferation medium were transferred to the shoot multiplication medium containing the best concentration of BA and different concentrations of IAA and IBA (Table 2).

Each treatment was replicated thrice and number of bottles per replication was 4. Observations were recorded on shoot length (cm) and percentage of response (%).

3.6.3 *In vitro* rooting

The composition of the rooting media was same as that of shoot multiplication medium (MS medium supplemented with best concentration of BA and different concentrations of IAA and IBA). Each treatment was replicated thrice and number of bottles per replication was 4. Observations were taken on days for root initiation, rooting percent (%), no. of roots per shoot and root length (cm).

3.7 HARDENING AND PLANTING OUT

The rooted plantlets of 4-6 cm length, were carefully removed from the culture bottles adhering agar was washed off in sterile distilled water and transferred to small protays containing autoclaved red soil, sand and coir pith compost (1:1:1 ratio). The plantlets were covered with porous polythene cover to maintain high humidity. After 15 days the plants were transferred to small pots for further hardening under greenhouse conditions. Observations were recorded on days to emergence of new leaf and survival percent (%).

3.8 STATISTICAL ANALYSIS

Completely randomized design (CRD) (Panse and Sukhatme, 1985) was followed for statistical analysis. The data were subjected to analysis of variance (ANOVA) and significant difference between treatments were calculated.

¶

Table 2. Composition of shoot multiplication medium

Treatments	Plant growth regulator con. (mg/L)	
	IAA	IBA
SM_1	0.1	-
SM_2	0.2	-
SM_3	0.3	-
SM_4	0.5	-
SM_5	1	-
SM_6	-	0.1
SM_7	-	0.2
SM_8	-	0.3
SM_9	-	0.5
SM_{10}	-	1

Result

4. RESULTS

Investigations were carried out on "Standardisation of micropropagation technique in ivygourd (*Coccinia grandis* (L.) Voigt.) variety Sulabha" at the Department of Vegetable science, College of Agriculture, Vellayani during 2017-2019. The results of the study are presented below.

4.1 SELECTION OF EXPLANTS

Two explants *viz.* shoot tips and nodal segments, collected from tender shoots of ivygourd were used for the experiment. On inoculation in culture initiation medium (MS medium supplemented with different concentrations and combinations of Kn and BA), nodal segments responded better (Table 3), *i.e.* 50 % and 75 % when treated with 0.1 % $HgCl_2$ for 5 and 3 minutes respectively. Cultures with shoot tip as explants showed higher fungal contamination (Plate 1). Hence further study was continued with nodal segments as explant. Nodal segments were surface sterilized using $HgCl_2$ 0.1 % for 3 minutes.

4.2 BUD PROLIFERATION AND MULTIPLE SHOOT INDUCTION

The different concentrations and combinations of Kinetin (Kn) and Benzyl adenine (BA) along with the basal MS medium were tried to study their effect on bud proliferation and shoot multiplication. Surface sterilized nodal explants were inoculated in the medium for culture initiation and multiplication.

4.2.1 Effect of kinetin on bud proliferation and multiple shoot induction

Significant difference was observed among the treatments with respect to days for shoot bud initiation, no. of shoots / explant, shoot length and percentage of response. (Table 4).

Table 3. Effect of surface sterilization of explants

Sl. no	Explant	Chemical	Concentration (%)	Time	Response (%)
1	Shoot tips	$HgCl_2$	0.1 %	3	Nil
2	Shoot tips	$HgCl_2$	0.1 %	2	Nil
3	Shoot tips	$HgCl_2$	0.08 %	3	Nil
4	Nodal segments	$HgCl_2$	0.1 %	5	37.5
5	Nodal segments	$HgCl_2$	0.1 %	3	62.5

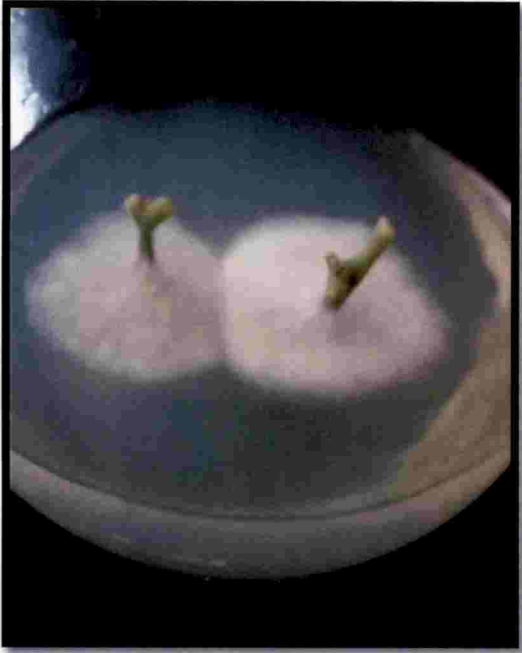

Plate 1. Fungal contamination

Table 4. Effect of Kinetin on bud proliferation and multiple shoot induction

Treatment No.	Kinetin (mg L^{-1})	Days to bud initiation	Shoots per explant	Shoot length (cm)
BP_1	0.1	17.17	0.67	0.38
BP_2	0.2	16.42	0.67	0.33
BP_3	0.3	12.33	1.33	1.04
BP_4	0.5	7.00	1.33	1.21
BP_5	1	6.75	1.58	2.25
SE m(±)		0.214	0.179	0.185
CD (0.05)		0.683	0.570	0.592

The earliest bud initiation was observed in 6.75 days in the treatment BP_5 (MS + Kn 1 mg L^{-1}) which was on par with the treatment BP_4, (MS+ Kn 0.5 mg L^{-1}) (7 days). The bud initiation was late (17.17 days) in the treatment BP_1 (MS +Kn 0.1 mg L^{-1}).

The treatment BP_5 (MS + Kn 1 mg L^{-1}) produced maximum number of shoots per explant *i.e.* 1.58, which was on par with the treatments BP_3 (MS + Kn 0.3 mg L^{-1}) and BP4 (MS + Kn 0.5 mg L^{-1}) (1.33). The least number of shoots per explant (0.67) was obtained in treatments BP_1 (MS + Kn 0.1 mg L^{-1}) and BP $_2$ (MS + Kn 0.2 mg L^{-1}).

Highest shoot length (2.25 cm) was obtained in the treatment BP_5 (MS + Kn 1 mg L^{-1}), while the lowest was observed in BP_2 (MS + Kn 0.2 mg L^{-1}) and which was on par with BP_1 (MS + Kn 0.1 mg L^{-1}).

Among the five treatments of Kinetin (BP_1 to BP_5), BP_5 (MS + Kn 1 mg L^{-1}) gave significantly higher response with respect to different observations *viz.,* Days for shoot bud initiation, Number of shoots per explant, shoot length and percentage of response (Plate 2).

4.2.2 Effect of BA on bud proliferation and multiple shoot induction

There was significant variation among the different treatments with respect to days for shoot bud initiation, no. of shoots per explant, shoot length and percentage of response (Table 5).

The earliest shoot bud initiation (5.50 days) was observed in BP_{10} (MS + BA 1 mg L^{-1}). The treatment was found to be significantly superior to all other treatments. Maximum number of days for shoot bud initiation (16.08 days) was taken by the treatment BP_6 (MS + BA 0.1 mg L^{-1}).

With respect to number of shoots per explant, maximum number of shoots (1.75) was obtained in the treatment BP_{10} (MS + BA 1mg L^{-1}) which was on par with the treatment BP_9 (MS + BA 0.5 mg L^{-1}) (1.42). Minimum number of shoots

Plate 2. Effect of kinetin (MS + Kn 1 mg L^{-1}) on bud proliferation and multiple shoot induction

Table 5. Effect of BA on bud proliferation and multiple shoot induction

Treatment No.	BA (mg L^{-1})	Days to bud initiation	Shoots per explant	Shoot length (cm)
BP_6	0.1	16.08	0.58	0.29
BP_7	0.2	15.08	0.67	0.38
BP_8	0.3	12.33	1.17	0.92
BP_9	0.5	8.50	1.42	2.08
BP_{10}	1	5.50	1.75	5.71
SE m(±)		0.129	0.149	0.169
CD (0.05)		0.412	0.476	0.539

(0.58) was observed in the treatment BP_6 (MS + BA 0.1 mg L^{-1}). It was on par with BP_7 (MS + BA 0.2 mg L^{-1}) (0.67).

Maximum shoot length (5.71 cm) was recorded by the treatment, BP_{10} (MS + BA 1 mg L^{-1}). Minimum shoot length (0.29 cm) was observed in the treatment BP_6 (MS + BA 1 mg L^{-1}), which was on par with BP_7 (MS + BA 0.2 mg L^{-1}).

Among the five different treatments containing BA (BP_6 to BP_{10}), the treatment BP_{10} (MS + BA 1 mg L^{-1}) was found to be significantly superior to other treatments with respect to days for bud initiation, number of shoots per explant and shoot length (Plate 3).

