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Formal and dead words cannot carry the fragrance of emotions with them still they are the only available means of expressing emotions in such formal acknowledgement.

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Place: Kolhapur (Veerendra Naik B)

Date:

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LIST OF ABBREVIATIONS

%	:	Per cent
/	:	Per
>	:	More than
<	:	Less than
±	:	Plus or minus
^0C	:	Degree Celsius
½ MS	:	Half strength Murashige and Skoog medium
2, 4-D	:	2, 4-Dichloro phenoxy acetic acid
2,4,5-T	:	2,4,5-Triphenoxyacetic acid
$2H_2O$	:	Dihydrogen monoxide
ABA	:	Abscisic Acid
$AdSO_4$	:	Adenine sulphate
$AgNO_3$	:	Silver nitrate
Av.	:	Average
BAP	:	Benzyl Adenine Purine
$CaCl_2$	:	Calcium chloride
$CaCl_2.2H_2O$	:	Calcium Chloride Dihydrate
CH	:	Casein hydrolysate
cm	:	Centimetre
CRD	:	Completely randomized design
DDW	:	Double distilled water
DNA	:	Deoxyribose Nucleic Acid
et al.	:	Et. Alibis (and associates)
fig.	:	Figure
FYM	:	Farm yard manure
g	:	gram
GA_3	:	Gibberellic acid
HCl	:	Hydrochloric acid
HEPA	:	High Efficiency Particulate Air
$HgCl_2$	:	Mercuric chloride
hr.	:	Hour
i.e.	:	That is
IAA	:	Indole 3-acetic acid

IBA	:	Indole 3- butyric acid
In-vitro	:	Culture of living material literally in glass *i.e.* on the artificial medium under ascetic condition
In-vivo	:	Processes accruing within the intact living organism
ISSR	:	Inter Simple Sequence Repeats
Kcal	:	Kilo calorie
Kn	:	Kinetin
lbs	:	Libra
Ltd	:	Limited
M	:	Molar
m	:	Meter
mg	:	Mili gram
Mg/l	:	Milligram per litter
mgl^{-1}	:	Miligram per litter
min.	:	Minutes
ml	:	Mili litter
mm	:	Milimetre
MS	:	Murashige and Skoog medium (1996)
MSHP	:	Murashige and Skoog high phosphate medium
N	:	Normality
NaOH	:	Sodium hydroxide
NaOCl	:	Sodium hypochlorite
PGR's	:	Plant growth regulators
ppm	:	Parts Per Million
Pvt.	:	Private
q/ha	:	Quintal per hector
RAPD	:	Random Amplified Polymorphic DNA
RSM	:	Response Surface Methodology
S.E.	:	Standard error of means
TDZ	:	Thiaduzurone
UV	:	Ultra violet
v/v	:	Volume by volume basis
Viz.	:	Videlicet (namely)
w/v	:	Weight by volume basis
α-NAA	:	Alpha-naphthalene acetic acid
µM	:	Micromolar

ABSTRACT

OPTIMIZATION OF MICROPROPAGATION TECHNIQUES IN SPINE GOURD BY USING RESPONSE SURFACE METHODOLOGY

by

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The present investigation entitled “Optimization of Micropropagation Techniques in Spine Gourd by using Response Surface Methodology” was conducted with the objective to optimize the various levels and combinations of media supplements on culture media for amount of callus growth on different explants *viz.,* leaf, petiole, internode and to standardize the protocol for *in-vitro* regeneration of female *Momodica dioica* genotype RMDSG-2 using nodal and shoot tip explants. D-optimal response surface methodology was used for experimental designing and to analyze the generated data for the effect of various levels of media supplements on the node and shoot tip explant at initiation, multiplication and at rooting stages. MS media was used as a basal media and was supplemented with various levels of media supplements as suggested by RSM. The best optimized combinations observed from initiation stage for each experiment were again compared in CRD and statistically observed best treatment was used for multiplication of shoots from nodal as well as shoot tip explants. The best regenerated plants were then transplanted for rooting study.

Among the leaf, petiole and internode explants, the leaf explants showed earliest callus initiation with high amount of callus on a MS media supplemented with 1.0 and 2.0 mgl^{-1} NAA. The white fragile callus was observed in the all treatments except 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} BAP (brown fragile callus) and 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kinetin (pink fragile callus).

For shoot initiation nodal and shoot tip explant were tried. As regards nodal explants, the early shoot initiation was observed in 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn and 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn supplemented media. The maximum number of shoots per explant were

observed in 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA added media while treatment 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} IAA produced shoots with maximum length. The shoot tip explants placed on media fortified with 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn observed best media for early initiation of shoot, The maximum number of shoots per explant were recorded in 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA treated media while 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA treated media registered maximum shoot length.

The best media supplement treatments as observed in various experiments conducted on statistical analysis were selected for further optimization in nodal as well as shoot tip explants separately. As regards nodal explants, 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn treated media gave early shoot initiation. The maximum number of shoots per explant were recorded in 1.8 mgl^{-1} BAP + 0.5 mgl^{-1} Kn supplemented media while maximum shoot length was registered in 0.46 mgl^{-1} BAP + 0.1 mgl^{-1}IAA treated MS media. In case of shoot tip explants, treatment 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn was found best for early shoot initiation while maximum number of shoots per explant were recorded in MS media treated with 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The media fortified with optimized combination of 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn recorded maximum shoot length.

The selected optimized combinations of media supplements were further used for the multiplication of plants from the regenerated nodal as well as shot tip explants. The optimized combination of 0.46 mgl^{-1} BAP + 0.10 mgl^{-1} IAA produced large number of plants with maximum number of shoots per explant as well as highest shoot length from the regenerated nodal explant shoot. As regards the shoot tip explant regenerated shoots, the media supplemented with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA recorded maximum number of plants. However, the maximum number of shoots per explant as well as maximum length of shoots in regenerated shoot tip explants were registered in the media supplemented with optimized combinations of 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA and 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn respectively.

Well regenerated plants using optimized media supplements were transplanted in rooting media optimized by using RSM. As regards the nodal explants, the earliest root initiation observed in 1.0 mgl^{-1} NAA supplemented media while MS media fortified with 2.0 mgl^{-1} IBA, recorded the maximum number of roots as well as length of root per regenerated shoot. The media treatment with 1.0 mgl^{-1} NAA recorded earliest root initiation with maximum number of roots per regenerated shoot from shoot tip explant. The maximum length of roots in shoot tip regenerated plants was observed in 2.0 mgl^{-1} IBA supplemented media.

Healthy and well rooted plants regenerated from nodal and shoot tip explants using optimized media supplements were transplanted in growing media consisting of cocopeat and soil (1:1) for hardening. The plants regenerated from the nodal explants showed 67.85 per cent survival rate within 20 days, whereas plants regenerated from shoot tip explants recorded 80.64% survival rate within 18 days.

Veerendra Naik B. (1 to 130)

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1. INTRODUCTION

Spine gourd (*Momordica dioica* Roxb. Ex. Wild) is a perennial, rhizomatous, dioecious herbaceous climbing creeper belongs to cucurbitaceae family. It is grown for its immature nutritious tender fruits. This high economic value and export oriented crop has been originated in tropical Asia *i.e.,* Indian sub-continent and distributed through-out the country up to an altitude of 1500 m (Shekhawat *et al*. 2011). Spine gourd has glabrous stem, broad ovate leaves, entire deeply 3-5 lobed; yellow solitary flowers, ovoid or ellipsoid fruits, 2.5-6.3 cm long, shortly beaked, densely echinate with soft spines; seeds slightly compressed, 67 mm long, irregularly corrugated. Spine gourd fruits have very good shelf life along with fruits, young leaves, flowers and seeds also edible (Ghive *et al*. 2006[a]). Spine gourd grows in warm and humid weather and tuberous roots are planted in pits. Plantation is done at the onset of summer with the first shower, in the month of May while flowering starts from Jun-July and fruiting ends in September to December. The plants undergoes dormant during winter season (Rasul, *et al*. 2007). It is a high demandable seasonal vegetable and medicinal plant, fruit yield varies at 75-100 q/ha at optimum management (Bharathi, 2007). It is free from cholesterol and has adequate amount of high energy (45.74 Kcal), water (84%), minerals and vitamins (Gopaln *et al*. 1994). Fruits are rich source of protein, calcium, phosphorus, iron and highest amount of carotene (162 mg/100g edible portion) among cucurbits (Ram, *et al*. 2001). Fruits are also a rich source of lectins, triterpinse of ursolic acid, momordic ursenol, vitamins, iodine, flavonoids and glycosides (Ali and Srivastava 1998; Singh, *et al*. 2009) and is described as "Good supplement for nutrient" (Aberoumand, 2010).

The medicinal properties of spine gourd are sex-specific and only female plants have medicinal values (Shastri *et al*. 1962). Fruits have its own value in preparation of medicines against ulcer, piles, sores and obstruction of liver and spleen as appetizer and astringent (Ghive *et al.* 2006[c]). Fruits are also used to cure the asthma, leprosy, tumor, excessive salivation and heart diseases. Ayurvedic practitioner prescribed spine gourd fruit to cure diabetes and fruit powder is used to induce sneezing leading to nasal clearance. Leaf decoction of female spine gourd is used as aphrodisiac, to eliminate parasites in intestine, fever and respiratory disorder (Kumar and Prajapati, 2003). Seeds are quite helpful for relieving chest problem and urinary discharge. The roots are used in bleeding piles, bowl infection, urinary complaint and also in headache, kidney stone, jaundice and root paste is applied on body as sedative (Jain and Singh, 2010). Root paste is applied on body as sedative (Chakravorty, 1959). Luo (1998) reported that in traditional days it was used in treatment of Snake bite, Scorpion sting and also used as abortifacient. Along with these medicinal properties, they also possess anti-bacterial, anti-oxidant, anti-inflammatory, anti-lipid, anti-cancerous, anti-fertility, anti-allergic, peroxidative and nematicidal. It has prominent position among cucurbitaceous vegetables owing to its good nutritional and medicinal value, rich

taste, high keeping quality, ability to withstand long distant transportation, high economic return and good export potential (Rasul, 2003).

This vegetable has immense potential for contribution to a particular pocket of food production because they are well adapted to existing as well as adverse climatic condition and are generally resistant to pests and pathogens. This popular vegetable has high demand in market but still remained as underutilized, underexploited and minor cucurbitaceous vegetable due to its dioecious nature and conventional method of vegetative propagation (Bharathi *et al*. 2007). The commercial methods of propagation in spine gourd is largely depends on tuberous root followed by stem cuttings and seeds. The commercial multiplication using tuberous roots is critically limited due to low rate of multiplication and dormancy, because of which it occupies a valuable cultivable land until next planting season. The problem arises with the stem cutting are late availability in fruiting season, along with stem cuttings containing 2-3 nodes from dark green vines of 2-3 months old plants are planted, but only 36 % of plants will sprout and survive. The difficulties in seed propagation are hard seed coat, prolonged seed dormancy (4-5 month), limited seed germination (10%) and unpredictable sex ratio in seedling progenies before flowering (Mondal, *et al.* 2006). Due to its dioecious nature, poor natural pollination of female flowers results in lower yield. To compensate this problem plantation needs 5-10 % male plants as pollinizer and it is imperative for good fruit set (Rasul, 2007). It is highly cross pollinated in nature and exhibit genetic variation for morphology and growth parameters.

As the conventional methods of spine gourd propagation impose several limitations for large scale production of sex specific plants, an efficient clonal propagation is must. Improvement of plant species via biotechnological approach depends on plant tissue culture. Micropropagation helps to overcome above said problems in great extent and systematic improvement is boon for horticulture, pharmaceutical industry and Ayurveda. High multiplication ratio achieved rapid multiplication of disease and pest free elite plant within short span of time and space (Ghive, 2006[b]). An *in-vitro* propagation system offers unlimited availability of planting material early in the planting season. It can be employed as an alternative means for genetic upgradation and its application largely depends on a reliable plant regeneration system. The application of micropropagation is well-established for rapid large-scale propagation of many crops including cucurbitaceous vegetables. Micropropagation has been reported in shoot tip and nodal explants of Cucurbitaceae such as *Citrullus lanatus* (Barnes *et al*. 1978) and *Cucumis sativus* (Vasudevan *et al*. 2001; Ahmed and Anis, 2005).

Unfortunately, so far not much systematic efforts have been made to overcome the problems in *Momordica dioica* and crop remained neglected from research point of view. Systematic approach can evolve some solutions to overcome these problems. Therefore, there is necessity to develop a protocol for multiplication of selected female plants. Response Surface

Methodology is useful for analysing and optimizing protocols involving multiple variables affecting a response. RSM uses various statistical, graphical and mathematical techniques to develop, improve and optimize a process. It also use for modeling and analysis of problem, if response variable is influenced by several independent variable. Therefore, in the present study it was proposed to use Response Surface Methodology, for optimizing the micropropagation protocol in *Momordica dioica* female plants. RSM was employed to determine the optimum concentration of plant growth regulators so as to obtain a rapid and efficient micropropagation protocol for large scale commercial propagation with the following specific objectives given below.

1) To study the amount of callus growth on various types of explants
2) To standardize efficient *in-vitro* regeneration and micro propagation protocol in spine gourd
3) To develop a protocol for *in-vitro* shoot multiplication and root induction in spine gourd

2. REVIEW OF LITERATURE

Spine gourd is dioecious and perennial creeper. It is conventionally propagated through seeds, vine cutting and dividing tubers. The conventional methods of propagation impose a severe limitation for commercial cultivation being low rate of seed germination, prolonged seed dormancy, hard seed coat, tubers multiplication, quality of tubers, storage of tubers, sprouting of cuttings and identification of specific sex during early stage of growth period. To overcome these drawbacks, the plant tissue culture is extremely desirable for commercial production of spine gourd with predetermined sex. High multiplication ratio of plants as well as true to type plants can be achieved by micro propagation technique, which enables rapid multiplication of disease and pest free plants within short space and time for commercial propagation. Apart from their use as a tool of research, plant tissue culture techniques in recent years, have become of major industrial importance in area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Considerable work had been done on micropropagation of majority of cucurbits. However very limited and scanty information is available in respect of micropropagation of spine gourd. The available literature on micropropagation techniques of important cucurbitaceous crops including spine gourd are briefly reviewed as under.