4.2.3 Effect of combination of Kinetin and BA on bud proliferation and multiple shoot induction

The best treatment of kinetin among the five different treatments for bud proliferation and multiple shoot induction was found to be BP_5 (MS + Kn 1 mg L^{-1}). The best treatment of BA among the five different treatments for bud proliferation and multiple shoot induction was found to be BP_{10} (MS + BA 1 mg L^{-1}). Combination of the above two treatments was taken as BP_{11} (MS + Kn 1 mg L^{-1} + BA 1 mg L^{-1}) (Plate 4). Percentage of response was calculated for all the treatments (Table 6). In the combination treatment BP_{11} (MS + Kn 1 mg L^{-1} + BA 1 mg L^{-1}), shoot buds were initiated within 7.08 days with 0.92 shoots per explant, shoot length of 3.04 cm and with a percentage of response of 91.67 %. Among all the eleven treatments tried, BP_{10} (MS + BA 1 mg L^{-1}) yielded significantly higher response with respect to different parameters *viz.,* days for shoot bud initiation (5.50), number of shoots per explant (1.75), shoot length (5.71 cm) and percentage of response (100.00).

4.3 SHOOT MULTIPLICATION

The multiple shoots obtained from the best treatment BP_{10} (MS + BA 1 mg L^{-1}) were transferred to shoot multiplication medium. Shoot multiplication medium used was BP_{10} (MS + BA 1 mg L^{-1}) along with different concentrations of IAA (0.1,

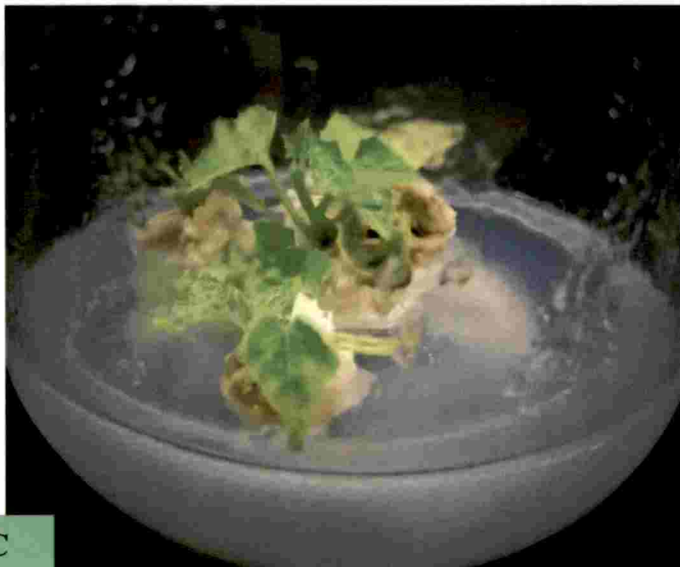

Plate 3. Effect of BA (MS + BA 1 mg L^{-1}) on bud proliferation and multiple shoot induction; A) One week after inoculation B) Two weeks after inoculation C) Three weeks after inoculation

Plate 4. Effect of combination of kn and BA on bud proliferation and multiple shoot induction (MS + Kn 1 mg L^{-1} + BA 1 mg L^{-1})

Table 6. Effect of Kinetin and BA individually and in combination on Bud proliferation and multiple shoot induction

Treatment No.	Plant growth regulators (mg L^{-1})		Days to bud initiation	Shoots per explant	Shoot length (cm)	Percentage of response (%)
	Kn	BA				
BP_1	0.1	-	17.17	0.67	0.38	33.33
BP_2	0.2	-	16.42	0.67	0.33	41.67
BP_3	0.3	-	12.33	1.33	1.04	66.67
BP_4	0.5	-	7.00	1.33	1.21	66.67
BP_5	1	-	6.75	1.58	2.25	83.33
BP_6	-	0.1	16.08	0.58	0.29	25
BP_7	-	0.2	15.08	0.67	0.38	33.33
BP_8	-	0.3	12.33	1.17	0.92	58.33
BP_9	-	0.5	8.50	1.42	2.08	83.33
BP_{10}	-	1	5.50	1.75	5.71	100
BP 11			7.08	0.92	3.04	91.67
SE m(±)			0.201	0.159	0.248	
CD (0.05)			0.593	0.469	0.731	

0.2, 0.3, 0.5 and 1 mg L^{-1}) and IBA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}). Results are presented in Table 7.

Significant difference was observed among the treatments for length of shoot. The treatments containing different concentrations of IAA did not show a good response for shoot multiplication except SM_4 (MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1}), which produced shoot having length of 4.06 cm. At high concentration of IAA *i.e.* SM_5 (MS + BA 1 mg L^{-1} + IAA 1 mg L^{-1}) basal callusing was observed, which affected further shoot multiplication (Plate 5).

Among the two sets of hormones tried, IBA showed significantly good response to shoot multiplication and elongation. Out of the five treatments containing different concentrations of IBA, three treatments produced shoots having more than 5 cm length after 3 weeks of transfer. They are SM_8 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}), SM_9 (MS + BA 1 mg L^{-1} + IBA 0.5 mg L^{-1}), SM_{10} (MS + BA 1 mg L^{-1}) with shoot length 8.67 cm, 5.83 cm, 6.88 cm respectively.

Among all the ten treatments tried for shoot multiplication, SM_8 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) was found to be the best shoot multiplication medium. It produced shoots having a length of 8.67 cm after 3 weeks of transfer from bud proliferation medium (Plate 6). The minimum length of shoot (1.08 cm) was obtained in SM_1 (MS + BA 1 mg L^{-1} + IAA 0.1 mg L^{-1}).

Percentage of response was calculated for all the treatments. All the treatments containing IAA responded less than 50 % except SM_4 (MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1}), which exhibited 50 % response. All the treatments containing IBA showed 50 % or more response except BP_6 (MS + BA 1 mg L^{-1} + IBA 0.1 mg L^{-1}), which showed a response of 41.67 %. Highest percentage of response for shoot multiplication was recorded in SM_8 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) (83.33 %).

4.4 ROOTING

Micro shoots were transferred to the best treatment for bud proliferation and multiple shoot induction *i.e.* BP_{10} (MS + BA 1 mg L^{-1}) supplemented with different

Plate 5. Basal callusing observed on the shoot multiplication medium (MS + BA 1 mg L^{-1}+ IAA 1 mg L^{-1})

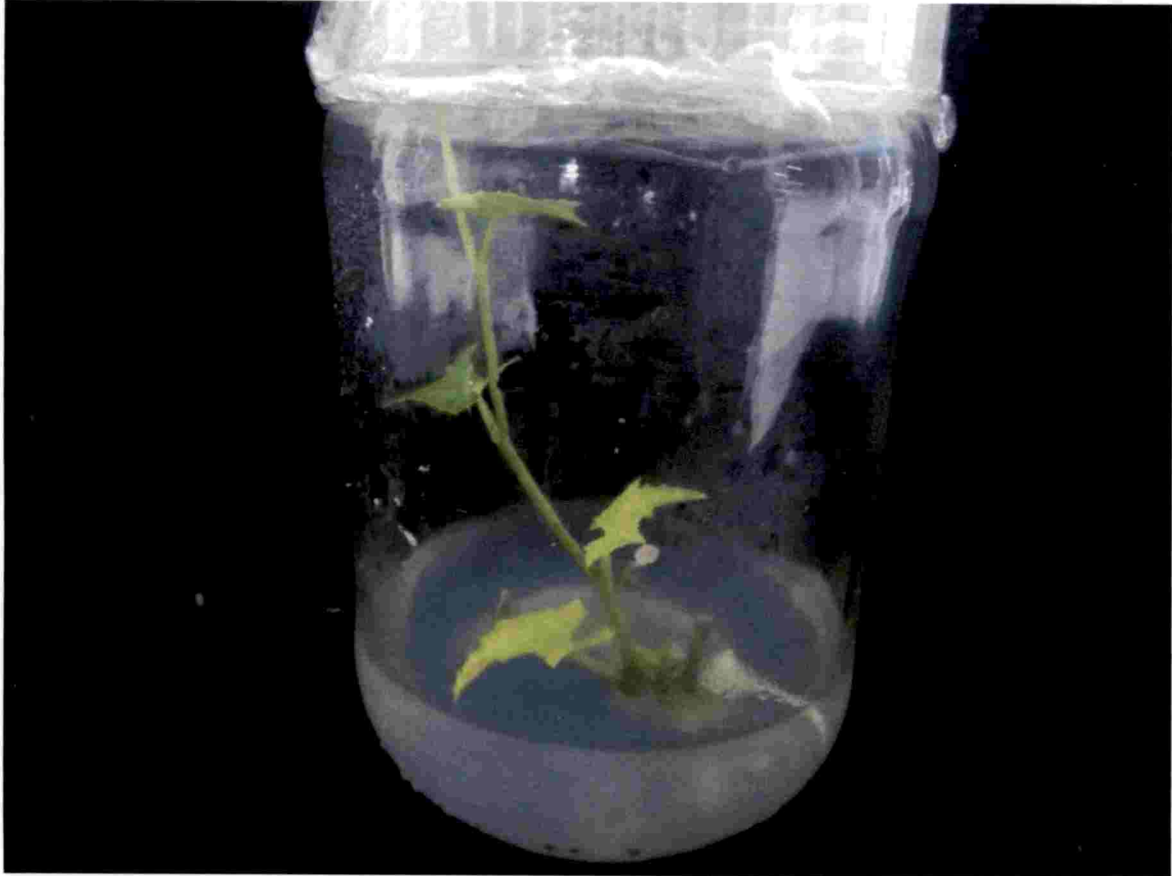

Plate 6. Effect of IBA (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) on shoot multiplication

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concentrations of IAA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) and IBA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) individually for root initiation.