2.1 Choice of explant

Explants are small pieces of plant parts or tissues that are aseptically cut and used to initiate a culture in a nutrient medium. Different parts of the plants are used as explants for micropropagation. The correct choice of explant material can have an important effect on the success of tissue culture. The choice of explant depends the kind of culture to be initiated, purpose of the proposed culture and the plant species to be use. Depending upon the goal or objective the choice of the explants differs. For the clonal propagation, the lateral or terminal bud or shoot will serve as better explant. However, for the callus induction, pieces of cotyledons, hypocotyl, stem, leaf or embryo may be used as explant depending upon the crop and genotype. The studies have been carried out in case of spine gourd for the choice of explants are summarized briefly hereunder suitable subheadings.

2.1.1 Apical bud

Apical bub is the one of standardized explant for the *in-vitro* propagation. In cucurbits many scientists have tried apical bud as an explant in their investigation and micropropagation studies *viz*., *Benincasa hispida* (Haque *et al.* 2008, Kausar *et al.* 2013), *Citrullus lanatus* (Compton and Grey. 1992, Khalekuzzaman *et al.* 2012, Vedat *et al.* 2002), *Cucumis hystrix* (Compton *et al*. 2001), *Cucumis melo* (Faria *et al*. 2013, Huda and Sikdar 2006, Venkateshwaralu. 2012), *Cucumis sativus* (Sangeetha *and* Venkatachalau. 2011), *Cucurbita maxima* (Mahazabin *et*

al. 2008), interspecific *Cucurbita* hybrid (Sarowar *et al.* 2003), *Trichosanthes dioica* (Abdul-Awal *et al.* 2005) and *Trichosanthes cucumerina* (Devendra *et al.*2008).

2.1.2 Axillary bud

Several reports in the literature have focused that shoots came be regenerated from the callus, however there is always a chance of somaclonal variation. Therefore, nodal explants are the perfect choice to ensure clonal uniformity among the regenerants (Bopanaan, S. 2008). Nodal explants have the higher capacity to regenerate and multiply in *in-vitro* condition. Many researchers tried axillary bud in their micropropagation investigations *viz*. *Citrullus lanatus* (Khatun *et al.* 2010[b]), *Cucumis anguria* (Margareate. 2014), *Cucumis melo* (Parvin *et al.* 2013, Venkateshawaralu *et al.* 2010), *Cucumis sativus* (Ahamad and Anis. 2005, Firoz Alam *et al.* 2015), *Cucurbita maxima* (Hoque *et al.* 2008), *Momordica balsamina* (Thakur *et al.* 2011), *Momordica charantia* (Sultana *et al.* 2003, Sultana *et al.* 2005, Verma *et al.* 2014), *Momordica cochinchinensis* (Debnath *et al*. 2013[a]), *Momordica cymbalarica* (Devi *et al.* 2017), *Momordica dioica* (Choudhary *et al.* 2017, Debnath. 2013[b], Ghive *et al.* 2006[b], Govind *et al.* 2012, Jadhav. 2015, Kapadia. 2018, Kulkarni. 1999, Mustapha *et al.* 2012, Mustapha *et al.* 2013, Patel and Kalpesh, 2015, and Shekhawat *et al.* 2011.), and *Trichosanthes dioica* (Komal. 2011[a], Komal 2011[b,] Komal 2011[c]).

2.1.3 Cotyledon

Cotyledon is one of the most interested explants by the researchers in micropropagation of cucurbits due its efficiency to produce more callus. In *Benincasa hispida* (Thomas *et al.* 2004) and *Citrullus colocynth* (Ram and Shashtri, 2015) found best result for rhizogenesis by cotyledon explant. *Citrullus lanatus* (Suratman *et al.* 2009, Chaturvedi and Bhantnagar. 2001, Dadauza *et al.* 1997, Khatun *et al.* 2010[a], Krug *et al.* 2005 and Li *et al.* 2011), *Cucumis figarei* (Yutaka *et al.* 1998), *Cucumis melo* (Chovelon *et al.* 2008, Grey *et al.* 1993, Bezirganoglu *et al*. 2014 and Randall *et al.* 1989), *Cucumis metuliferus* (Yutaka *et al.* 1998), *Cucumis sativus* (Yutaka *et al.* 1998, Nanasato *et al.* 2013, Hisajima and Arai. 1989), *Cucurbita ficifolia* (Kim *et al.*2010), *Cucurbita moschata* (Valdez-Melara *et al.* 2009), *Cucurbita pepo* (Paula. 1992), *Lagenaria siceraria* (Han *et al.* 2004), *Luffa acutangula* (Umamaheshwari *et al.* 2014), Zohura *et al.* (2013), *Luffa cylindrica* (Singh *et al.* 2011), *Trichosanthes cucumerina* (Kawale and Choudhary, 2009), *Trichosanthes dioica* (Malex *et al.* 2010) and *Momordica dioica* (Hoque et al. 2000, Karim, 2013, Karim and Ullah. 2011, Nabi *et al.* 2002[a], Nabi *et al.* 2002[b], and Karim. 2011). All the scientist used cotyledon as a explant in spine gourd on a MS media fortified with 1.0 mgl^{-1}BAP + 0.1 mgl^{-1}NAA. However, Arekar (2012) used BAP (4.44 µm and 8.88 µm for spine gourd.

2.1.4 Leaf

Various scientists used leaf explants for micropropagation studies. Few of them who have worked in cucurbits are listed here viz., *Citrullus colocynth* (Devendra. 2009, Guma *et*

al. 2015), *Citrullus lanatus* (Moideen and Prabha. 2013), *Coccinia abyssinica* (Raju *et al.* 2015, reported molecular confirmation of sex by leaf explant), *Cucumis anguria* (Saima malik *et al*. 2007), *Cucumis melo* (Satapathy *et al.* 2014), *Cucumis sativus* (Savitha *et al*. 2010), *Cucumis trigonus* (Shashtree *et al*. 2014), *Luffa acutangula* (Sourab *et al*. 2017), *Luffa cylindrica* (Srivastava and Roy. 2012), *Momordica charantia* (Sultana *et al*. 2004, Swamy *et al.* 2015), *Momordica dioica* (Thiruvengadam *et al.* 2006*,* Thiruvengadam *et al.* 2013, Usman *et al.* 2011), *Trichosanthes dioica* (Rahman *et al*. 2012).

2.1.5 Other explants

Some of the other explants like leaf node, somatic embryo, hypocotyle, petiole, cutting etc. are also used by scientists in their research. *Cucumis melo* (Rahaman *et al.* 2012, used leaf node), *Cucumis sativus* (Ikram-ul haq *et al.* 2013, used cuttings, Selvaraj *et al.* 2006, used hypocotyle, Claveria *et al.* 2005, parthenogenic embryo, Elmeer *et al.* 2009 somatic embryo, Jesmine and Mian 2016 and Kiełkowska and Havey. 2011 used the stem as explant), *Cucurbita pepo* (Pal *et al.* 2007 used hypocotyle), *Lagenaria siceraria* (Hasbullah. 2017, used stem fragments), *Luffa acutangula* (Moideen and Prabha. 2014 and Vellivella *et al.* 2016 used petiole as explant), *Momordica charantia* (Thiruvengadam *et al.* 2012[b]. Used petiole)*, Momordica dioica* (Thiruvengadam *et al.* 2012[a]. used encapsulated shoot tip, Ghive *et al.* 2006[a] healthy shoots, Hoque *et al.* 2007. immature embryo, Karim and Ahamad. 2010 internode, Jamatia. 2016 node and leaf, Thiruvengadam *et al.* 2007 Used leaf), *Momordica sahyadrica (*Rajashekharan *et al.* 2012 Seedlings), *Sechium edule (*Abdelnour *et al.* 2002 Stem) and *Trichosanthes cucumerina* (Kawale and Choudhary. 2009 used cotyledonary node).

2.2 Effect of plant growth regulators in direct regeneration

2.2.1 Apical bud

Thiruvengadam *et al*. (2012[a]) developed an efficient protocol for regeneration of encapsulated shoot tip explants of spine gourd. Shoot tip explants excised from *in- vivo* proliferated shoots were encapsulated in calcium alginate beads. They opined that the gelling matrix of 3% sodium alginate and $CaCl_2$, $2H_2O$ was the most suitable for formation of ideal calcium alginate beads. Further, they reported that maximum response (100%) for conversion of encapsulated shoot tip explants into plantlets could be obtained on 0.7% agar solidified full-strength MS medium containing 0.5 µM BAP. Encapsulated shoot tips could be stored at low temperature (4°C) up to 10 weeks with a survival frequency of 50%. In addition, they also reported that, well-developed regenerated and hardened plantlets acclimatized and established in field with 90% survival frequency, where they grew well without any detectable variations.

Rajashekharan *et al*. (2012) conducted investigations on the *in-vitro* propagation and conservation of *Momordica sahyadrica* species. The various explants from *in-vitro* grown seedling were cultured on modified MS medium (BAP, BAP+NAA/IBA). They observed shoot

differentiation on MS medium supplemented with BAP. Further, shoot as well as root differentiation was obtained on medium containing BAP + IBA/NAA. Multiple shoots with roots were formed on MS medium without hormones. Rooting was induced on shoots in medium containing IBA and 40% of the plants survived successfully when transferred to the field. *In-vitro* grown shoots were conserved for six months without subculture.

Kausar *et al.* (2013) in *Benincasa hispida* used shoot tip and node as explant among which shoot tip showed the highest rate of multiple shoots at 1.5 mgl^{-1} BAP + 0.2 mgl^{-1} GA_3, where average number of shoots per culture was 5.55 and showed that lower concentration of GA_3 found to be effective in multiple shoot induction.

Huda and Sikdar (2006) used IBA in addition to the combination of BAP and GA3 for micropropagation studies in cucurbits and found good shoot initiation and elongation when explants are placed on MS media supplemented with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IBA + 0.3 mgl^{-1} GA_3. Shoot proliferation rate, shoot quality, and other parameters showed best result at the combination of MS with BAP 0.4 µM.

Abdul-Awal *et al.* (2005) reported that in *Trichosanthes cucumerina* the rate of shoot multiplication was maximum 12.00±0.70 after 4^{th} sub culture at concentration of 1.0 mgl^{-1} BAP in combination with lower amount of 0.1 mgl^{-1} NAA. Out of different chemical combinations used, 100% multiple shoot formation was noticed in 1.0 mgl^{-1} BAP + 0.2 mgl^{-1} NAA.

Khalekuzzaman *et al.* (2012) used shoot tip as an explant on MS media fortified with 3.0 mgl^{-1} BAP but in *Citrullus lanatus* they used MS + 5.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. They reported that shoot tip showed maximum frequency (73%) for growth response and 72% of the regenerated plantlets were acclimatized and survived in the soil condition successfully.

Venkateshwaralu (2012) reported that for multiple shoots proliferation MS augmented with 0.5 mgl^{-1} IAA + 2.0 mgl^{-1} BAP was best for induction of shoots for shoot tip explants.

Efficient cloning of *Cucumis hystrix* using 1.0 mm shoot-tip explants. Establishment of Stage I cultures was greatest (83%) when shoot tips were cultured on (per liter) 30 gml^{-1}. sucrose, 0.1 gml^{-1} myo-inositol, and 5.0 gml^{-1} Agargelplus, 1.7 µM IBA, 0.5 µM Kinetin and 0.3 µM GA_3. BAP 5 µM proved best for Stage II shoot proliferation. Plantlet height influenced acclimatization. Over 72% of plantlets survived (Compton *et al.* 2001).

Most of the research scientists found that the range of 1.0-3.0 mgl^{-1} BAP gave good results by using shoot tip as explant in *Cucumis sativus, Cucumis melo, Cucurbita maxima* (Sangeetha *et al.* 2011, Faria *et al*. 2013, Mahazabin. 2008) but some scientist reported that usage of BAP in combination with NAA and IAA in the range of 0.1-0.5 mgl^{-1} helps in establishment of the plant (Abdul-Awal *et al.* 2005, Devendra *et al.* 2008, Khalekuzzaman *et al.* 2012, Venkateshwaralu. 2012).

2.2.2 Axillary bud

In plant tissue culture technique, most of the axillary buds were used to get a multiple shoots due to absence of apical dominance.

Ghive *et al*. (2006[a]) showed that axillary bud was a better explant than the shoot tip for micropropagation of 3 local genotypes of *Momordica dioica*, comprising 2 females (AKSG-5 and AKSG-35) and one male genotype (AKSGM-1). Further they reported that basal MS medium containing $AdSO_4$ (70 or 80 mgl^{-1}) and supplemented with 1.0 mgl^{-1} benzyladenine and 1.0 mgl^{-1} NAA gave the maximum multiple shoots (>5 per culture) with better growth response.

Ghive *et al*. (2006[b]) tried different explants for standardizing the protocol for micropropagation of Spine gourd (*Momordica dioica* Roxb) and opined that axillary buds were the best explant for *in-vitro* propagation. Further, they concluded that combination of MS + 1.5 mgl^{-1} BAP + 10 mgl^{-1} NAA was found to be the best for establishment and initiation of explant. The treatment combination of MS + $AdSO_4$ (70 mgl^{-1} or 80 mgl^{-1}) + BAP (1.0 mgl^{-1}) + NAA (1.0 mgl^{-1}) was adjudged to be superior for multiple shoot development. In addition to this, they opined that MS + AdSO4 (80 mgl^{-1}) + IBA (1.0 mgl^{-1}) was found to be the best treatment for induction and development roots. The maximum survival of plantlets in primary hardening (54.44) was observed on soilrite: cocopeat (3:1) while as regards the local genotypes under study, during secondary hardening, they found that 95 per cent plantlets were survived in case of AKSG-35 followed by AKSG-5 (90%) and AKSGM-1 (75%).

Jamatia (2016) developed an efficient plant regeneration protocol in spine guard by using two explants *viz, in-vivo* nodal and leaf as well as *in-vitro* nodal and leaf explants respectively. Among the different treatments of TDZ and BAP studied, she reported maximum shoot induction (71.6%) in a media containing 1.5 mgl^{-1} TDZ while the highest shoot induction response (83.3%) was noticed in 1.5 mgl^{-1} BAP supplemented media. The 100% shoot regeneration was obtained from *in-vitro* nodal segment in TDZ (0.5-2.5 mgl^{-1}) alone, BAP (1.0-2.0 mgl^{-1}) alone and NAA fortified media. Further she opined that for shoot multiplication, 0.5 mgl^{-1} BAP alone and in combination with 1.0 mgl^{-1} kinetin were most suitable.

Mustafa *et al.* (2012) reported that good amount of compact and green callus from nodal cultures of *Momordica dioica*, could be achieved on MS medium fortified with 2.0 mgl^{-1} 2,4-D + 1.0 mgl^{-1} BAP. They also reported that high frequency of regeneration of plantlets on the BAP + 1.0 mgl^{-1} IAA supplemented medium after the sub culture.

Jadhav (2015) carried out research on four different genotypes of Spine Gourd (*Momordica dioica* Roxb.) to standardize a reliable procedure for shoot and root initiation. The cultures were initiated using nodal explants of genotypes R1P17, R2P5, R9P7 and R11P5. He found that shoot initiation and shoot proliferation were greatly affected by the genotypes, medium combinations and their interactions. Further he reported MS + 1.0 mgl^{-1} BAP + 0.2 mgl^{-1} NAA

medium for genotype R1P17 for initiation of early shoot while genotype R11 P5 produced a greater number of shoots in the same medium. Among all the genotypes under study, highest shoot length was produced by the R11P5 genotypes. The results revealed that the MS medium containing 0.5 mgl^{-1} NAA was the most effective medium for the root initiation in regenerated shoots of all the genotypes. The highest root length was reported by R1P17 genotype on MS + 1.0 mgl^{-1} IBA. The medium MS + 1.5 mgl^{-1} NAA recorded significantly minimum number of day to initiation of root and MS + 0.5 mgl^{-1} IBA recorded significantly maximum root length. The rooted shoots were successfully established in polythene bags containing sand, soil and FYM in 1:2:1 ratio. The established plants were finally transplanted in the field conditions.

Shekhawat *et al.* (2011) reported bud breaking occurrence of nodal explants of female plants of *Momordica dioica* Roxb. on MS media. The nodal segments were harvested and the cut ends of the explants were sealed with wax and then surface sterilized and cultured, bud breaking occurred on Murashige and Skoog's (MS) media. The cultures were amplified by passages on MS medium supplemented with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. Further they observed shoot amplification (29.2 shoots per vessel). The root induction with 89% success rate was recorded in micropropagated shoots when transferred on ½MS medium + 2.0 mgl^{-1} IBA. The *in-vitro* regenerated shoots were also rooted *ex-vitro* with 34% success.

Rai *et al*. (2012) developed an efficient protocol for rapid *in-vitro* clonal propagation of spine gourd (*Momordica dioica* Roxb.) of genotypes RSR/DR15 (female) and DR/NKB-28 (male) enhanced axillary shoot proliferation from nodal segments. Maximum shoot proliferation of 6.2 shoots per explant with 100% shoot regeneration frequency was obtained from the female genotype on Murashige and Skoog's medium supplemented with 0.9 µM BAP and 200 mgl^{-1} casein hydrolysate (CH). While from the male genotype the optimum shoot regeneration frequency (86.6%) and 6.4 shoots per explant were obtained on MS medium supplemented with 2.2 µM BAP. CH induced vigorous shoots, promoted callus formation, and proved inhibitory for shoot differentiation and shoot length, especially in explants from male genotype. Rooting was optimum on ½MS medium (male 92.8 %, female 74.6 %) containing 4.9 µM IBA.

Choudhary *et al*. (2017) reported an improved and efficient micropropagation method for wild female *Momordica dioica* using nodal explants. Shoot amplification was achieved using sub-culturing of *in-vitro* raised shoots on MS medium supplemented with various concentrations of BAP alone or in combination with IAA. The maximum number of shoots (45.30±3.83) with an average length 6.52±0.89 cm were differentiated on MS medium containing 0.5 mgl^{-1} BAP, 0.1 mgl^{-1} IAA and additives (50 mgl^{-1} ascorbic acid, 25 mgl^{-1} each of adenine sulphate, citric acid and L-arginine). The cloned shoots were rooted *ex-vitro*. Each shoot treated with 250 mgl^{-1} IBA for 5 min, produced 12.3±1.33 roots with a mean length 5.4±0.73 cm. More than 85% (46 plants) of *ex-vitro* rooted plantlets were successfully hardened in a greenhouse with

normal growth characteristics. In order to evaluate the genetic stability of micropropagated plants, the two PCR-based techniques, Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) were used. The amplification patterns of the micropropagated and mother plant were monomorphic thus depicting genetic stability of the micropropagation system.

Kulkarni (1999) conducted micropropagation studies in Kartoli by using nodal segment as an explant and developed a proper method of *in-vitro* regeneration and multiplication. MSHP + 80 ppm $AdSO_4$ + 10 ppm BAP + 5.0 ppm IBA +100 mgl^{-1} myo-inositol + 0.8 % Agar agar + 3% sucrose gave the best results (75%). The same medium gave the maximum multiple shoots per culture (81±1.28) at the end of 4^{th} subculture. It was found that the nodal segment cultures of Kartoli initiated maximum rooting response (86.66%) to the medium, MS basal+ 3.0 ppm NAA + 0.8% Agar agar + 3% sucrose + 0.2% activated charcoal. Among the different potting mixture compositions tried for hardening of the *in-vitro* developed plantlets vermiculite alone gave maximum (77.33%) survival and the lowest survival was observed by using FYM (20%) alone.

Kapadia (2018) optimized the culture medium for higher multiplication and efficient micropropagation of spine gourd by using nodal explants. He reported that that supplementation of 1.0 mgl^{-1} NAA along with 1.0 mgl^{-1} BAP induced vigorous and healthy shoots with highest (4.75±0.25) mean number of shoots with average shoot length of around 5.10±0.04 cm. Further, the regenerated shoots of 4 cm in length were used for rooting purpose. The highest rooting percent (86.67%), number of roots per culture (9.6±0.50) and root length (4.59±0.09 cm) with lowest percent callusing at cut ends (33.33%) was reported in the treatment M2 (½MS + IBA 2.0 mgl^{-1}). The well-developed shoots with roots were deliberately transferred to the polythene small size glass containing equal mixture of soil and vermicompost. The established plants were finely transplanted in the field conditions.

Most of the pioneer investigators used the BAP in the range of 0.5, 1.0, 1.5, and 2.0 mgl^{-1} alone or in combination with the other growth regulators for nodal explants (Verma *et al.* 2014, Ahamad and Anis. 2005, Margareate. 2014, Thakur *et al.* 2011, Venkateshawaralu *et al.* 2010, Khatun *et al.* 2010^{b}, Firoz Alam *et al.* 2015, Sultana *et al.* 2005, Hoque *et al.* 2008, Parvin *et al.* 2013, Sultana *et al.* 2003) but Keng and Hoong (2005) reported that multiple shoots could be induced on MS medium supplemented with 8.0 mgl^{-1} BAP in musk melon cv. Honey dew (*Cucumis melo*).

When all were used the full strength MS medium for their research purpose, Verma *et al*. (2014) used half strength MS with 0.5 mgl^{-1} BAP in monoecious Bitter melon, after 3^{rd} sub culture more shoots and shoot length was 3.4 cm and 2.7 cm respectively.

Addition of 200 mgl^{-1} casein hydrolysate to the shoot induction medium (MS + BAP) significantly enhanced the number of multiple shoots in *Cucumis sativus L.* but 200 mgl^{-1} casein hydrolysate + 0.9 µM BAP enhanced axillary shoot proliferation from nodal segments of

Spine gourd. Maximum shoot proliferation of 6.2 shoots per explant with 100% shoot regeneration frequency, CH induced vigorous shoots, promoted callus formation, and proved inhibitory for shoot differentiation and shoot length, especially in explants from male genotype (Ahamad and Anis. 2005).

In *Cucumis melo var utillisimus* highest concentration of 15 mgl^{-1} Adenine sulphate in combination with BAP were found to be best for multiple shoot induction (Venkateshawaralu *et al.* 2010).

In case of *Citrullus lunatus* and *Momordica charantia* 1.0 or 2.0 mgl^{-1} BAP in combination with 0.1 or 0.2 mgl^{-1} NAA for early shoot initiation, establishment and maximum shoot multiplication with significantly more height and good percentage of acclimatized and successful survivalence of rooted plants in *ex-vitro* condition (Khatun *et al.* 2010[b], Sultana *et al.* 2003).

Sultan (2005) conducted experiment to know the effect of different concentration of sucrose, pH and agar by using nodal explants of *Momordica charantia*. For maximum shoot induction, medium containing MS + 2.0 mgl^{-1} BAP + 0.2 mgl^{-1} NAA, with 30 mgl^{-1} sucrose, 7.0 mgl^{-1} agar and 5.5-6.0 level pH was found to be best. 30 mgl^{-1} sucrose gave 100% shoot proliferation with shoot number and length of 5.1±0.8 and 5.6±0.4 cm respectively. MS medium having 7.0 mgl^{-1} agar showed 100% frequency in shoot proliferation.

Margareate (2014) reported that high frequency of multiple shoot regeneration was achieved on MS medium containing 1.0 mgl^{-1} BAP + 0.2 mgl^{-1} NAA + 20 mgl^{-1} L - glutamine while elongation of shoots were achieved by adding 0.5 mgl^{-1} GA_3 in *Cucumis anguria*.

A minimal medium has been formulated in order to reduce the cost and time of *in-vitro* raised plants of *Trichosanthes dioica* Roxb. Semi solid MS with coconut water 15% showed the highest percentage of plantlet regeneration (99%), when explants are cultured on this medium rhizogenesis was observed in 5-6 days, followed by shoot formation in 8-10 days (Komal 2011[c]).

2.2.3 Cotyledons

In micropropagation, cotyledons play a vital role In giving the successful plantlets in this regard most of the scientist were used the BAP alone or in combination with the other growth hormone for regeneration of plant in that most of investigators were concluded that BAP in the range 1.0, 1.5, 2.0 mgl^{-1} showed to be best for optimum plant regeneration (Malex *et al.* 2010, Zohura *et al.* 2013, Li *et al.* 2011).

Karim and Ullah (2011) tested four explants *viz.* internodes, nodes, leaves, shoot tips and cotyledons for *in-vitro* culture of spine gourd. They observed better performance of cotyledon explants for callus, shoot and root induction of spine gourd (98.33%) at 1.0 mgl^{-1} BAP. Callus initiation was induced by 1.0 mgl^{-1} BAP supplemented with 0.5 mgl^{-1} NAA from internode explants within shortest time (14 days). Proliferated calli were further cultured on MS medium for

shoot initiation. The cotyledon explants produced highest frequency of shoot formation (89.67%). MS medium containing 1.0 mgl^{-1} BAP resulted in maximum number of shoots (5.33%) and longest shoots (0.9 cm). The shoots were sub-cultured on MS medium supplemented with IBA and IAA for rooting and sufficient roots were induced on half MS medium containing only 0.3 mgl^{-1} IBA.

Arekar (2012) concluded that non-conventional methods of propagation *i.e.*, tissue culture offers an efficient method for propagation of *Momordica dioica*. He cultured the decoated seeds of *Momordica dioica* on MS basal medium supplemented with 4.44 µM and 8.88 µM BAP and reported to give rise to maximum number of shoots in 7-8 weeks. Further he opined that MS medium with 0.049 µM IBA showed rooting in 45 days and the regenerated plantlets were successfully hardened in vermiculite.

Zohura *et al.* (2013) reported best organogenesis in *Luffa acutangula* by using cotyledon as explant, when the media is supplemented with 1.5 mgl^{-1} BAP plus 1.0 mgl^{-1} NAA.

When Water melon is treated with the lower concentration of BAP 20 µM, the highest mean number of shoots reported were 9.83±0.81, whereas another scientist used cotyledons excised from 7-day-old aseptic seedlings the Sugar baby variety of *Citrullus lunatus* Thumb, Matsum and Nakai and reported the highest percentage of shoots on MS + 3.0 µM BAP + 3.0 µM 2iP and MS + 3.0 µM BAP + 3.0 µM IAA. Finally 55% plants showed success in field (Suratman. 2009, Chaturvedi and Bhantnagar. 2001).

Indole 3-Acetic acid is used by the several scientist in *Cucurbita ficifolia, Citrullus lanatus, Citrullus colocynth* for getting plantlet but use MS + 2.0 mgl^{-1} IAA + 1.5 mgl^{-1} IBA in *Citrullus colocynth,* rhizogenesis was reported by Rama Krishna and Shashtri (2015), along with that MS + 0.1 mgl^{-1} IAA + 1.0 mgl^{-1} zeatin was also found to be efficient shoot regeneration medium for *Cucurbita ficifolia* (Kim *et al*. 2009).

Randall *et al.* (1989) in their studies placed cotyledonary explants of *Cucumis melo* on MS medium supplemented with 5.0 µM IBA and 5.0 µM BAP and cultured at 25-29°C under low light intensity (5-30 $\mu molm^{-2}s^{-2}$). They reported that the presence of abscisic acid significantly increased the number of explants producing shoot buds. Further they opined that bud initiation was affected by genotype, seedling age, light intensity, and temperature. Addition of Gibberellic acid, Thidiazuron or Silver nitrate to regeneration medium did not improve either bud initiation or shoot regeneration.