The treatments exhibited significant difference for days for root initiation, no. of roots per shoot and root length and rooting percent (Table 8.).

Earliest root initiation (11.08 days) was observed in SM_7 (MS + BA 1 mg L^{-1} + IBA 0.2 mg L^{-1}). Root initiation was late (21 days) in SM_5 (MS + BA 1 mg L^{-1} + IAA 1 mg L^{-1}) which was on par with SM_4 (MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1}).

With respect to number of roots per shoot, maximum number of roots per shoot (7) was recorded in SM_7 (MS + BA 1 mg L^{-1} + IBA 0.2 mg L^{-1}). The least number of roots per shoot (0.08) was produced in treatment SM_5 (MS + BA 1 mg L^{-1} + IAA 1 mg L^{-1}), which was on par with SM_1 (MS + BA 1 mg L^{-1} + IAA 0.1 mg L^{-1}) (0.33), SM_2 (MS + BA 1 mg L^{-1}+ IAA 0.2 mg L^{-1}) (0.58), SM_3 (MS + BA 1 mg L^{-1}+ IAA 0.3 mg L^{-1}) (0.25), SM_4 (MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1}) (0.25) and SM_{10} (MS + BA 1 mg L^{-1}+ IBA 1 mg L^{-1}) (0.50).

The longest root (9.69 cm) was obtained in the treatment SM_7 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) (Plate 7). The treatments containing IAA did not produce roots having length more than 1 cm.

None of the treatments containing IAA (SM_1 to SM_5) exhibited 50 % rooting. Maximum of 33.33 % response was obtained in the treatment SM_3 (MS + BA 1 mg L^{-1} + IAA 0.3 mg L^{-1}) and minimum of 8.33 % in the treatment SM_5 (MS + BA 1 mg L^{-1}).

Rooting percentage was calculated for all the treatments. Among different treatments containing IBA all the treatments exhibited a response of 50 % or more except SM_{10} (MS + BA 1 mg L^{-1} + IBA 1 mg L^{-1}), which showed a response of 41.67 %. Maximum rooting percentage (83.33 %) was obtained in SM_7 (MS + BA 1 mg L^{-1} + IBA 0.2 mg L^{-1}).

Plate 7. *In vitro* on MS + BA 1 mg L^{-1} + IBA 0.2 mg L^{-1}

Table 7. Effect of IAA and IBA on Shoot multiplication

Treatments	Plant growth regulators		Shoot length(cm)	Percentage of response (%)
	IAA	IBA		
SM_1	0.1	-	1.08	25
SM_2	0.2	-	2.08	25
SM_3	0.3	-	1.55	33.33
SM_4	0.5	-	4.06	50
SM_5	1	-	2.58	41.67
SM_6	-	0.1	1.91	41.67
SM_7	-	0.2	3.08	50
SM_8	-	0.3	8.67	83.33
SM_9	-	0.5	5.83	66.67
SM_{10}	-	1	6.88	58.33
SE m(±)			0.130	
CD (0.05)			0.385	

Table 8. Effect of IAA and IBA on rooting

Treatments	Plant growth regulators(mg L^{-1})		Days to root initiation	No. of roots per shoot	Root length(cm)	Rooting percentage (100%)
	IAA	IBA				
SM_1	0.1	-	15.00	0.33	0.33	25
SM_2	0.2	-	14.42	0.58	0.50	25
SM_3	0.3	-	17.67	0.25	0.25	33.33
SM_4	0.5	-	19.92	0.25	0.25	25
SM_5	1	-	21.00	0.08	0.08	8.33
SM_6	-	0.1	14.67	3.08	4.16	50
SM_7	-	0.2	11.08	7.00	9.69	83.33
SM_8	-	0.3	16.00	2.50	2.72	58.33
SM_9	-	0.5	15.92	2.42	1.74	50
SM_{10}	-	1	15.42	0.50	0.50	41.67
SE m(±)			0.407	0.197	0.099	
CD (0.05)			1.208	0.586	0.294	

4.5 HARDENING AND PLANTING OUT

Well rooted plantlets were carefully removed from the culture bottles, adhering agar was washed off in sterile distilled water and transferred to protrays containing autoclaved red soil, sand and coir pith compost (1:1:1 ratio). The plantlets were covered with porous polythene cover to maintain high humidity. Within 6-7 days new leaf emerged in most the plantlets. After 15 days the plants were transferred to small pots for further hardening under greenhouse conditions. The plants showed a survival rate of 72% after two weeks (Plate 8).

Plate 8. Hardening and planting out

A) Plantlets planted on portrays

B) Protray covered with polythene cover

C) Planted out in small pot

Discussion

5. DISCUSSION

The present study, "Standardisation of micropropagation technique in ivygourd (*Coccinia grandis* (L.) Voigt.) variety Sulabha" was conducted at the Department of Vegetable science, College of Agriculture, Vellayani during 2017-2019. The results obtained are discussed in this chapter.

5.1 SELECTION OF EXPLANTS

Nodal segments collected from tender shoots of ivy gourd were used as explant in the present study. Rapid micropropagation in ivygourd using nodal explants was attempted earlier by Gulati (1988), Sundari *et al.,* (2011) and Patel and Ishnava (2015).

The cardinal importance of choice of explants for successful induction of *in vitro* regeneration for crop improvement was emphasized by Kumar *et al.* (2003). Direct regeneration from axillary buds or shoot tips, which are pre existing meristems, is essential to obtain plantlets with uniform growth characteristics of the mother plant. In the present study, Sulabha variety of ivygourd has been attempted.

Komalavalli and Rao (2000) observed that among various explants tested, only nodal explants and shoot tips showed positive morphogenetic response and readily developed multiple shoots, whereas other explants produced only callus. Maximum bud sprouting and shoot number without callus formation occurred in nodal explants compared to shoot tip explants.

Explants collected from the field may contain various microorganisms, so surface sterilization of the explant is an essential operation to establish healthy and disease free cultures. Hence nodal segments were surface sterilized using 70 % alcohol followed by $HgCl_2$ 0.1 % for 3 minutes.

Josekutty *et al.* (1993) reported that detergent washed nodal explants of ivygourd were treated with 0.2% mercuric chloride solution for 1.5 to 2 min and rinsed several times with sterile distilled water for surface sterilization.

Nodal segments of *C. grandis* was sterilized with freshly prepared 70% alcohol for 30 sec; 3% sodium hypochlorite for 3 min and with 0.1% mercuric chloride for 3 min. The explants were washed thrice with sterile distilled water after every treatment (Sundari *et al.*, 2011).

5.2 BUD PROLIFERATION AND MULTIPLE SHOOT INDUCTION

MS basal medium supplemented with different concentrations and combinations of Kinetin (Kn) and Benzyl adenine (BA) was used as bud proliferation and multiple shoot induction medium.

5.2.1 Effect of Kinetin on Bud Proliferation and Multiple Shoot Induction

All individual concentrations of Kn showed varied response with respect to days for bud initiation, no. of shoots / explant, shoot length (Fig 1) and percentage of response.