2.3 Effect of plant growth regulators on somatic embryogenesis

Plant regeneration via somatic embryogenesis follows the initiation of embryonic culture, proliferation of embryonic culture, prematuration of somatic embryo, maturation of somatic embryo and plant development on nonspecific media. So many interested scientists worked on plant regeneration by using somatic embryogenesis.

2.3.1 Cotyledon

Hoque *et al.* (2007) showed that higher percentage of adventitious shoots could be regenerated from the immature embryo explant than that of immature cotyledon of the crosses made between female and female (pollen was collected from induced bisexual flower) of tetraploid Kakrol (*Momordica dioica*). Further they suggested that the best response of shoot proliferation was obtained in embryo explants grown in supplemented with 10.8 mgl^{-1} BAP, 1.08 mgl^{-1} NAA and 0.54 mgl^{-1} GA3; whereas, shoot regeneration on cotyledon was achieved on a MS supplemented with 16.2 mgl^{-1} BAP, 2.7 mgl^{-1} NAA and 0.54 mgl^{-1} GA_3. In both cases shoot primordia emerged continuously. Regenerated shoots were excised from both sources and rooted in MS supplemented with different concentrations of IAA. Rooted plantlets were acclimatized successfully and later established in the field for the production of female plant and also evaluation of somaclonal variation.

High frequency somatic embryogenesis that is 3.3 somatic embryos were noticed in *Cucumis melo* on 5 mgl^{-1} 2, 4-D and 0.1 mgl^{-1} TDZ, 3% sucrose highly significant in embryo induction and development (Gray *et al*. 1993). Random Amplified Polymorphic DNA (RAPD) markers to evaluate genetic stability of regenerants of cucumber plants obtained through somatic embryogenesis were carried out by using five primers OP-C10, OP-G14, OP-H05, OP-Y03 and OP-AT01 and found that there is no any significant visual differences between the somatic embryo plants and F1 hybrids (Elmeer *et al.* 2009). The multiple shoots were observed in a media supplemented with 2.5-5.0 µm BAP in *Cucumis sativus* by using cotyledons as explant (Hisajima and Arai, 1989). Somatic embryogenesis was successfully achieved in *Cucurbita pepo* by using shoot tip and cotyledon at various combination and concentration of 1.2 mgl^{-1} 2,4,5-T + 0.8 mgl^{-1} BAP + 0.1 mgl^{-1} Kinetin and 4.7 µm 2.,4,5-T + 4.0 µm BAP + 0.5 µm Kn, respectively where best callus was noticed in cotyledon derived explant (Paula *et al.* 1990 and Paula, 1992). Friable embryogenic calli was produced from zygotic embryos (53-56%) and from cotyledonary seedlings (70%) of *Cucurbita moschata* cv. Sello de Oro cultured on callus induction medium (CIM) supplemented with 0.5 mgl^{-1} or 3.5 mgl^{-1} 2,4-D. Embryogenic calli induction was achieved in 75% *C. moschata* pure lines and when further evaluated reported calli percentage frequency range from 5% to 34%. Regenerated plants appeared morphologically normal and set flowers and fruits with seeds that could germinate normally (Valdez-Melara *et al.* (2009). Different main cucurbits such as cucumber, watermelon, squash, and melons were studied to build a protocol for somatic embryogenesis out of several explants used cotyledons and hypocotyls given the best result. Genetic constitution of mother plants seems to play a key role in somatic embryogenesis. Somatic embryos can exhibit developmental abnormalities, particularly when they arise from protoplast-derived cultures (Debeaujon and Branchard. 1993).

2.3.2 Leaf

Thiruvengadam *et al.* (2013) evaluated an efficient method of somatic embryogenesis using exogenous polyamines through suspension culture and developed a protocol for leaf derived callus of spine gourd. Embryogenic callus was originated on MS medium supplemented with 4.4 µm 2,4-D, with addition of poly amines (Putriscine, Spermidine and Leprine). Putriscine at 1.0 µm increased fresh weight of embryogenic calli and maximum somatic embryogenesis observed on MS medium + 3% sucrose + 3.3% µm 2,4-D for three weeks of culture.

Raju *et al.* (2015) revealed that highest percentage (85%) of embryogenic callus was obtained from MS medium supplemented with 2.0 mgl^{-1} each of 2, 4-D and BAP in leaf explants of spine gourd. Further they observed maximum number of shoots (12.15±1.51 shoots) on MS medium augmented with 4.0 mgl^{-1} BAP in combination with 2.0 mgl^{-1} L-glutamine from leaf derived embryogenic callus of spine gourd.

Usman *et al*. (2011) recorded the maximum callus induction of 94.16% in leaf disc explants of cucumber on MS medium supplemented with 2.0 mgl^{-1} 2, 4-D. The calli induced in leaf disc on the 5.0 mgl^{-1} 2, 4-D yielded the highest embryo formation i.e., 23%.

2.3.3 Petiole

Karim and Ahamad (2010) reported that *In-vitro* somatic embryogenesis and subsequent plant regeneration was achieved in callus cultures derived from internode, node, shoot tip, petiole and leaf explant. Highest callus induction was noticed in intermodal explant of teasle gourd plant on semi-solid Murashige and Skoog (MS) basal salts and growth regulators supplemented with 1.0 mgl^{-1} BAP, 0.1 mgl^{-1} NAA and 30 gml^{-1} (w/v) sucrose. Somatic embryos proliferated rapidly by somatic embryogenesis after 4 weeks. The embryogenic callus germinated on MS salts and growth regulators supplemented with 1.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA. The embryo-derived plantlets were transferred on ½MS media with 0.3 mgl^{-1} IBA and sufficient rooting was achieved. Plantlets were acclimatized in the controlled environment.

Punja *et al.* (1990) achieved regeneration in six inbreed lines or F_1 hybrids of *Cucumis sativus* on MS medium containing various concentration of 2,4-D/BA, NAA/BA, NAA/Z, NAA/K. The range of regeneration frequency for cotyledon, leaf and petiole explants was 0-38, 0-75 and 14-96% respectively, after 6-8 weeks in culture. Highest frequency of plantlet formation occurred with petiole explants incubated on NAA/BA (5.0/2.5 µM), NAA/Z (5.0/5.0 µM) or 2,4-D/BA (5.0/5.0 µM). Approximately 80% of these plantlets survived after transplanting to the green house soil, and they flowered and set fruit. F_1 hybrids endeavor given highest regeneration frequency of 91% on 2,4-D/BA at (5.0/5.0 µM). Formation of the somatic embryos was observed on 2,4-D/BA, while organogenesis and embryogenesis both were evident on NAA/BA, NAA/Z. Cotyledon explants yielded lowest (7%) frequency of plantlet formation.

2.3.4 Others explants

In cell suspension culture, single or small aggregates of cells multiply while suspended in agitated liquid medium. Thiruvengadam is one of the pioneer research scientist who worked more and more on the cell suspension culture of some cucurbits to achieve somatic embryogenesis. In all his findings he used the 2,4-D either single or in combination with the other plant hormones, in the range of 2.2 µm and 2.0 µM 2,4-D with 0.5 µm L- glutamine and found to be the best for somatic embryogenesis by using petiole and leaf explants in *Momordica dioica* and *Cucumis anguria* respectively.

2.4 Effect of plant growth regulators on organogenesis

Organogenesis is the development of adventitious organs or primordia from undifferentiated cell mass called callus in tissues culture by the process of differentiation. The regeneration of plant or plant organs only taken place by the expression of cellular totipotancy of the callus tissue. In the process of organogenesis a good quality of callus initiation will play a vital role for the further regeneration.

2.4.1 Apical bud

Patel (2015) developed an efficient protocol for *in-vitro* shoot multiplication and regenerations of spine gourd as well as to check its antidiabetic activity by using nodal segments as explants. MS agar-gelled medium with optimum concentration of 1.5 mgl^{-1} BAP + 0.1 mgl^{-1} NAA and 0.5 mgl^{-1} NAA + 0.5 mgl^{-1} NB_6 had an effect on callus production. Shoot multiplication was found best in 0.5 mgl^{-1} NB_6 + 0.5 mgl^{-1} BAP. After 15 days, shoot length of 5.2±0.37cm and shoot numbers 10±1.4 were observed. In the investigations, he developed a novel method by which multiple shoot can be induced on MS medium supplemented with cytokinins (BAP, NAA and NB_6). This is the first report in *Momordica dioica,* that he used the NB_6 growth hormones for induction of callus and multiplication of shoot.

Mustafa *et al.* (2012) reported that good amount of compact and green callus from nodal cultures could be achieved on MS medium fortified with 2.0 mgl^{-1} 2,4-D + 1.0 mgl^{-1} BAP. High frequency of regeneration of plantlets was observed on the 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} IAA medium after the sub culture. Best rooting was observed on 3.0 mgl^{-1} IBA.

Agarwal and Kamal (2004) investigated on *in-vitro* clonal propagation of *Momordica charantia.* The explants from *in-vitro* grown seedlings were cultured on modified MS medium. Shoot differentiation was obtained on MS medium supplemented with BAP. Root, callus were formed on IBA and 2,4-D, respectively. Shoot as well as root differentiation was obtained on medium containing BAP+IBA/NAA. Multiple shoot with roots were formed on MS medium without hormones. Rooting occurred on grown shoot on medium containing IBA and 40% of the plants survived successfully, when transferred to the field.

2.4.2 Axillary bud

Coconut milk is the one of the very good organic source of growth hormones mainly for cytokinins. In this regards, Debnath *et al* (2013[b]) by using nodal explants of *Momordica dioica* And *Momordica cochinchinensis* had reported that highest percentage of callusing and organogenesis, in both the species, was observed by keeping coconut milk and 2,4-D constant i.e. 15% v/v and 2.0 mgl^{-1} respectively. Further, along with both the concentrations in *Momordica dioica* they added BAP 0.5 mgl^{-1} while in case of *Momordica cochinchinensis* didn't use the BAP with agar gelled MS as basal medium. In another treatment of *Momordica dioica*, coconut milk was avoided and good amount of compact and green callus was obtained on 2.0 mgl^{-1} 2, 4-D + 1.0 mgl^{-1} BAP (Debnath. 2013[a], Debnath *et al*. 2013[b], Mustafa *et al.* 2012). Direct organogenesis was reported in *Momordica cymbalarias* on BAP 2.0 mgl^{-1} for shoot regeneration (Devi *et al.* 2017).

2.4.3 Cotyledon

Nabi *et al*. (2002[b]) studied different types of explants like node, shoot tip, leaf and the cotyledon in case of spine gourd. Among all the explants used, the cotyledon showed the best performance. The combination of 1.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA was found most suitable in callus induction followed by 0.2 mgl^{-1} NAA. The highest number of multiple and tallest shoots were obtained on MS medium fortified with 1.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA. For rooting, ½MS supplemented with IBA proved to be better than IAA, although on ½ MS supplemented with IAA tallest shoot were observed.

Karim (2013) conducted research on organogenic callus induction and high frequency shoot regeneration from cotyledon explants of Teasel gourd. About 94.8% of cotyledon explants derived from 18 day old cotyledon produced green, compact nodular organogenic callus in MS medium containing 1.0 mgl^{-1} BAP after 51 days with 95% shoot induction. The elongated shoots rooted in MS medium supplemented with 0.3 mgl^{-1} IBA rooted plants were acclimatized in growth room and subsequently established in soil with 100% survivability. This protocol produced an average of 19.8 shoots cotyledon per explant in 22.2 days of culturing.

Hoque *et al*. (2000) reveled that high frequency callus formation occurred on MS medium supplemented with 0.2-1.0 mgl^{-1} 2, 4-D alone, or with 0.2-1.0 mgl^{-1} NAA alone or in combination with 1.0-2.0 mgl^{-1} BAP or 1.0-2.0 mgl^{-1} Kinetin. Growth, morphological nature and organogenic potentiality of the calluses varied with the growth regulator supplements. The medium containing both 2.0 mgl^{-1} BAP and 0.5 mgl^{-1} NAA elicited the highest shoot regeneration rate (58.1% from the apical and 71.3% from the basal half of cotyledon explants). However, the maximum number of shoots per cotyledon explant regenerated on medium containing 2.0 mgl^{-1} BAP and 0.2 mgl^{-1} NAA. The *in-vitro* regenerated shoots were rooted on MS medium supplemented with 1.0 mgl^{-1} IAA and successfully transferred to soil.

Karim (2011) generated a complete reproducible protocol for large scale propagation and *in-vitro* regeneration from different organs of teasel gourd. Internodes, nodes, leaves, shoot tips and cotyledons were used as explants. Cotyledons showed higher percentage of callus induction (98.33%) at 1.0 mgl^{-1} BAP in 15.33 days whereas callus initiation was induced by 1.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA from internode explant within shortest time (14.21 days). Proliferated calli were cultured on MS medium for shoot initiation. The cotyledon explants had produced highest frequency of shoot formation (89.67%). MS medium containing 1.0 mgl^{-1} BAP resulted maximum number of shoots (5.33) and longest shoots (0.9 cm). The shoots were sub-cultured on MS medium supplemented with IBA and IAA for rooting. Sufficient roots were induced on ½ MS medium containing only 0.3 mgl^{-1} IBA. Thus a protocol of regeneration of teasel gourd has been developed via callus formation using cotyledon explants.

Organogenesis in water melon was studied and best result were obtained in cotyledon segments from the proximal region, explants were cultivated in medium MS supplemented with 1.0 mgl^{-1} BAP and 10% coconut water. The histological study showed that the organogenesis occurs directly, without callus formation, on epidermal and sub-epidermal layers of the explants (Krug *et al*. 2005).

In the process of organogenesis BAP supplemented with the other growth hormones found best for callusing. In this regards, so many persons used the 1.0-2.0 mgl^{-1} BAP and supplemented with the lower concentration of NAA 0.1 mgl^{-1} to higher concentration of 0.5 mgl^{-1} in *Lagenaria siceraria* by using cotyledon and stem as explant (Hoque *et al*. 2000, Hasbullah. 2017).

Umamaheshwari *et al.* (2014) regenerated multiple shoots via indirect organogenesis in *Luffa acutangula* L. Roxb. from cotyledon explants. Further they reported that about 78.34% cotyledon derived callus produced 10.3 shoots/ explants on MS + BAP 1.0 mgl^{-1} + 0.2 mgl^{-1} Zeatin + 0.2 mgl^{-1} NAA + 0.6 mgl^{-1} 2,4-D + 0.1 mgl^{-1} Picloram + 20 mgl^{-1} $AdSO_4$.

Han *et al.* (2004) reported that maximum shoot regeneration observed in proximal parts of cotyledons from 4-day-old seedlings of bottle gourd when cultured on MS medium with 3.0 mgl^{-1} BAP and 0.5 mgl^{-1} $AgNO_3$ under a 16 hr photoperiod. Flow cytometric analysis revealed that most of the $AgNO_3$ derived culture were diploid.

Moon *et al.* (2000) carried the experiment to find out the effects of plant growth regulators on callus formation, rooting and shooting from cotyledon explant in oriental melon. Various combinations of 0.1 mgl^{-1} auxins (IAA, NAA) and 0.5, 1.0, 1.5, 2.0 mgl^{-1} cytokinins (BA, kinetin, zeatin) were treated to the MS basal medium, respectively. Callus was induced most effectively as 2,437.0 mg (FW)/ explant in MS medium supplemented with 0.1 mgl^{-1} NAA and 2.0 mgl^{-1} BA, but that was non-embryogenic callus as colored yellow white and broke easily. Root was induced most effectively at a frequency of 98.0% in MS medium supplemented with 0.1

mgl^{-1} NAA and 0.5 mgl^{-1} kinetin.

In most of the findings, researchers used cotyledon as an explant in various cucurbit crops and they used BAP as good callus inducing growth hormone either single or in combination with various kind of growth regulators. In all the best callusing reports, BAP range is 1.0 1.5, 2.0 and 3.0 mgl^{-1} (Krug *et al.* 2005, Compton and Grey, 1992, Karim, 2013, Yutaka *et al.* 1998, Hasbullah, 2017, Han *et al.* 2004,). Callus induction and subsequent regeneration potentiality of watermelon from cotyledon and internode was also studied. Greenish compact callus was achieved from cotyledon on MS with 1.0 mgl^{-1} 2, 4-D within one week of inoculation (Khatun *et al*. 2010[a]).

In-vitro organogenesis from hypocotyle explant of *Cucumis melo* var Poinsett, calli were induced on MS + 87.64 µM Sucrose + 0.8% agar + 3.62 µM 2,4-D + 2.22 µM BAP, and regeneration of adventitious shoot from these calli (25 shoots per explant) were achieved on MS + 8.88 µM BAP + 2.5 µM zeatin + 10% coconut water (Selvaraj *et al*. 2006).

The 2,4-Dichlorophenoxy acetic acid at 2.5 mgl^{-1} gave best callusing percentage in hypocotyle explants of *Cucurbita pepo* and the highest percentage of shoot regeneration (85%) was obtained at 0.5 mgl^{-1} TDZ. About 70% of regenerated plantlets survived under *ex-vitro* conditions (Pal *et al.* 2007). MS + BAP (1.0-6.0 µM) + 0.2 and 0.5 µM NAA were observed as a media which gave best response when cotyledon explant of *Benincasa hispida* was used. (Thomas *et al.* 2004).

2.4.4 Leaf

Nabi *et al.* (2002[a]) used leaf explants for *in-vitro* culture of spine gourd. They observed swelling of the leaf in MS supplemented with 2.0 mgl^{-1} BAP + 0.10 mgl^{-1} NAA. A high callus growth was obtained in the MS augmented with 2.0 mgl^{-1} BAP + 0.2 mgl^{-1} NAA and 3.0 mgl^{-1} BAP + 0.2 mgl^{-1} NAA. However, leaf explants had showed no response to shoot initiation.

Swamy *et al*. (2015) developed an efficient protocol for *in-vitro* regeneration in *Momordica dioica* with leaf explants. The leaf explants were inoculated on MS medium supplemented with different combinations and concentration of 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. After 3 weeks, cultures were observed for proliferated callus. The callus was turned to white friable and green compact callus on a media with high concentration with cytokinine 1.0 mgl^{-1} BAP. After sub culture of same callus in 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, 80% of regenerated plantlets were found. With low concentration of cytokinine 0.5 mgl^{-1} BAP, no callus induction was observed.

Devendra (2009) established efficient plant regeneration via organogenesis in *Momordica dioica* (Roxb.) Wild. Using leaf derived calli. The maximum morphogenic callus induction rate (80%) was observed from leaf explant by culturing in MS medium supplemented with 1.0 mgl^{-1} 2, 4-D + 2.0 mgl^{-1} BAP. Calli size and fresh weight increased substantially through sub-culturing. The highest percentage of shoot regeneration (70%) and highest mean number of shoots (12.33) per culture were obtained with 1.5 mgl^{-1} BAP + 1.5 mgl^{-1} Kn. Leaf explants were

more responsive than node explants in terms of callus induction and subsequent regeneration. Regenerated shoots were rooted in MS medium supplemented with 1.0 mgl^{-1} IBA. About 70% of regenerated plantlets survived.

For the production of callus, TDZ is one of the major source for callusogenesis, in single or in combination. Most of the scientists used combination form of TDZ with 2, 4-D and Kinetine, Best callusogenic response was observed in 2, 4–D + 2.0 mgl^{-1} TDZ and MS +2.0 mgl^{-1} Kn + 1.0 mgl^{-1} TDZ in *Luffa acutangula* and *Citrullus colocynth* respectively by using leaf as explant (Moideen and Prabha, 2013, Shashtree *et al.* 2014).

Guma *et al.* (2015) conducted study to develop efficient protocol for sterilization and callus induction for *Coccinia abyssinica*, Maximum clean survival explants were obtained (82.5±0.5) at 5% NaOCl with 10 minutes and best maximum callus induction (80±2) was achieved from the combination 5.0 µm of BAP and 2, 4-D.

During the course of organogenesis callusing is the first step to induce a good quality callus. In some many investigations, best callusing range of 2,4-D is 0.5, 1.0, 1.5 and 2.5 mgl^{-1} with BAP 0.5, 1.0 and 2.0 mgl^{-1} in leaf explants of *Citrullus lanatus, Citrullus colocynth, Trichosanthes dioica* (Sultana *et al.* 2004, Savitha *et al.* 2010, Sourab *et al.* 2017). Some of the scientists used either BAP alone or BAP with IAA to get the callus followed by organogenesis. If BAP is used single, the most desirable concentration was 1.0 and 1.5 mgl^{-1} or if used with IAA then MS + 1.0 mgl^{-1} BAP + IAA 0.25 mgl^{-1} was found to be best in *Cucumis trigonus* leaf explants (Satapathy *et al.* 2014). In case of *Luffa cylindrica,* BAP 1.0, 1.5 and 3.0 mgl^{-1} in combination with the NAA 0.1, 0.5 and 1.0 mgl^{-1} was found to be the best source of callusing and organogenesis. In some cases, use of 1.0 mgl^{-1} BAP + 1.0 mgl^{-1} NAA was reported for multiple shoot regeneration from callus leaf explants (Srivastava and Roy. 2012, Swamy *et al.* 2015).

2.4.5 Petiole

Thiruvengadam *et al.* (2007) studied on efficient protocol for plantlet regeneration from cell suspension culture of spine gourd through somatic embryogenesis. Petiole derived calli were cultured on MS medium with 4.5 µm 2, 4-D and 10% coconut milk. Maximum frequency of somatic embryos (36.3%) was observed on MS supplemented with 2.2 µm 2, 4-D. Embryo development was asynchronous and strongly influenced by 2, 4-D concentration. The MS liquid medium augmented with 2.2 µm 2, 4-D and 0.5 µm L-glutamine was effective in achieving highest somatic embryos induction (44.5%). Sucrose was found to be effective as carbon source for callus induction, embryo maturation and embryo germination. Relatively, only few number of embryos developed into shoots/ roots when transferred to 1/10 MS solid medium containing 0.5 µm abscisic acid (ABA), 2% (w/v) sucrose, 0.2% (w/v) Gelrite. About 11% somatic embryo germinated morphologically normal fertile plants within 2 weeks. Regenerated plants were successfully hardened, with a survival rate of approximately 60%, and established in the field.

Thiruvengadam *et al.* (2012[b]) reported that addition of polyamines in culture media enhanced the percentage of callus induction in organogenesis of bitter melon by using petiole as explant, 3.0 µM NAA, 1.0 µM TDZ and 1.0 µM putrecine induced (95.0%) callus induction and Regeneration of adventitious shoots from callus (53 shoots per explant) was achieved on 3.0 µM TDZ with 1.0 µM NAA and 1.0 µM Spermidine.

Callus induction and multiplication was tried on *Luffa acutangula* from node, leaf and petiole explant. Out of all explants, petiole showed the best callusing percentage in 1.5 mgl^{-1} 2, 4–D + 1.5 mgl^{-1} TDZ (Moideen and Prabha. 2014). IISR marker techniques were used to find out the clonal fidelity from the callus derived regenerated plant of *Luffa acutangula.* In this 2.0 mgl^{-1} BAP and 0.2 mgl^{-1} NAA were used for highest callus (Vellivella. 2016).

2.4.6 Others

Vedat *et al.* (2002) used the 0.5 mgl^{-1} BAP and got 50% more number of shoots in *Citrullus lunatus* for higher organogenesis. The effect of commercial fruit juices were tested for callus induction, its proliferation and plant regeneration in cucumber. Orange, apple, strawberry and red grapes were used in the place of 3% sucrose. Out of these, MS supplemented with Orange juice was found to be the best source of callusing (Ikram-ul Haq *et al.* 2013).

2.5 In-vitro root induction

Ghive *et al*. (2006[b]) reported that healthy shoots of spine gourd having their own root system were able to survive and became complete plantlets. The highest rooting was observed in AKSGM-1(93%) and obtained on RM3 (MS+IBA 1.0 mgl^{-1}). Further, they reported AKSG-5 with a greater number of primary roots (4.33) per culture and AKSG-35 with maximum root length (4.17 cm).

Table 1: Review of literatures in table form					
Sr. No.	**Crop**	**Explant**	**Best treatments (mgl^{-1})**	**Result**	**Author**
1	*Sechium edule*	Stem part	MS + BAP (0.1)	Full plantlet in soil	Abdelnour, *et al.*(2002)
2	*Trichosanthes dioica*	Shoot tip	MS + BAP (1.0) + NAA (0.2)	Full plantlet in soil	Abdul-Awal, *et al.* (2005)
3	*Cucumis sativus L.*	Node	MS + BAP (1.0 µM) + Casein hydrolysate (200)	Full plantlet in soil	Ahamad and Anis (2005)
4	*Momordica dioica*	Cotyledon	MS + BAP (4.44 and 8.88 µm)	Full plantlet in soil	Arekar (2012)
5	*Cucumis melo L.*	Cotyledon	Bacteria concentration of OD_{600} 0.6, inoculation for 30 min,	Genetic transformation	Bezirganogalu, *et al*. (2014)
6	*Citrullus lanatus*	Cotyledon	MS + BAP (3.0 µM) + 2iP (3.0 µM)	Full plantlet in soil	Chaturvedi, *et al.* (2001)
7	*Momordica dioica*	Node	MS + BAP (0.5) + IAA (0.1) + Ascorbic acid (50) +Adenine sulphate, Citric Acid , L-arginine (25)	Full plants in soil, monomorphic, genetic stability	Choudhary, *et al.* (2017)
8	*Cucumis melo L.*	Cotyledon	MS + BAP + 2.0-iP	Agrobacterium mediated Genetic transformation	Chovelon, *et al.* (2008)
9	*Cucumis sativus L.*	Parthenogen ic embryo	500 gamma radiation, Co 60 Ý- rays source	Haploid production	Claveria, *et al.* (2005)
10	*Citrullus lanatus*	Shoot tip	MS + BAP (1.0)	Full plantlet in soil	Compton and Grey (1992)
11	*Cucumis hystrix*	Shoot tip	MS + Sucrose (30 g) + myo-inositol (0.1 g) + Agargelplus (5 g) + IBA (1.7 µM) + Kinetin (0.5 µM) + GA_3 (0.3 µM)	Full plantlet in soil	Compton, *et al*. (2001)
12	*Citrullus lanatus*	Cotyledon	Agrobacterium tumefacie ns LBA4404 + vector pBI121 + r gene β–glucur onidase (gus) + neomycin phosphotransferase(nptII)	For transgenic	Dabauza, *et al.* (1997)
13	*Cucurbitaceae*	Cotyledon		Reviewed somatic embryogenesis	Debeaujon and Brancherd (1993)
14	*Momordica dioica*	Node	MS + 2, 4-D (2.0) + BAP (0.5) / Coconut milk (15% v/v).	Organogenesis	Debnath *et al.* (2013^{b})