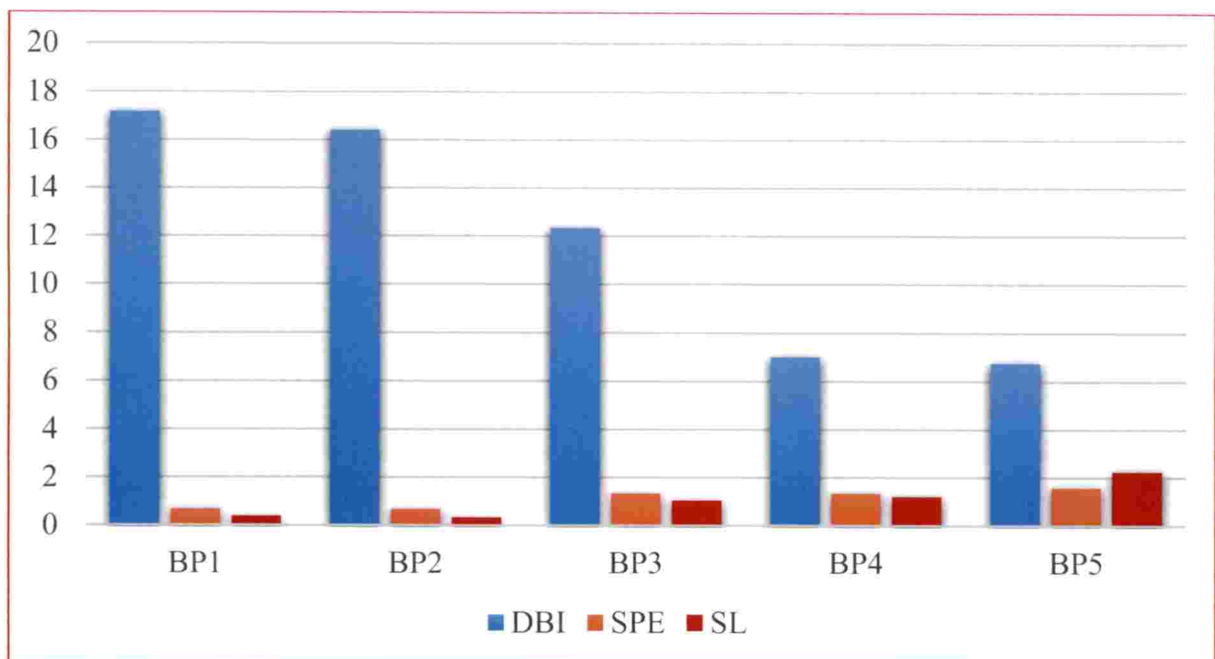
According to Sundari *et al.* (2011), MS basal medium supplemented with Kn 0.5 mg L^{-1} initiated buds in nodal explants of *C.grandis* within 12 days with cent percent bud proliferation, maximum number of shoots/ explant (3) and highest shoot length (8.2 ± 0.5 cm).

Patel and Ishnava (2015) reported that shoot bud induction was obtained in *C.grandis* in MS + Kn 0.2 mg L^{-1} after 5 days of inoculation, with maximum number of shoots.

In contrast to these observations, in the present study, among all the five treatments of Kn, earliest bud initiation (6.75 days), maximum number of shoots per explant (1.58), shoot length (2.25 cm) and percentage of response (83.33 %) was observed in the treatment BP_5 (MS + Kn 1 mg L^{-1}).

In vitro plant regeneration of cucumber (*Cucumis sativus* (L.) from cotyledon and hypocotyl explants was attempted by Ugandhar *et al.* (2011) on MS medium supplemented with BAP (1.0-5.0 mg L^{-1}) and Kn (1.0-5.0 mg L^{-1}) individually and in combination. Shoot bud induction was found to decrease at high levels of Kn.

Fig 1. Effect of Kinetin on bud proliferation and multiple shoot induction



DBI – Days for shoot bud initiation **SPE** – Shoots per explant **SL** – Shoot length

5.2.2 Effect of BA on Bud Proliferation and Multiple Shoot Induction

Among all the treatments of BA tried for bud proliferation and multiple shoot induction, the treatment BP_{10} (MS + BA 1 mg L^{-1}) was found to be significantly superior to all other treatments with respect to days for bud initiation (5.50 days), number of shoots per explant (1.75), shoot length (5.71 cm) and percentage of response (100 %) (Fig 2). The results are in line with the reports of Sundari *et al.* (2011) in ivygourd.

Patel and Ishnava (2015) reported the effect of BA on shoot proliferation from nodal explants of *C.grandis*. Shoot buds were obtained after 5 days of inoculation in MS + BA 0.5 mg L^{-1}.

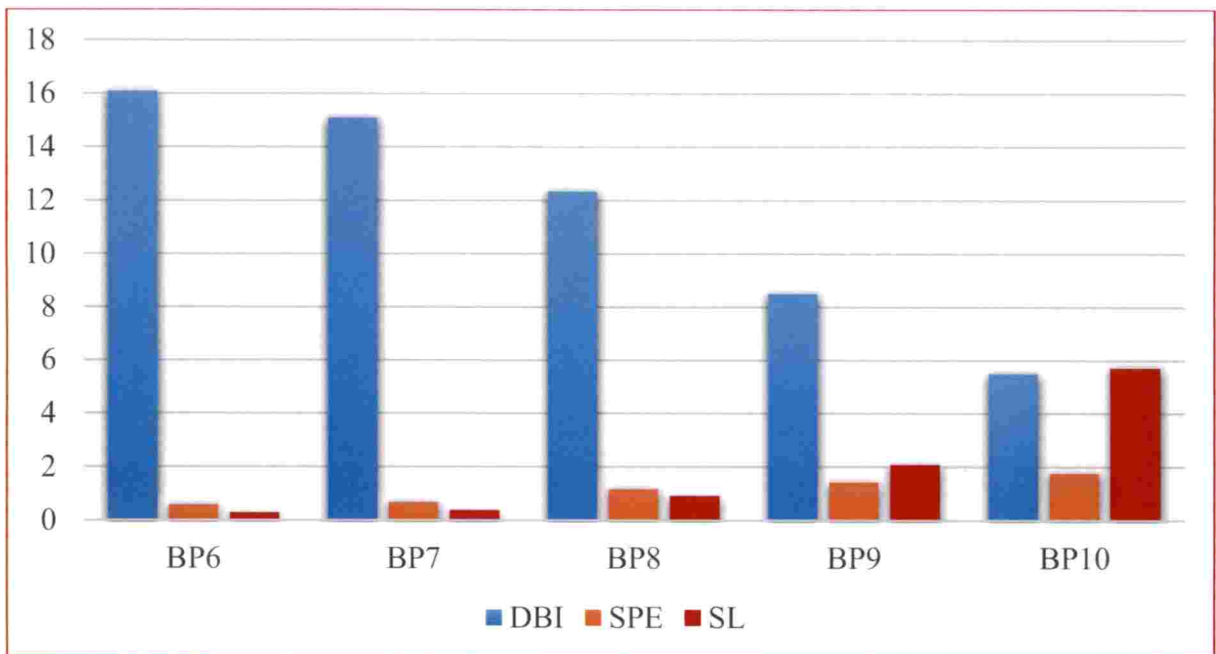
In micropropagation of *cucurbita maxima* Duch. through shoot tip culture, longest shoots (6.1±0.85 cm) and the highest percentage of shoot formation (90.45%) were observed in MS medium supplemented with 3.0 mg L^{-1} BA after 30 days of culture. But highest number of shoots per explant (16.5±0.95) was observed in the media containing 2.0 mg L^{-1} BA. Higher concentration of BA did not improve the shoot size and number (Mahzabin *et al.*, 2008).

Cytokinins are adenine derivatives which are mainly concerned with cell division, modification of apical dominance and shoot differentiation in tissue culture. Lateral buds are released from dormancy and shoot formation is promoted by cytokinins.

5.2.3 Effect of Combination of Kinetin and BA on Bud Proliferation and Multiple Shoot Induction

Kathal *et al.* (1988) observed that combination of two cytokinins, BAP and 2ip in regeneration of plants from leaf explants of *Cucumis melo* was effective for multiple shoot induction. The synergistic effect of cytokinins, BA and Kn for multiple shoot induction was also reported by Komalavalli and Rao (2000) and Sujatha and Kumari (2007) in medicinal plants *Gymnema sylvestre* and *Artemisia vulgaris* respectively. Hence in the present study, the synergistic effect of Kn and BA was tested. In the combination treatment BP_{11} (MS + Kn 1 mg L^{-1} + BA 1 mg

Fig 2. Effect of BA on bud proliferation and multiple shoot induction



DBI – Days for shoot bud initiation **SPE** – Shoots per explant **SL** – Shoot length

L^{-1}), shoot buds were initiated within 7.08 days with 0.92 shoots per explant, shoot length of 3.04 cm and with a percentage of response of 91.67 % (Fig 3) (Fig 4).

Among the treatments containing BA alone, Kinetin alone and combination of BA and Kinetin, BP_{10} (MS + BA 1 mg L^{-1}) yielded significantly higher response with respect to different parameters *viz,* days for shoot bud initiation (5.50), number of shoots per explant (1.75), shoot length (5.71 cm) and percentage of response 100.00) *i.e* the individual concentration of BA exhibited best results than individual concentration of kinetin and combination of BA and Kn.

BA was the most effective cytokinin for bud proliferation and multiple shoot induction in many plants of the family Cucurbitaceae *viz. Trichosanthes dioica* (Kumar *et al.,* 2003), *Cucurbita maxima* (Mahzabin *et al.,* 2008) and *Lagenaria siceraria* (Saha and Kazumi, 2007). Single use of BA was found to be more effective than combination treatment with NAA and Kinetin for direct organogenesis from shoot tips in *Cucurbita maxima* by Mahzabin *et al.* (2008).

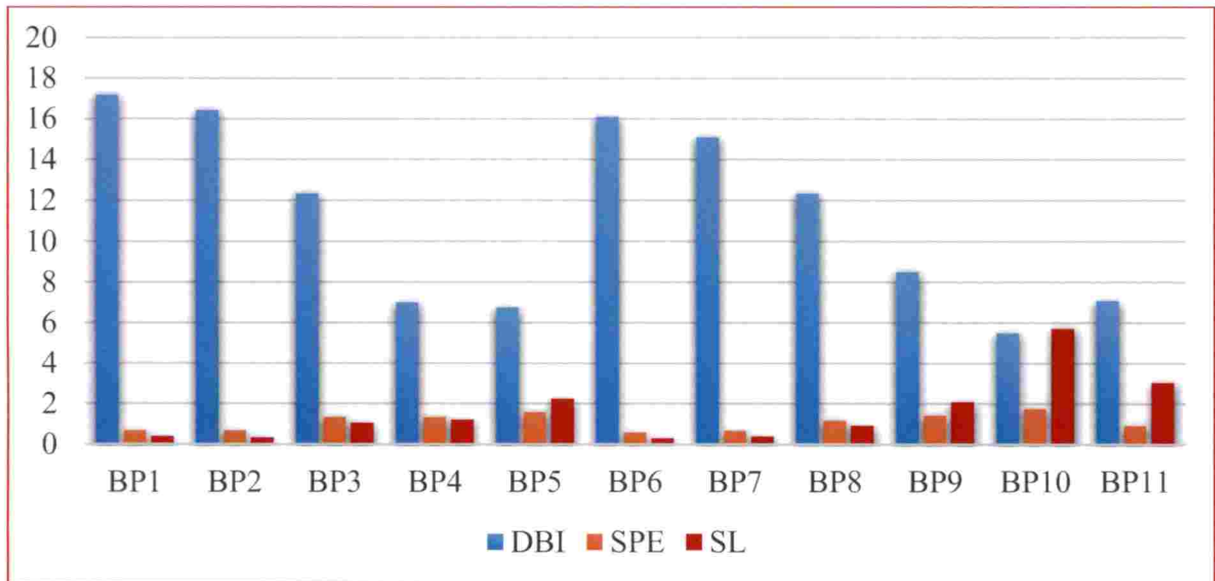
According to Saha and Kazumi (2007), BA combined with NAA or Kinetin resulted in a lower shoot regeneration as well as shoot number per explant in bottlegourd.

Saha and Gosh (2014) also reported reduced shoot induction and multiplication rates by 50 % in the combination treatment of BA and Kinetin in ridge gourd.

Higher concentrations of BA producing callus and suppressing shoot elongation was reported by Kumar *et al.* (2003) in poined gourd. Mahzabin *et al.* (2008) also reported reduction in shoot length and promotion of massive base callus in *Cucurbita maxima*, when the concentration of BA was increased in the media. Deliterious effect of growth regulators at higher concentrations and ineffectiveness at lower concentrations, both results in poor performance.

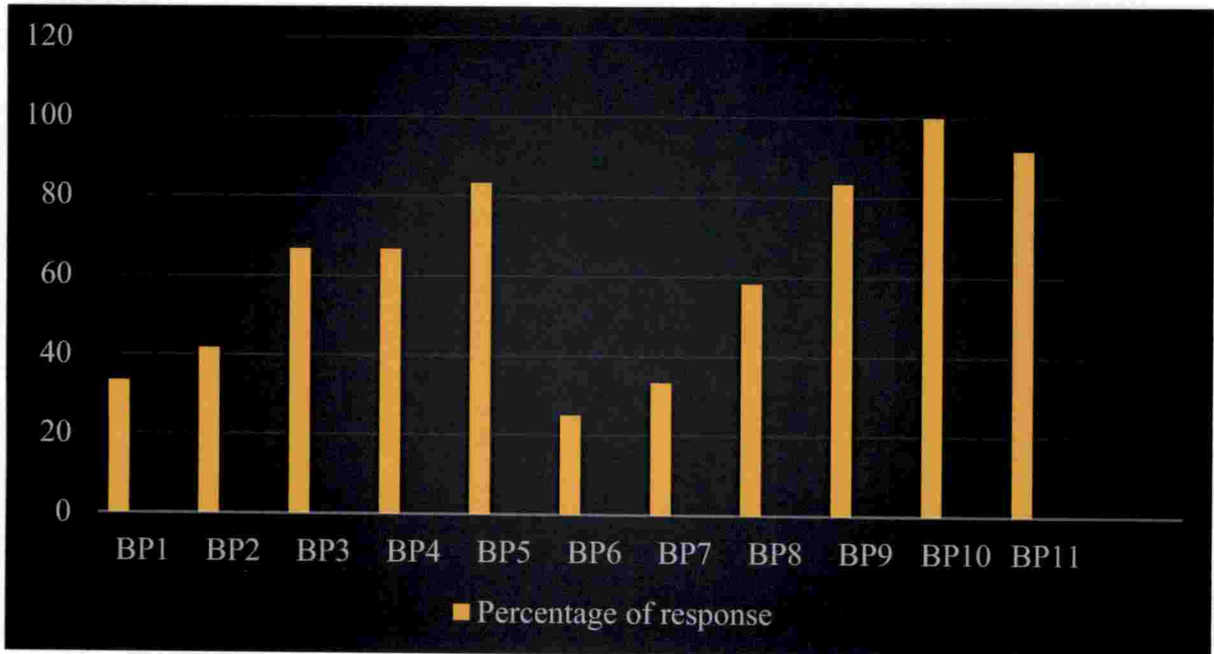
The differences observed in the response of different hormone combinations on plant regeneration can be due to either the presence of different hormone receptors with varying affinity to different auxins (Starling *et al.,* 1986) or to specific enzyme systems that catabolize cytokinins with particular side groups (McGaw and Horgan, 1983).

Fig 3. Effect of Kinetin and BA individually and in combination on Bud proliferation and multiple shoot induction



DBI – Days for shoot bud initiation **SPE** – Shoots per explant **SL** – Shoot length

Fig 4. Percentage of response of cytokinins on bud proliferation and multiple shoot induction



But in the present study, multiplication rate *i.e* number of shoots per explant was low. For increasing the multiplication rate, addition of different additives could be suggested.

Ahmad and Anis (2005) observed that addition of casein hydrolysate to the multiple shoot induction medium (MS + BA) enhanced the number of multiple shoots in cucumber. Optimum shoot regeneration was observed on Murashige and Skoog (MS) medium containing 1.0 μM BA and 200 mg L^{-1} casein hydrolysate

The effect of different additives *viz.,* chitosan, adenine sulphate and thidiazuron on multiplication rate of axillary buds were assessed in medicinal plants by Soumya (2017) and Deepa (2018) in chethikoduveli (*Plumbago rosea* L.) and koovalam (*Aegle marmelos* L. Corr.) respectively and observed that adenine sulphate was best for enhancement of multiple shoot induction *in vitro*.

5.3 SHOOT MULTIPLICATION

The multiple shoots obtained from the best treatment BP_{10} (MS + BA 1 mg L^{-1}) were transferred to shoot multiplication medium containing different concentrations of IAA and IBA. The effect of IAA on shoot multiplication in terms of shoot length and percentage of response was studied. In contrast to the report of Sundari *et al.* (2011) on the *in vitro* propagation of ivy gourd, IAA did not show a good response for shoot multiplication except in SM_4 (MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1}), which produced shoot having a length of 4.06 cm with a percentage response of 50 %. The same treatment (MS + BA 1 mg L^{-1}+ IAA 0.5 mg L^{-1}) studied by Sundari *et al.* (2011) produced shoot having a length of 4.3 ± 0.06 cm with 80 % response.

At high concentration of IAA *i.e* SM_5 (MS + BA 1 mg L^{-1} + IAA 1 mg L^{-1}) basal callusing was observed. Similar problem of basal callusing was observed by Lakshmanan *et al.* (1997) in the *in vitro* mass propagation of a woody ornamental *Ixora coccinea*. The presence of IAA in the shoot multiplication medium was detrimental to shoot proliferation. The production of large amounts of

basal callus and vitrification of shoots were the major problems in proliferating shoot cultures in the presence of IAA and BA.

Sharma and Thorpe (1990) observed that addition of activated charcoal (0.05 or 0.1%) to the shoot proliferation medium speeded up the production of adventitious roots by at least 2 weeks and inhibited the formation of callus at the basal end of the shoots in *in vitro* propagation of mulberry (*Morus alba* L.) through nodal segments.