15	*Momordica cochinchinensis*	Node	MS agar gelled + 2, 4-D (2.0) + Coconut milk (15% v/v)	Callus	Debnath, *et al*. (2013[a])
16	*Momordica dioica*	Leaf	MS + 2, 4-D (1.0) + BAP (2.0)	Organogenesis	Devendra *et al*. (2009)
17	*Trichosanthes cucumerina*	Shoot tip	MS + BAP (1.0) + NAA (0.1)	Full plantlet in soil	Devendra, *et al.*(2008)
18	*Momordica cymbalarica*	Node	MA + BAP (2.0)	Full plantlet in soil	Devi, *et al.* (2017)
19	*Cucumis sativus L.*	Somatic embryo	Primers (OP-C10, OP-G14, OP-H05, OP-Y03 and OP-AT01)	Genetic stability by RAPD	Elmeer, *et al.*(2009)
20	*Cucumis melo L.*	Shoot tip	MS + BAP (2.0)	Full plantlet in soil	Faria, *et al*. (2013)
21	*Cucumis sativus L.*	Node	MS + BAP (1.5)	Full plantlet in soil	Firoz Alam, *et al.* (2015)
22	*Momordica dioica*	Healthy shoots	MS + IBA (1.0)	Highest percent of rooting	Ghive, *et al.* (2006[a])
23	*Momordica dioica*	Node	MS + $AdSO_4$ (70/ 80) + BAP (1.0) + NAA (1.0)	Multiple shoots	Ghive, *et al.* (2006[b])
24	*Momordica dioica*	Node	MS + BAP (0.6 µm) + Casein hydrolysate (200)	Assesse d genetic stability by RAPD	Rai, *et al.* (2012)
25	*Cucumis melo L.*	Cotyledon	MS + 2,4-D (5) + TDZ (0.1)	Somatic embryogenesis	Grey, *et al.* (1993)
26	*Coccinia abyssinica*	Leaf	5% NaOC with 10 Minutes	Sterilization	Guma, *et al.* (2015)
27	*Lagenaria siceraria*	Cotyledon	MS + BAP (3) +$AgNO_3$ (0.5)	$AgNO_3$ derived plants are diploid	Han, *et al.* (2004)
28	*Benincasa hispida*	Shoot tip	MS + BAP (1.5)	Full plantlet in soil	Haque, *et al.* (2008)
29	*Lagenaria siceraria*	stem	MS + BAP (2.0) + NAA (0.5)	Full plantlet in soil	Hasbullah (2017)
30	*Cucumis sativus L.*	Cotyledon	MS + BAP (2.5-5 µm)	Multiple shoots	Hisajima and Arai (1989)
31	*Momordica dioica*	Cotyledon	MS + BAP (2.0) + NAA (0.5)	Organogenesis	Hoque. *et al.* (2000)
32	*Momordica dioica*	Immature embryo	MS + IBA (10.8) + NAA (1.08) + GA_3 (0.54)	Full plantlet in soil	Hoque, *et al.* (2007).
33	*Cucurbita maxima*	Node	MS + BAP (2.0)	Full plantlet in soil	Hoque, *et al.* (2008)
34	*Cucumis melo L.*	Shoot tip	MS + BAP (1.0) + IBA (0.1) + GA_3 (0.3)	Full plantlet in soil	Huda and Sikdar (2006)
35	*Cucumis sativus L.*	Cuttings	MS + Orange juice	Callus	Ikram-ulhaq, *et al.* (2013)

36	*Momordica dioica*	Node	MS + BAP (1.0) + NAA (0.2)	Full plantlet with Genotypes response	Jadhav (2015)
37	*Momordica dioica*	Node	MS + BAP (1.5)	Full plantlet in soil	Jamathia (2016)
38	*Cucumis sativus L.*	Stem	MS + BAP (0.5) + NAA (1.0)	Callus	Jesmine and Mian (2016)
39	*Momordica dioica*	Node	MS + BAP (1.0) + NAA (1.0)	Full plantlet in soil	Kapadia (2018)
40	*Momordica dioica*	Cotyledon	MS + BAP (1.5)	Full plantlet in soil	Karim (2011)
41	*Momordica dioica*	Cotyledon	MS + BAP (1.0)	Full plantlet in soil	Karim (2013)
42	*Momordica dioica*	Cotyledon	MS + BAP (1.0)	Plantlet regenerated from calli	Karim and Ullah (2011)
43	*Momordica dioica*	Internode	MS + BAP (0.1) + NAA (0.1) + Sucrose (30 g/l w/v)	Somatic embryogenesis	Karim and Ahamad (2010)
44	*Benincasa hispida*	Shoot tip	MS + BAP (1.5) + GA_3 (0.2)	Full plantlet in soil	Kausar, *et al.* (2013)
45	*Trichosanthes cucumerina L.*	Cotyledonary node	Kinetin (0.1) and BAP (2.0)	Full plantlet in soil	Kawale and Choudhary (2009)
46	*Cucumis melo L.*	Node	MS + BAP (8.0)	Full plantlet in soil	Keng and Hoong (2005)
47	*Citrullus lanatus*	Shoot tip	MS + BAP (5.0) + IAA (0.1)	Full plantlet in soil	Khalekuzzama, *et al.* (2012)
48	*Citrullus lanatus*	Cotyledon	MS + 2, 4-D (1.0)	Callus	Khatun, *et al.*(2010[a])
49	*Citrullus lanatus*	Node	MS + BAP (1.0) + NAA (0.2)	Full plantlet in soil	Khatun, *et al.*(2010[b])
50	*Cucumis sativus L.*	Stem fragments	MS + Kinetine (6.0 µm)	Flower and pollen production	Kiełkowska and Havey (2011)
51	*Cucurbita ficifolia*	Cotyledon	MS + zeatin (1.0) + IAA (0.1)	Full plantlet in soil	Kim, *et al.*(2010)
52	*Trichosanthes dioica*	Node	MA + BAP (2.0) + NAA (0.3)	Full plantlet in soil	Komal (2011[a])
53	*Trichosanthes dioica*	Node	MS + BAP (2.5)	Callus	Komal (2011[b])
54	*Trichosanthes dioica*	Node	Semi solid MS + Coconut milk (15%)	Full plantlet in soil	Komal (2011[c])
55	*Citrullus lanatus*	Cotyledon	MS + BAP (1) + coconut water (10%).	Organogenesis	Krug, *et al.* (2005)
56	*Momordica dioica*	Node	MSHP + $AdSO_4$ (80 ppm) + BAP (10 ppm) + IBA (5 ppm) + myo-inositol (100) + Agar agar (0.8%) + Sucrose (3%)	Full plantlet in soil	Kulkarni (1999)

57	*Cucumis melo L.*	Cotyledon	MS + BA (2.0) + IAA (0.2)	Full plantlet in soil	Li, *et al.* (2011)
58	*Cucurbita maxima*	Shoot tip	MS + BAP (3.0)	Full plantlet in soil	Mahazabin (2008)
59	*Trichosanthes dioica*	Cotyledon	MS + BAP (1.0)	Full plantlet in soil	Malex, *et al.* (2010)
60	*Cucumis angurea*	Node	MS + BAP (1) + NAA (0.2) + L - glutamine (20)	Full plantlet in soil	Margareate (2014)
61	*Cucumis sativus L.*	Shoot tip	MS + BAP (0.4 µm)	Full plantlet in soil	Mohammadi and Siveritepe (2007)
62	*Luffa acutangula*	Leaf	2, 4 – D + TDZ – (2.0)	Callusogenesis	Moideen and Prabha (2013)
63	*Luffa acutangula*	Petiole	MS + 2, 4-D + TDZ (1.5)	Callus	Moideen and Prabha (2014)
64	*Momordica dioica*	Node	MS + 2, 4-D (2.0) + BAP (1.0)	Organogenesis	Mustapha, *et al.* (2012)
65	*Momordica dioica*	Node	MS + BAP (2.0) + L- glutamic (2.0)	Callus and shoot buds	Mustapha, *et al.* (2013)
66	*Momordica dioica*	Cotyledon	MS + BAP (1.0) + NAA (0.1)	Multiple shoots	Nabi, *et al.* (2002[a])
67	*Momordica dioica*	Cotyledon	MS + BAP (1.0) + NAA (0.1)	Organogenesis	Nabi *et al.* (2002[b])
68	*Cucumis sativus L.*	Cotyledon	Kanamycin resistance and green fluorescent protein (GFP) fluorescence,	Genetic transformation	Nanasato, *et al.* (2013)
69	*Cucurbita pepo*	Hypocotyle	MS + Thidiazuron (0.5)	Full plantlet in soil	Pal, *et al.* (2007)
70	*Cucumis melo L.*	Node	MS + BAP (2.0)	Full plantlet in soil	Parvin *et al.* (2013)
71	*Momordica dioica*	Node	MS + NB6 + BAP (0.5+0.5)	Shoot multiplication from callus	Patel and Kalpesh (2015)
72	*Cucurbita pepo*	Cotyledon	2.,4,5-T (4.7 µm)+ BAP (4.0 µm) + Kinetine (0.5 µm)	Somatic embryos	Paula (1992)
73	*Cucurbita pepo*	Shoot tip	MS + 2,4,5-T (1.2) + BAP (0. 8) + Kinetin (0.I)	Somatic embryogenesis	Paula, *et al.* (1990)
74	*Cucumis melo L.*	Leaf node	MS + BAP (1.0)	Full plantlet in soil	Rahaman, *et al.* (2012)
75	*Momordica sahyadrica*	Seedlings	MS + BAP	Full plantlet in soil and conservation	Rajashekharan, *et al.* (2012)
76	*Momordica dioica*	Leaf	MS + 2, 4-D (2.0) + BAP (2.0)	Molecular confirmation of sex	Raju, *et al.* (2015)

77	*Citrullus colocynth*	Cotyledon	MS + IAA (2.0) + IBA (1.5)	Rhizogenesis	Ram and Shashtri (2015)
78	*Cucumis melo L.*	Cotyledon	MS + IBA (5.0 µM) + BAP (5.0 µM) + 25-29° C + light intensity (5-30 $\mu molm^{-2}s^{-2}$)	Factors of influence	Randall, *et al.* (1989)
79	*Momordica charantia*	Leaf	MS + BAP (1.5)	Callusogenesis	Saima malik, *et al.* (2007)
80	*Cucumis sativus L.*	Shoot tip	MS + BAP (1.0)	Full plantlet in soil	Sangeetha, *et al.* (2011)
81	*Interspecific Cucurbita hybrid*	Shoot tip	MS + BAP (3.0)	Full plantlet in soil	Sarowar, *et al.* (2003)
82	*Cucumis trigonus*	Leaf	MS + BA (1.0) + IAA (0.25)	Full plantlet in soil	Satapathy, *et al.* (2014)
83	*Citrullus colocynth*	Leaf	MS + 2,4-D (1.5) + BAP (1.0)	Callus	Savitha, *et al.* (2010)
84	*Cucumis sativus L.*	Hypocotyle	MS + Sucrose (87.64 µM) + agar (0.8%) + 2,4-D (3.62 µM) + BAP (2.22 µM)	Organogenesis	Selvaraj, *et al.* (2006)
85	*Citrullus colocynth*	Leaf	MS + Kn (2.0) + TDZ (1.0)	Callusogenesis	Shashtree, *et al.* (2014)
86	*Momordica dioica*	Node	MS + BAP (2.0) + IAA (0.1)	Full plantlet in soil	Shekhawat, *et al.* (2011)
87	*Luffa cylindrica*	Cotyledon	MS salts + B5 + BAP (10 µM)	Resistant GUS (β-Glucuronidase)	Singh, *et al.* (2011)
88	*Trichosanthes dioica*	Leaf	MS + BAP (0.5) + 2,4-D (0.5)	Callus	Sourab, *et al.* (2017)
89	*Luffa cylindrica*	Leaf	MS + BAP (1.5) + NAA (1.0)	Callus	Srivastava and Roy (2012)
90	*Momordica charantia*	Node	MS + BAP (2.0) + NAA (0.2)	Full plantlet in soil	Sultana, *et al.* (2003)
91	*Citrullus lanatus*	Leaf	MS + 2, 4-D (2.5)	Organogenesis callus	Sultana, *et al.* (2004)
92	*Momordica charantia*	Node	MS + BAP (2) + NAA (0.2) + Sucrose 30 gl^{-1} + Agar 7.0 gl^{-1}+ pH (5.5- 6.0)	Effects of sucrose, agar pH	Sultana, *et al.* (2005)
93	*Citrullus lanatus*	Cotyledon	MS + BAP (20.0 µM)	Full plantlet in soil	Suratman, *et al.* (2009)
94	*Momordica dioica*	Leaf	MS + BAP (3.0) + NAA (0.5)	Organogenesis	Swamy, *et al.* (2015)
95	*Momordica balsamina*	Node	MS + BAP (1.0)	Full plantlet in soil	Thakur, *et al.* (2011)
96	*Momordica charantia*	Leaf	MS + 2,4-D (1.0)	Embryogenesis	Thiruvengadam, *et al.* (2006)
97	*Momordica dioica*	Petiole	MS + 2,4-D (2.2 µm) + L- glutamine (0.5 µm)	Somatic emryoids	Thiruvengadam, *et al.* (2007)

98	*Momordica dioica*	Encapsulated Shoot tip	MS (0.7% agar solidified) + BAP (0.5 µm)	Full plant let in soil without variation	Thiruvengadam, *et al.* (2012[a])
99	*Momordica charantia*	Petiole	MS and Gamboge + NAA (3.0 µm) + TDZ (1.0 µm) + Putrecine (1.0 µm)	Plantlet from organogenesis	Thiruvengadam, *et al.* (2012[b])
100	*Momordica dioica*	Leaf	MS + 2, 4-D (3.3 µm) + Putrescine (0.5 µm)	Somatic embryogenesis	Thiruvengadam, *et al.* (2013)
101	*Benincasa hispida*	Cotyledon	MS + BAP (1–6 µM) + NAA, 0.2 and 0.5 µM	Full plantlet in soil	Thomas, *et al.* (2004)
102	*Luffa acutangula*	Cotyledon	MS + BAP (1.0) + Zeatin (0.2) + NAA (0.2) + 2,4-D (0.6) + Picloram (0.1) + AdS (20).	Full plantlet in soil	Umamaheshwari, *et al.* (2014)
103	*Cucumis sativus L.*	Leaf	MS + 2,4-D (5) + TDZ (0.1)	Somatic embryogenesis	Usman, *et al.* (2011)
104	*Cucurbita moschata*	Cotyledon	Callus induction medium (CIM) + 2,4-D (0.5 or 3.5)	Somatic embryogenesis	Valdez-Melara, *et al.* (2009)
105	*Citrullus lanatus*	Shoot tip	MS + BAP (0.5)	Full plantlet in soil	Vedat, *et al.* (2002)
106	*Luffa acutangula*	Petiole	MS + BAP (2.0) + NAA (0.2)	genetic stability by IISR	Vellivella, *et al.* (2016)
107	*Cucumis melo var utillisimus*	Node	MS + BAP (1.0) + Adenine sulphate (15)	Multiple shoots	Venkateshawaralu, *et al.*(2010)
108	*Cucumis melo L.*	Shoot tip	MS + IAA (0.5) + BAP (2.0)	Multiple shoots	Venkateshwaralu (2012)
119	*Momordica charantia*	Node	½ MS + BAP (0.5)	Full plantlet in soil	Verma, *et al.* (2014)
110	*Cucumis figarei*	Cotyledon	MS + BAP (1.0) + ABA (1.0 or 2.0)	Full plantlet in soil	Yutaka, *et al.* (1998)
111	*Cucumis metuliferus*	Cotyledon	MS +BAP (1.0) + IAA. (0.2)	Full plantlet in soil	Yutaka, *et al.*(1998)
112	*Luffa acutangula*	Cotyledon	MS + BAP (1.5) + NAA (1.0)	Organogenesis	Zohura, *et al.* (2013)

3. MATERIAL AND METHODS

The present investigations on the optimization of micropropagation techniques in spine gourd by using response surface methodology were conducted with the laboratory facilities of Department of Botany, Shivaji University, Kolhapur and hardening studies were conducted at the Department of Botany and Instructional cum research farm of horticulture section at Rajarshi Chhatrapati Shahu Maharaj College of Agriculture, Kolhapur during 2018-19. The details of materials used, methods adopted and the statistical procedures followed during the course of investigation are described below.

3.1 Materials

3.1.1 Plant material

The various explants of spine gourd genotype RMDSG-2 were collected from the well-established mother plants grown at Instructional-cum-research farm of Horticulture Section of Rajarshi Chathrapati Shahu Maharaj College of Agriculture, Kolhapur.

3.1.2 Collection of explants from plant material

The healthy tops of 4-5 months old spine gourd genotype RMDSG-2 were collected immediately 1-2 hr. before using the plant materials for research and kept in cool place. The five different types of explants were prepared from the collected material.

3.1.3 Selection and Preparation of explant

3.1.3.1 Leaf

Matured young leaf explants of spine gourd were collected from upper parts of the field grown female plants. After surface sterilization using 0.1% mercuric chloride, leaf explants were prepared of 0.5-1.0 cm size by giving a small cut at center of leaf from surgical blade. Such leaf explants were inoculated on MS basal media supplemented with the various PGR's concentration in alone or in combination for studying the effect of various PGR's on regeneration studies.

3.1.3.2 Petiole

The matured young leaves with the good length of Petiole were selected for preparing petiole explants. The petioles having 2.0 cm length were separated from leaf and subjected for surface sterilization using 0.1% mercuric chloride. After sterilization, the final length was reduced to 1.6 cm by cutting 0.2 cm at both cut ends. Petioles were cultured on the various PGR's combination to investigate the effect of various combination of PGR's on amount of callus production, morphology of callus and time period required for callus initiation.

3.1.3.3 Internode

The matured young vines of field grown spine gourd were collected for preparation of internode explant. The internodes were prepared after separating leaves from vein

and used for inoculation in various PGR's combination after sterilization using 0.1% mercuric chloride. An Internode is part of stem between two nodes. The internodes of 1.5-2.0 cm in length were used in this research. During inoculation on the various combination of media supplements, the small portion of both the ends of the internode were removed to avoid the adverse effect of mercuric chloride.

3.1.3.4 Node

A stem part of 3.0 cm length containing an axillary bud was separated from the vein of matured field grown spine gourd vine. After sterilizing it, the final length was reduced to 2.6 cm by cutting 0.2 cm at both the cut ends without injuring the buds for further inoculation on culture media.

3.1.3.5 Shoot tip

The young top most shoot tips were collected along with the apical buds. The small leaves and tendrils are carefully removed from shoot tip without damaging the apical bud. Shoot tip of 3.0 cm were taken and after sterilization only 0.2 cm of bottom portion of the shoot tip are removed and further used for inoculation on culture media containing various combination of growth regulators.

3.1.4 Surface sterilization of explant

The selected plant materials brought from natural environment may contain the contaminants (micro-organisms) on surfaces. Thus in order to make explants free from contaminants, surface sterilization was carried out using 0.1% mercuric chloride. The selected explants *viz*., leaf, petiole, internode, axillary bud and apical bud were thoroughly washed in tap water and then distilled water containing 1-2 drops of Dettol and kept for 10-15 minutes. Then are again rinsed 4-5 times in double distilled water. Thereafter, explants are disinfected by treating with freshly prepared 70% alcohol for 30 seconds and then in 0.1% $HgCl_2$ solution for 2 minutes respectively with constant swirling. The explants were then rinsed 2-3 times with sterile distilled water. After sterilization explants were blotted on sterile blotting paper and inoculated on prepared media.

3.1.5 Chemicals and reagents

All chemicals and reagents used for the present study were of experimental grade and were procured from Hi-Media laboratories Pvt. Ltd., Mumbai. Media used in the present study with different combinations and concentration are described hereunder in experimental details.

Table 2: Plant growth regulators used

Sr. no	Plant growth regulators	
1.	**Auxins:**	Indole 3- Butyric Acid (IBA)
		Indole 3-Acetic Acid (IAA)
		α-Naphthalene Acetic Acid (NAA)
		2, 4-Dichlorophenol Acetic Acid (2, 4-D)
2.	**Cytokinins:**	6-Benzyl Amino Purine (BAP)
		Kinetine (Kn)
3.	**Others**	Casein hydrolysate (CH)
		Thidiazuron (TDZ)

3.1.6 Glasswares and equipment

The Glasswares used for all the experiment were of laboratory grade purchased from Borocil Company. Test tubes of 25×150 mm size with 55 ml capacity, 250 ml conical flasks and culture bottles were used.

The various equipment's *viz.,* refrigerator, electrical oven, autoclave, magnetic stirrer, pH meter, weighing balance, Horizontal laminar air flow cabinet with an HEPA (High Efficiency particulate Air) filter, glass double distillation assembly for double distilled water etc. were used for during experimental procedure.

3.1.7 Preparation of glasswares

3.1.7.1 Cleaning

It is important to use cleaned glasswares for *in-vitro* tissue culture. This was achieved by washing all the glasswares in liquid neutral detergent laboline, followed by rinsing thoroughly with tap water. Then glasswares were rinsed with the double distilled water and swept with the dry and clean cloth.

3.1.7.2 Maintenance of aseptic condition by sterilization

Sterilization was done to make media, glasswares and instruments free from microorganisms. For this, all glasswares *i.e.* conical flask, test tubes, glass bottles, petridishes, vessels etc., were packed in autoclavable plastic bags. Also, forceps, scalpels, absorbent papers were individually wrapped in Aluminum foil and autoclaved at 121^0C with 15 lbs pressure for 20 min. After sterilization they are taken out from the autoclave and kept in clean and dust proof place up to further use.

Aseptic conditions were maintained by performing all operations in laminar air flow chamber. For the maintenance of working balance of laminar air, HEPA filters were cleaned after every 6 months. Before starting any operation, the floor/walls of the laminar airflow were sterilized with 70% alcohol and UV exposure for 30-60 min.

Before transfer of the explants, all the surgical instruments were first dipped in rectified spirit or steripot then flamed on spirit lamp and cooled.

3.1.8 Basal media

For culturing of the selected explants of spine gourd, Murashige and Skoog (1962) basal media was used. Basically, MS media is composed with basic nutrients, vitamins, sucrose and agar etc. With the addition of designed ingredient and various concentrations and combinations of phytohormones, the basal media was used for *in-vitro* cultures' inoculation.

3.1.9 Preparation of culture media

The ready to use powder of MS media was weighed (41.41 gml^{-1}) and the step wise procedure followed for preparing one liter of culture medium as given in Fig. After the preparation of basal media, the various combinations of media supplements *i.e.* phytohormones were added. The digested media, were then dispersed in culture test tubes and mouth were corked with the nonabsorbent cotton. Finally the medium was autoclaved by using vertical autoclave at 121^0 C temperature at 15 lbs pressure for 20 min. Autoclaved media was cooled at 20-22^0C and preserved in dust proof cabinets until further use.

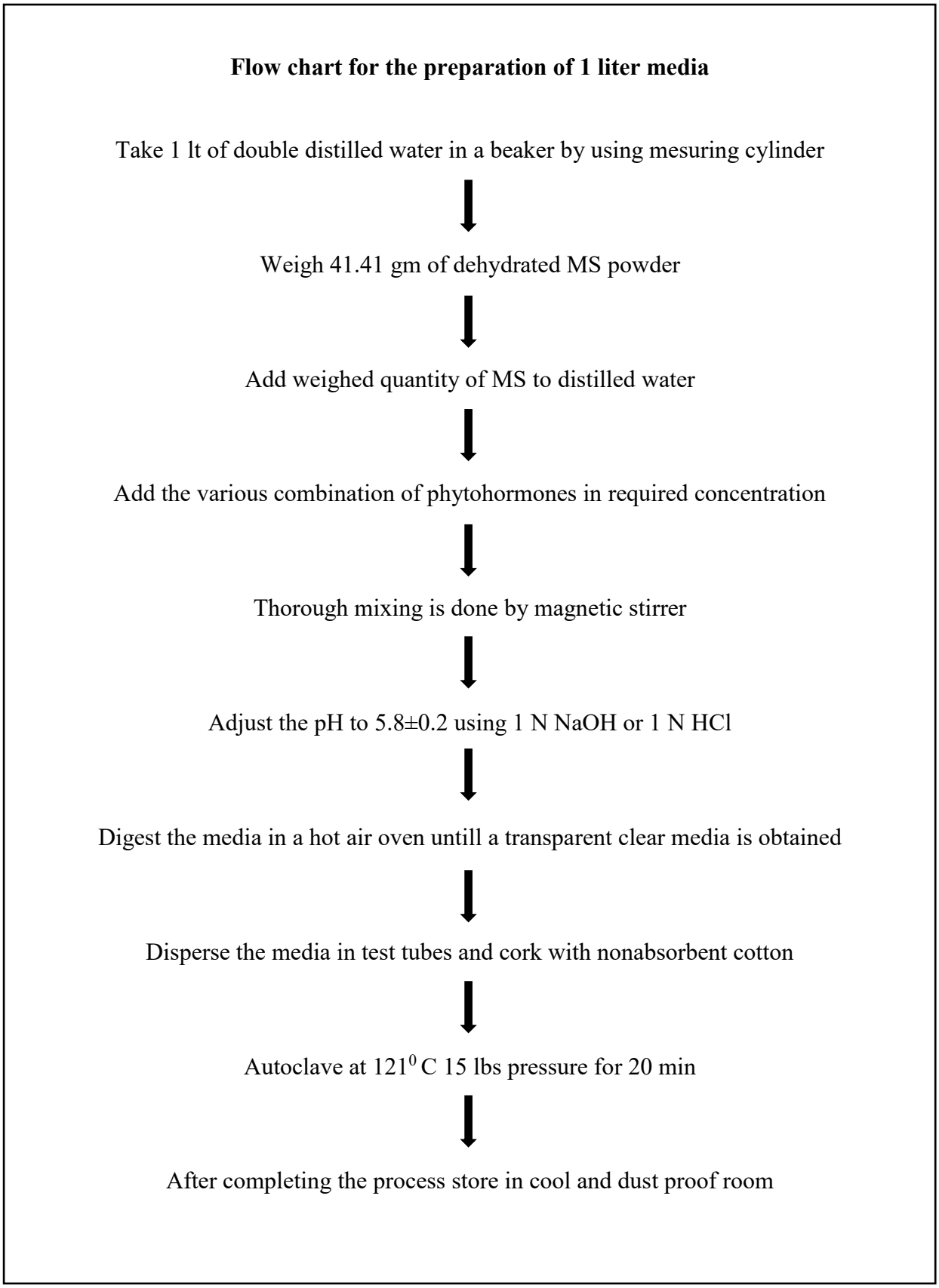
3.1.10 Preparation of stock solution

Stock solutions of all the standard growth hormones were prepared by dissolving 100 mg of growth hormone into few drops of specific solvents. NAA, IBA, BAP, 2.4-D, TDZ were dissolved in 1N NaOH while Kinetin and Casein hydrolysate were dissolved in distilled water then final volume was adjusted to 100 ml by adding distilled water. The final concentration of the stock is 1.0 mg per ml. Prepared stock was stored in amber coloured bottles at 8-10^0 C in refrigerators.

3.1.11 Inoculation of culture and maintenance

After sterilization of explants and media preparation the explants were inoculated on culture media under aseptic conditions of laminar air flow. Inoculated cultures were maintained in incubation room at 25±2^0 C temperature with the relative humidity of 60-80%. The illumination required for culture is provided by cool white florescent tube lights at an intensity of 2000 to 3000 lux and a photoperiod of 16:8 hr light and dark regime was maintained respectively.

Fig 1: Flow chart for the preparation of media



3.2 Experimental details

3.2.1 Explants for Callus study: -

- Leaf
- Petiole
- Internode

Table 3: Treatment details for callus regeneration using leaf, petiole and internode explants.