Among all the ten treatments tried for shoot multiplication, SM_8 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) was found to be the best treatment with a shoot length of 8.67 cm after 3 weeks of transfer from bud proliferation medium (Fig 5). Highest percentage of response for shoot multiplication was also recorded in SM_8 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) (83.33 %) (Fig 6). These findings were in accordance with the report of Sundari *et al.* (2011) in ivy gourd.

According to Huda and Sikdar (2006), shoot initiation with elongation were obtained in MS medium supplemented with 1.0 mg L^{-1} BA + 0.1 mg L^{-1} IBA + 0.3 mg L^{-1} Gibberellic acid (GA_3) in *in vitro* propagation of bittergourd through apical meristem. In pummelo (*Citrus grandis),* addition of 5.8 Mm GA_3 in shoot-proliferation medium during the second subculture improved shoot elongation and shoot multiplication in each successive subculture (Paudyal and Haq, 2000).

5.4 ROOTING

In vitro rooting of regenerated micro shoots were observed on rooting medium *i.e* BP_{10} (MS + BA 1 mg L^{-1}) supplemented with different concentrations of IAA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) and IBA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) individually for root initiation.

According to Sundari *et al.* (2011), highest percentage of root formation (100%) and maximum number of roots per shoot (7 ± 1.5) and root length (3.9 ± 0.2 cm) in ivygourd was achieved on MS medium supplemented with 0.1 mg L^{-1} IBA. The regenerated shoots of ivy gourd through indirect organogenesis from nodal explants were rooted *in vitro* on MS medium supplemented with 0.1 mg L^{-1}

Fig 5. Effect of IAA and IBA on Shoot multiplication

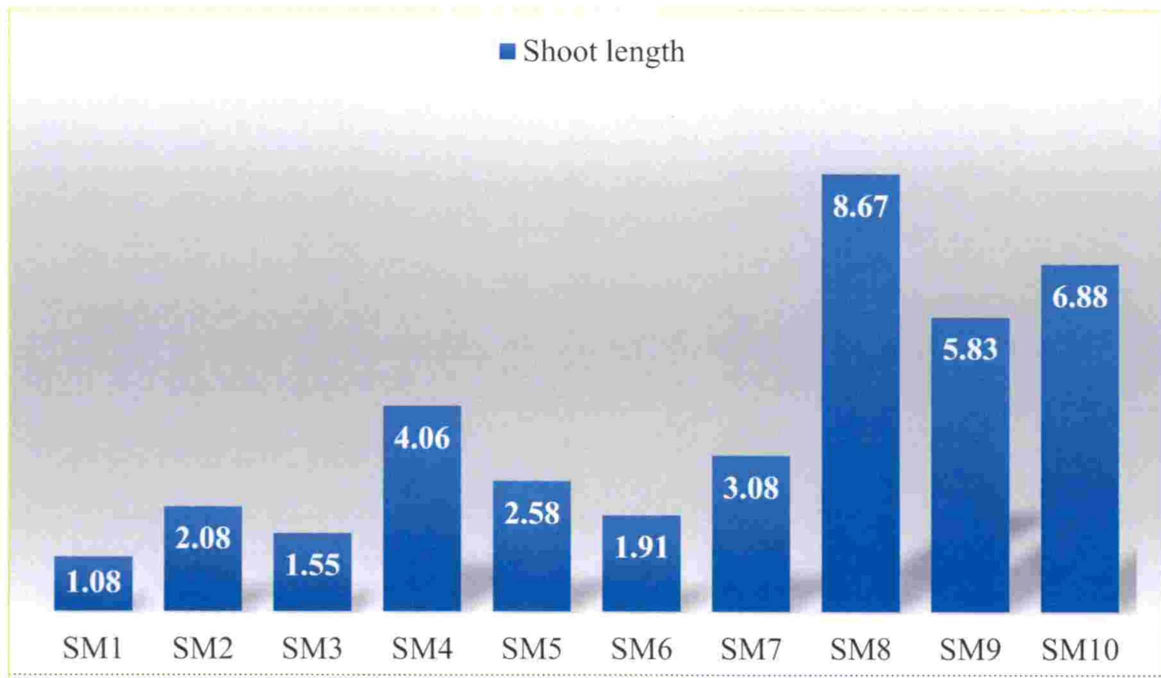
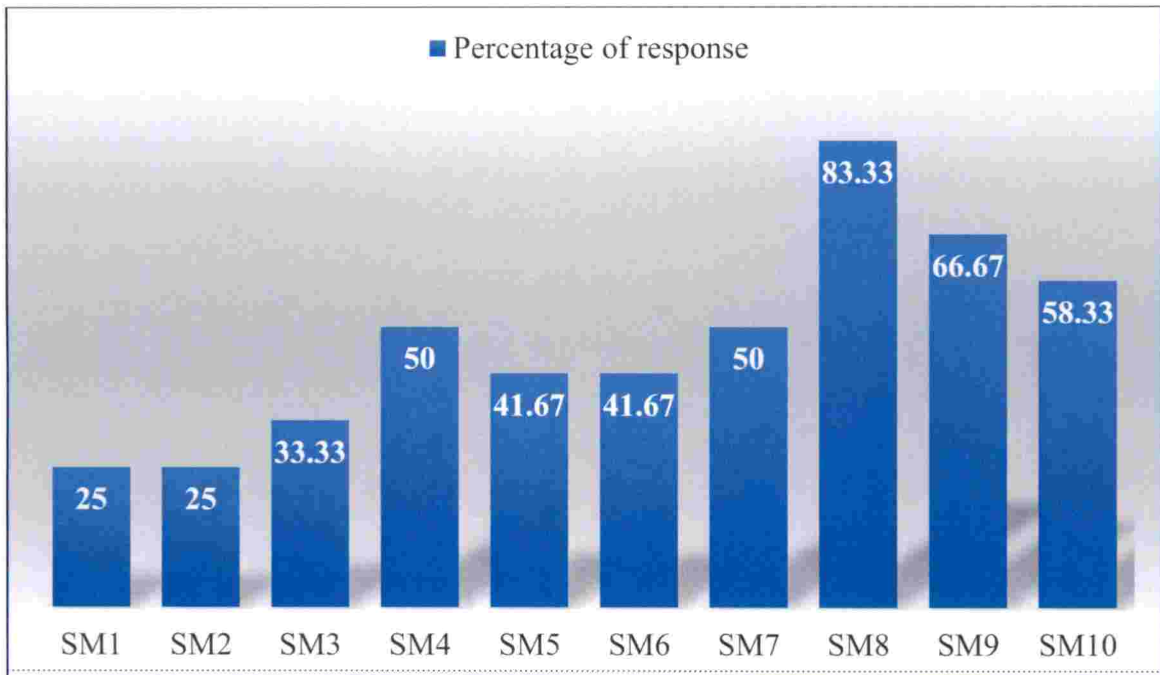


Fig 6. Percentage of response of shoot multiplication



IBA (Thiripurasundari and Rao, 2012). In contrast to these findings, in the present study, earliest root initiation (11.08 days), maximum number of roots per shoot (7) with longest root (9.69 cm) (Fig 7) and maximum rooting percentage (83.33 %) (Fig 8) was obtained in the treatment SM_7 (MS + BA 1 mg L^{-1} + IBA 0.2 mg L^{-1}). Root development was slow at higher concentrations of IBA (0.3, 0.5 & 1 mg L^{-1}).

The most commonly used auxin for root induction is IBA. The effectiveness of IBA for root induction has been proved by many workers. Mahzabin *et al.* (2008) reported that shoots of pumpkin (*Cucurbita maxima*) were rooted most effectively *in vitro* in half MS medium supplemented with 1.0 mg L^{-1} IBA. IBA favours the conjugation between endogenous IAA and aminoacids that leads to the synthesis of the specific protein necessary for root initiation (Devi *et al.,* 2017).

Josekutty *et al.* (1993) achieved root formation on half-strength MS medium without any hormone supplementation.

The regenerated shoots of cucumber (*Cucumis sativus* (L.) from cotyledon and hypocotyl explants (Ugandhar *et al.*, 2011) and muskmelon (*Cucumis melo* (L.) from shoot tip explants (Venkateshwarlu, 2012) were successfully rooted on MS medium supplemented with 1.0 mg L^{-1} IAA. Bhardwaj *et al.* (2017) obtained root initiation in half MS + 0.25mg L^{-1} IAA for *in vitro* regeneration of parthenocarpic cucumber. But in the present study, the treatments containing different concentrations of IAA did not show good response for *in vitro* rooting. None of the treatments containing IAA responded more than 50 % to rooting of micro shoots.

5.5 HARDENING AND PLANTING OUT

The ability to transfer plants out of culture on a large scale at low cost and with high survival rate is the ultimate success of micropropagation on a commercial scale (Chandra *et al.,* 2010).

Deb and Imchen (2010) opined that microshoots being transferred to *ex vitro* conditions are exposed to biotic and abiotic stresses, requiring acclimatization for successful establishment and survival of plantlets.

Fig 7. Effect of IAA and IBA on rooting

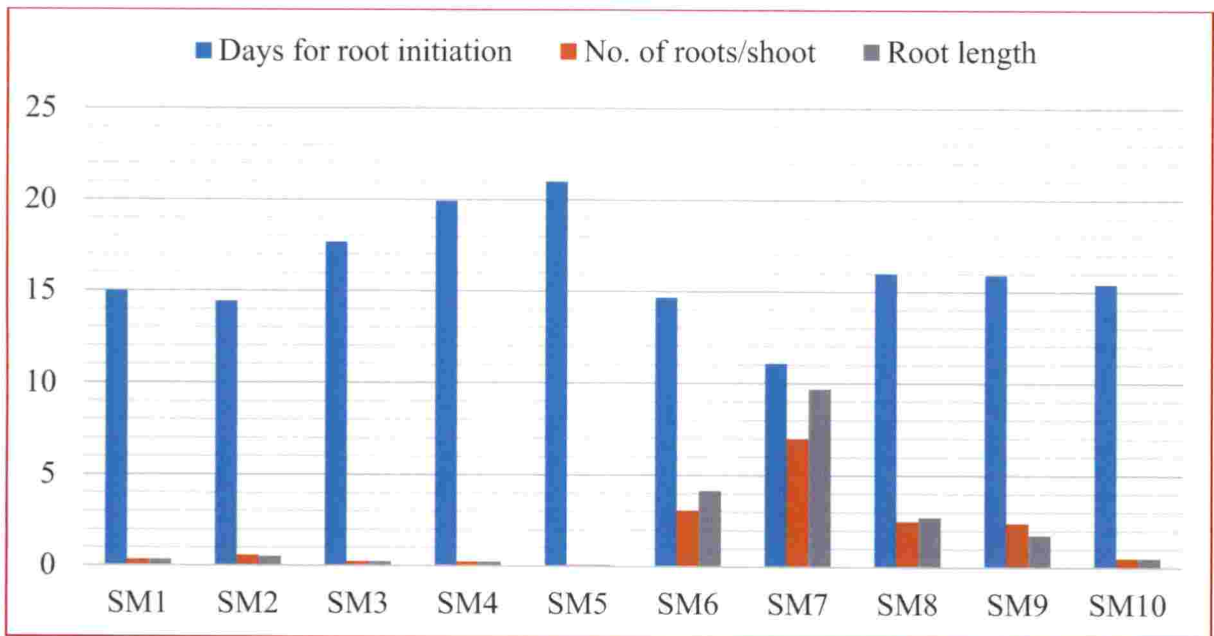
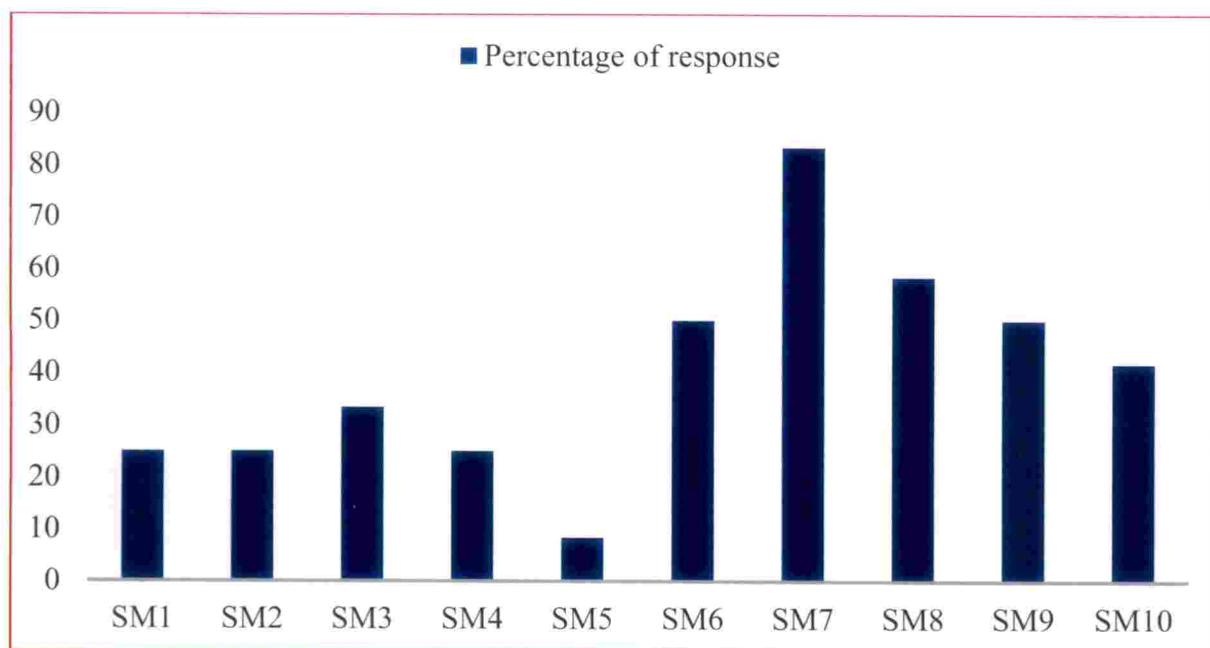


Fig 8. Percentage of response of *in vitro* rooting.



In the present study, new leaf emerged in most of the plantlets within 6-7 days. The plants were transferred to small pots after 15 days for further hardening under greenhouse conditions. The plants showed a survival rate of 72 % after 2 weeks.

Sarkar *et al.* (2008) reported 70 % survival rate in *in vitro* raised plantlets of ivygourd after acclimatization and three weeks thereafter, while Sunadri *et al.* (2011) reported 80 % survival of acclimated plantlets.

Rooted plantlets (8-10 cm tall) of *Trichosanthes cucumerina* var. *cucumerina* were transferred to soil, sand and farmyard manure mixture in 1:1:1 ratio. About 90% of the rooted plantlets were acclimatized and transferred to the greenhouse and successfully transferred to the field with 80% survival rate. The acclimatized plants exhibited normal growth (Devendra et al., 2008).

Saha *et al.* (2016) tried an acclimatization strategy for bittergourd plantlets by using two types of structures i.e glass jar with PP cap and plastic pot with polythene cover. The glass jar with PP cap was found to be comparatively better than plastic pot with polythene covering for hardening with respect to survival percentage, number of leaves per plantlet and plant height. Survival percentage of 80.94 % was obtained for nodal segments in glass jar with PP cap.

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Summary

6. SUMMARY

A study on "Standardisation of micropropagation technique in ivygourd (*Coccinia grandis* (L.) Voigt.) variety Sulabha" was carried out at the Department of Vegetable science, College of agriculture, Vellayani during 2017- 2019. The objective of the study was to develop an efficient, large scale multiplication protocol for the regeneration of C. *grandis* from shoot tips/ nodal explants.

The explants for the study were collected from the experimental plot of Department of Vegetable Science. The experiment was carried out in four phases *viz.,* bud proliferation and multiple shoot induction, shoot multiplication, rooting and hardening and planting out.

Shoot tips and nodal segments were taken as explants, and were surface sterilized by mercuric chloride at different concentrations for different duration. Nodal segments responded better to surface sterilization with mercuric chloride 0.1 % for 3 minutes than shoot tips. So further study was continued with nodal segments as explants.

Surface sterilized explants were inoculated on the medium for culture initiation and multiplication. Murashige and Skoog (MS) basal medium supplemented with five different concentrations and combinations each of Kinetin (Kn) and Benzyl adenine (BA) were tried to study the effect on bud proliferation and multiple shoot induction.

Among the five different treatments of Kinetin (BP_1 to BP_5), BP_5 (MS + Kn 1 mg L^{-1}) recorded significantly higher response with respect to different observations *viz.,* earliest shoot bud initiation (6.75 days), maximum number of shoots per explant (1.58) and shoot length (2.25 cm).

There was significant variation among the different treatments containing BA (BP_6 to BP_{10}), the treatment BP_{10} (MS + BA 1 mg L^{-1}) being earliest bud initiation (5.50 days), maximum number of shoots per explant (1.75) and maximum shoot length (5.71 cm).

Best of the above two sets of treatments were tried in combination as eleventh treatment for studying the synergistic effect of cytokinins for bud proliferation and multiple shoot induction. The combination treatment BP_{11} (MS + Kn 1 mg L^{-1} + BA 1 mg L^{-1}) exhibited shoot bud initiation within 7.08 days with 0.92 shoots per explant and shoot length of 3.04 cm. Percentage of response of cultures were calculated for all the treatments. Among the eleven treatments, tried for the study, BP_{10} (MS + BA 1 mg L^{-1}) yielded significantly higher response with respect to different characteristics *viz.,* days for shoot bud initiation (5.50 days), number of shoots per explant (1.75), shoot length (5.71 cm) and percentage of response (100 %).

The multiple shoots were transferred to shoot multiplication medium *i.e.* BP_{10} (MS + BA 1 mg L^{-1}) supplemented with different concentrations of IAA (0.1,0.2,0.3,0.5 and1 mg L^{-1}) and IBA (0.1,0.2,0.3,0.5 and 1 mg L^{-1}). The treatments containing different concentrations of IAA did not show a good response for shoot multiplication, except the treatment SM_4 (MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1}). Basal callusing was observed at high concentration of IAA *i.e.* SM_5 (MS + BA 1 mg L^{-1} + IAA 1 mg L^{-1}), which affected further shoot multiplication.

Among all the ten treatments tried for shoot multiplication, SM_8 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) produced highest shoots having a length of 8.67 cm after 3 weeks of transfer from bud proliferation medium, with high percentage of response (83.33 %).

The microshoots were transferred from shooting media to rooting media *i.e* BP_{10} (MS + BA 1 mg L^{-1}) along with different concentrations of IAA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) and IBA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}). Earliest root initiation (11.08 days), maximum number of roots per shoot (7), highest root length (9.69 cm) and maximum rooting percentage (83.33 %) was recorded by the treatment SM_7 (MS + BA 1 mg L^{-1} + IBA 0.2 mg L^{-1}).

None of the treatments of IAA (SM_1 to SM_5) exhibited more than 50 % rooting. Among different treatments containing IAA, maximum of 33.33 %

response was obtained in SM_3 (MS + BA 1 mg L^{-1} + IAA 0.3 mg L^{-1}) and minimum of 8.33 % in the treatment SM_5 (MS + BA 1 mg L^{-1} + IAA 1 mg L^{-1}).

Finally, the well rooted plantlets were transferred to portrays containing autoclaved red soil, sand and coir pith compost in 1:1:1 ratio. The plantlets were covered with porous polythene cover to maintain high humidity. New leaf emerged in 6-7 days in most of the plantlets. After 15 days, the plants were transferred to small pots for further hardening in greenhouse conditions. The plants showed a survival rate of 72% after two weeks.

Future line of work

- Enhancement of shoot multiplication rate could be attempted by the addition of different additives.
- Field evaluation of the current protocol could be done for future studies.

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Appendices

APPENDIX I

MS medium						
Stock	**Chemical**	**Quantity for 1L of MS (mg)**	**Conc. of stock**	**Volume of stock (mL)**	**Quantity for preparing stock (mg)**	**Volume for 1L MS media (mL)**
Macro nutrients						
A	NH_4NO_3	1650	5X	250 mL	8250	50 mL
	KH_2PO_4	1900			9500	
	KNO_3	370			1850	
	$MgSO_4.7H_2O$	170			850	
B	$CaCl_2.\ 2\ H_2O$	440	5X	100 mL	2200	20 mL
Micro nutrients						
C	KI	0.83	100X	100 mL	83	1 mL
	H_3BO_3	6.2			620	
	$MnSO_4.\ H_2O$	16.9			1690	
	$ZnSO_4.\ 7\ H_2O$	8.6			860	
	$Na_2MoO_4.\ 2\ H_2O$	0.25			25	
D	$FeSO_4.\ 7\ H_2O$	27.8	5X	100 mL	139	20 mL
	Na_2 EDTA. 2 H_2O	37.3			186.5	
E	$CuSO_4.\ 5\ H_2O$	0.025	1000X	100 mL	25	0.1 mL
	$CoCl_2$. 6 H2O	0.025			25	
Organic supplements						
F	Nicotinic acid	0.5	250X	100 mL	125	0.4 mL
	Pyridoxine – HCl	0.5			125	
	Thiamine – HCl	0.1			25	
	Glycine	2			500	
Components directly added						
	MS supplement	3.3g				
	Myoinositol	100 mg				
	Sucrose	30g				
	Agar	6.5g				

STANDARDISATION OF MICROPROPAGATION TECHNIQUE IN IVYGOURD (*Coccinia grandis* (L.) Voigt.) VARIETY SULABHA

by

THASNI A.
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ABSTRACT

Submitted in partial fulfillment of the requirements for the degree of

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DEPARTMENT OF VEGETABLE SCIENCE
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VELLAYANI, THIRUVANANTHAPURAM - 695 522
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ABSTRACT

The study entitled "Standardisation of micropropagation technique in ivygourd (*Coccinia grandis* (L.) Voigt.) variety Sulabha" was conducted at the Department of Vegetable Science, College of Agriculture, Vellayani, during 2017-2019 with the objective to develop an efficient, large scale multiplication protocol for the regeneration of *C. grandis* var. Sulabha from shoot tips and nodal explants.

The experiment was laid out in completely randomized design with three replications and four cultures per replication. Culture media tried for the experiment were bud proliferation medium, shoot multiplication medium and rooting medium. Shoot tips and nodal segments from tender shoots of *Coccinia grandis* var. Sulabha, maintained in the experimental field of Department of Vegetable Science, were taken as the explants. The explants were surface sterilized using 0.1 % mercuric chloride for 3 minutes.

Murashige and Skoog (MS) medium supplemented with Kinetin (Kn) and Benzyl adenine (BA) individually and in combination was used as bud proliferation medium, which consisted of eleven treatments, BP_1 to BP_5 [(MS + Kn (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1})], BP_6 to BP_{10} [(MS + BA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1})] and BP_{11} (MS + Kn 1 mg L^{-1} + BA 1 mg L^{-1}, the best of the above two sets of treatments in combination). The treatment BP_{10} (MS + BA 1 mg L^{-1}) exhibited significantly higher response for days to bud initiation (5.50), number of shoots per explant (1.75), and shoot length (5.71cm), with cent percent response. Minimum number of shoots (0.58) was observed in the treatment BP_6 (MS +BA 0.1 mg L^{-1}), which was on par with BP_7 (MS + BA 0.2 mg L^{-1}) (0.67). Minimum shoot length (0.29 cm) was observed in the treatment BP_6 (MS + BA 1 mg L^{-1}), which was on par with BP_7 (MS + BA 0.2 mg L^{-1}). Bud proliferation was not obtained, when shoot tip was used as explant.

The shoots obtained from the best treatment BP_{10} (MS + BA 1 mg L^{-1}) were transferred to shoot multiplication medium consisting of BP_{10} (MS + BA 1 mg L^{-1}) along with different concentrations of IAA (0.1, 0.2, 0.3, 0.5 and1 mg L^{-1}) and IBA

(0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}). The treatment SM_8 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) exhibited maximum shoot length of 8.67 cm after 3 weeks of transfer from bud proliferation medium. The minimum length of shoot (1.08 cm) was obtained in SM_1 (MS + BA 1 mg L^{-1} + IAA 0.1 mg L^{-1}). Percentage of response was calculated for all the treatments. All the treatments containing IAA responded less than 50 %, except SM_4 (MS + BA 1 mg L^{-1} + IAA 0.5 mg L^{-1}), which exhibited 50 % response. All the treatments containing IBA showed 50 % or more response, except BP_6 (MS + BA 1 mg L^{-1} + IBA 0.1 mg L^{-1}), which showed a response of 41.67 %. Highest percentage of response for shoot multiplication was recorded in SM_8 (MS + BA 1 mg L^{-1} + IBA 0.3 mg L^{-1}) (83.33 %).

Shoots were transferred to different rooting media *i.e* MS + BA 1 mg L^{-1} supplemented with different concentrations of IAA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) and IBA (0.1, 0.2, 0.3, 0.5 and 1 mg L^{-1}) individually for root initiation. The treatments exhibited significant difference for days to root initiation, no. of roots per shoot, root length and rooting percent. Earliest root initiation (11.08 days), maximum number of roots per shoot (7), longest root (9.69 cm) and maximum rooting percentage (83.33 %) were recorded in SM_7 (MS + BA 1 mg L^{-1} + IBA 0.2 mg L^{-1}).

Rooted plants were transferred to protrays containing autoclaved red soil, sand and coir pith compost in 1 : 1 : 1 ratio for hardening under high humidity and subsequently to small pots, after 15 days under greenhouse conditions for further establishment. Emergence of new leaves was observed within 6-7 days.

The study revealed that bud proliferation in nodal explants of *Coccinia grandis* var. Sulabha was achieved in MS medium supplemented with BA 1.0 mg L^{-1}, shoot multiplication in MS + BA 1.0 mg L^{-1} + IBA 0.3 mg L^{-1} and rooting in MS + BA 1.0 mg L^{-1} + IBA 0.2 mg L^{-1} among the different treatments tried.

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