Sr. No.	Treatments (mgl^{-1})
1	1.0 mgl^{-1} NAA
2	2.0 mgl^{-1} NAA
3	3.0 mgl^{-1} NAA
4	4.0 mgl^{-1} NAA
5	2.0 mgl^{-1} TDZ
6	2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D
7	2.0 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP
8	1.0 mgl^{-1} BAP + 200 mgl^{-1} CH
9	1.5 mgl^{-1} BAP + 200 mgl^{-1} CH
10	2.0 mgl^{-1} BAP + 200 mgl^{-1} CH
11	2.5 mgl^{-1} BAP + 200 mgl^{-1} CH
12	3.0 mgl^{-1} BAP + 200 mgl^{-1} CH
13	1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn
14	2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn
15	2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn
16	1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn
17	2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn
18	2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn
19	0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA
20	0.50 mgl^{-1} BAP + 0.1 mgl^{-1} IAA
21	0.75 mgl^{-1} BAP + 0.1 mgl^{-1} IAA
22	1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA

3.2.2 Optimization of regeneration protocol using RSM

Response Surface Methodology was used for the optimization of micropropagation techniques in spine gourd (*Momordica dioica*) using nodal and shoot tip explants in MS media supplemented with different combinations of PGRs. Investigation was focused on optimization of initiation, multiplication, rooting and hardening stages of spine gourd. Various combinations of experiments were conducted using RSM, each with two independent factors (PGR) and their 13 formulations obtained through Design Expert version 11.0.5.0 software. (www.statease.com). Single factor experiments were conducted by giving optimal customization command whereas two factor experiments were conducted by using central composite design with 5 central points for both the commands. The data generated from

all experiments were analyzed using Design-Expert Software and for all the responses generalized polynomial equations were obtained.

3.2.2.1 Initiation stage

Experimental design was constructed using RSM for optimizing various combinations of PGRs and to study their influence on the various responses in each growth stage for both the nodal and shoot tip explants. For this experiment Murashige and Skoog's (1962) basal media was kept constant and supplemented with various levels of plant growth regulators as generated by RSM. The explants were inserted in the middle of the test tubes and aseptic conditions were maintained during inoculation process. The explants are incubated in a standard culture condition for growth and development.

Table 4: Treatment details of initiation stage

Explants	Treatment details
Node: -	1) 1.0 mgl^{-1} to 3.0 mgl^{-1} BAP
	2) 1.0 mgl^{-1} to 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA
	3) 1.0 mgl^{-1} to 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH
	4) 1.5 mgl^{-1} to 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn
	5) 1.5 mgl^{-1} to 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn
	6) 0.25 mgl^{-1} to 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA
Shoot tip :-	1) 1.0 mgl^{-1} to 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA
	2) 1.0 mgl^{-1} to 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH
	3) 1.5 mgl^{-1} to 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn
	4) 1.5 mgl^{-1} to 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn
	5) 0.25 mgl^{-1} to 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA

3.2.2.1.1 Experimental Design for optimizing various levels of PGRs for nodal explants of spine gourd using D Optimal Response Surface Methodology at initiation stage

The experimental design consisting of 13 different runs obtained using D-optimal RSM for optimizing various combinations/levels of PGRs and to study their effect on responses like average number of days required for shoot initiation, average number of shoots/ explant and average length of shoot is given in Table. A stem part of 2.6 cm length containing an axillary bud was used for the inoculation on MS media supplemented with various levels of plant growth regulators to know their relative effect on various responses of spine gourd.

Table 5: Experimental design for optimizing various levels of BAP on nodal explants

RUN	Factor 1 A: BAP (mgl^{-1})
1	3.0 mgl^{-1} BAP
2	1.0 mgl^{-1} BAP
3	2.5 mgl^{-1} BAP
4	2.0 mgl^{-1} BAP
5	2.0 mgl^{-1} BAP
6	1.5 mgl^{-1} BAP
7	1.0 mgl^{-1} BAP
8	2.0 mgl^{-1} BAP
9	3.0 mgl^{-1} BAP
10	2.5 mgl^{-1} BAP
11	3.0 mgl^{-1} BAP
12	2.0 mgl^{-1} BAP
13	1.5 mgl^{-1} BAP

Table 6: Experimental design for optimizing various levels of BAP and NAA on nodal explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: NAA (mgl^{-1})
1	2.5 mgl^{-1}	0.5 mgl^{-1}
2	3.0 mgl^{-1}	0.5 mgl^{-1}
3	1.0 mgl^{-1}	0.5 mgl^{-1}
4	2.0 mgl^{-1}	0.5 mgl^{-1}
5	2.0 mgl^{-1}	0.5 mgl^{-1}
6	1.5 mgl^{-1}	0.5 mgl^{-1}
7	3.0 mgl^{-1}	0.5 mgl^{-1}
8	1.5 mgl^{-1}	0.5 mgl^{-1}
9	3.0 mgl^{-1}	0.5 mgl^{-1}
10	2.5 mgl^{-1}	0.5 mgl^{-1}
11	1.0 mgl^{-1}	0.5 mgl^{-1}
12	1.0 mgl^{-1}	0.5 mgl^{-1}
13	2.0 mgl^{-1}	0.5 mgl^{-1}

Table 7: Experimental design for optimizing various levels of BAP and CH on nodal explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B : CH (mgl^{-1})
1	1.0 mgl^{-1}	200 mgl^{-1}
2	2.0 mgl^{-1}	200 mgl^{-1}
3	3.0 mgl^{-1}	200 mgl^{-1}
4	2.0 mgl^{-1}	200 mgl^{-1}
5	2.0 mgl^{-1}	200 mgl^{-1}
6	1.0 mgl^{-1}	200 mgl^{-1}
7	1.5 mgl^{-1}	200 mgl^{-1}
8	1.0 mgl^{-1}	200 mgl^{-1}
9	1.5 mgl^{-1}	200 mgl^{-1}
10	3.0 mgl^{-1}	200 mgl^{-1}
11	2.5 mgl^{-1}	200 mgl^{-1}
12	2.5 mgl^{-1}	200 mgl^{-1}
13	3.0 mgl^{-1}	200 mgl^{-1}

Table 8: Experimental design for optimizing various levels of BAP and 0.5 mgl^{-1} Kn on nodal explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: Kn (mgl^{-1})
1	1.5 mgl^{-1}	0.5 mgl^{-1}
2	1.5 mgl^{-1}	0.5 mgl^{-1}
3	2.0 mgl^{-1}	0.5 mgl^{-1}
4	2.0 mgl^{-1}	0.5 mgl^{-1}
5	2.5 mgl^{-1}	0.5 mgl^{-1}
6	2.0 mgl^{-1}	0.5 mgl^{-1}
7	1.5 mgl^{-1}	0.5 mgl^{-1}
8	2.0 mgl^{-1}	0.5 mgl^{-1}
9	2.5 mgl^{-1}	0.5 mgl^{-1}
10	2.0 mgl^{-1}	0.5 mgl^{-1}
11	2.5 mgl^{-1}	0.5 mgl^{-1}
12	1.5 mgl^{-1}	0.5 mgl^{-1}
13	2.5 mgl^{-1}	0.5 mgl^{-1}

Table 9: Experimental design for optimizing various levels of BAP and 1.0 mgl^{-1} Kn on nodal explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: Kn (mgl^{-1})
1	1.5 mgl^{-1}	1.0 mgl^{-1}
2	2.0 mgl^{-1}	1.0 mgl^{-1}
3	2.0 mgl^{-1}	1.0 mgl^{-1}
4	2.5 mgl^{-1}	1.0 mgl^{-1}
5	2.0 mgl^{-1}	1.0 mgl^{-1}
6	1.5 mgl^{-1}	1.0 mgl^{-1}
7	2.5 mgl^{-1}	1.0 mgl^{-1}
8	2.0 mgl^{-1}	1.0 mgl^{-1}
9	2.5 mgl^{-1}	1.0 mgl^{-1}
10	1.5 mgl^{-1}	1.0 mgl^{-1}
11	2.0 mgl^{-1}	1.0 mgl^{-1}
12	2.0 mgl^{-1}	1.0 mgl^{-1}
13	2.0 mgl^{-1}	1.0 mgl^{-1}

Table 10: Experimental design for optimizing various levels of BAP and IAA on nodal explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: IAA (mgl^{-1})
1	1.00 mgl^{-1}	0.10 mgl^{-1}
2	1.00 mgl^{-1}	0.10 mgl^{-1}
3	0.50 mgl^{-1}	0.10 mgl^{-1}
4	1.00 mgl^{-1}	0.10 mgl^{-1}
5	0.50 mgl^{-1}	0.10 mgl^{-1}
6	0.25 mgl^{-1}	0.10 mgl^{-1}
7	1.00 mgl^{-1}	0.10 mgl^{-1}
8	0.25 mgl^{-1}	0.10 mgl^{-1}
9	0.50 mgl^{-1}	0.10 mgl^{-1}
10	0.75 mgl^{-1}	0.10 mgl^{-1}
11	0.25 mgl^{-1}	0.10 mgl^{-1}
12	0.75 mgl^{-1}	0.10 mgl^{-1}
13	0.75 mgl^{-1}	0.10 mgl^{-1}

3.2.2.1.2 Experimental Design for optimizing various levels of PGRs for shoot tip explants of spine gourd using D Optimal Response Surface Methodology at initiation stage

The experimental design consisting of 13 different runs obtained using D-optimal RSM for optimizing various combinations/levels of PGRs and to study their effect on responses like average number of days required for shoot initiation, average number of shoots/ explant and average length of shoot is given in Table. The top most shoot tips are taken along with the apical bud. The shoot tips of 3.0 cm length were used for inoculation on MS media supplanted with various combinations and concentrations of plant growth regulators to know their relative effects on the above said responses.

Table 11: Experimental design for optimizing various levels of BAP and NAA on shoot tip explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: NAA (mgl^{-1})
1	1.5 mgl^{-1}	0.5 mgl^{-1}
2	2.5 mgl^{-1}	0.5 mgl^{-1}
3	3.0 mgl^{-1}	0.5 mgl^{-1}
4	2.0 mgl^{-1}	0.5 mgl^{-1}
5	1.0 mgl^{-1}	0.5 mgl^{-1}
6	3.0 mgl^{-1}	0.5 mgl^{-1}
7	2.5 mgl^{-1}	0.5 mgl^{-1}
8	2.5 mgl^{-1}	0.5 mgl^{-1}
9	1.5 mgl^{-1}	0.5 mgl^{-1}
10	1.0 mgl^{-1}	0.5 mgl^{-1}
11	2.5 mgl^{-1}	0.5 mgl^{-1}
12	2.5 mgl^{-1}	0.5 mgl^{-1}
13	3.0 mgl^{-1}	0.5 mgl^{-1}

Table 12: Experimental design for optimizing various levels of BAP and CH on shoot tip explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: CH (mgl^{-1})
1	2.0 mgl^{-1}	200 mgl^{-1}
2	2.0 mgl^{-1}	200 mgl^{-1}
3	2.0 mgl^{-1}	200 mgl^{-1}
4	2.0 mgl^{-1}	200 mgl^{-1}
5	1.0 mgl^{-1}	200 mgl^{-1}
6	1.5 mgl^{-1}	200 mgl^{-1}
7	2.5 mgl^{-1}	200 mgl^{-1}
8	2.0 mgl^{-1}	200 mgl^{-1}
9	3.0 mgl^{-1}	200 mgl^{-1}
10	2.0 mgl^{-1}	200 mgl^{-1}
11	3.0 mgl^{-1}	200 mgl^{-1}
12	1.0 mgl^{-1}	200 mgl^{-1}
13	2.0 mgl^{-1}	200 mgl^{-1}

Table 13: Experimental design for optimizing various levels of BAP and 0.5 mgl^{-1} Kn on shoot tip explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: Kn (mgl^{-1})
1	2.0 mgl^{-1}	0.5 mgl^{-1}
2	2.0 mgl^{-1}	0.5 mgl^{-1}
3	2.0 mgl^{-1}	0.5 mgl^{-1}
4	1.5 mgl^{-1}	0.5 mgl^{-1}
5	2.0 mgl^{-1}	0.5 mgl^{-1}
6	2.0 mgl^{-1}	0.5 mgl^{-1}
7	2.5 mgl^{-1}	0.5 mgl^{-1}
8	2.0 mgl^{-1}	0.5 mgl^{-1}
9	1.5 mgl^{-1}	0.5 mgl^{-1}
10	2.0 mgl^{-1}	0.5 mgl^{-1}
11	1.5 mgl^{-1}	0.5 mgl^{-1}
12	2.5 mgl^{-1}	0.5 mgl^{-1}
13	2.5 mgl^{-1}	0.5 mgl^{-1}

Table 14: Experimental design for optimizing various levels of BAP and 1.0 mgl^{-1} Kn on shoot tip explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: Kn (mgl^{-1})
1	2.5 mgl^{-1}	1.0 mgl^{-1}
2	2.0 mgl^{-1}	1.0 mgl^{-1}
3	2.0 mgl^{-1}	1.0 mgl^{-1}
4	1.5 mgl^{-1}	1.0 mgl^{-1}
5	2.5 mgl^{-1}	1.0 mgl^{-1}
6	2.0 mgl^{-1}	1.0 mgl^{-1}
7	2.5 mgl^{-1}	1.0 mgl^{-1}
8	2.0 mgl^{-1}	1.0 mgl^{-1}
9	2.0 mgl^{-1}	1.0 mgl^{-1}
10	2.0 mgl^{-1}	1.0 mgl^{-1}
11	2.0 mgl^{-1}	1.0 mgl^{-1}
12	1.5 mgl^{-1}	1.0 mgl^{-1}
13	1.5 mgl^{-1}	1.0 mgl^{-1}

Table 15: Experimental design for optimizing various levels of BAP and IAA on shoot tip explants

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: IAA (mgl^{-1})
1	0.25 mgl^{-1}	0.1 mgl^{-1}
2	0.25 mgl^{-1}	0.1 mgl^{-1}
3	0.50 mgl^{-1}	0.1 mgl^{-1}
4	0.50 mgl^{-1}	0.1 mgl^{-1}
5	0.50 mgl^{-1}	0.1 mgl^{-1}
6	0.75 mgl^{-1}	0.1 mgl^{-1}
7	0.75 mgl^{-1}	0.1 mgl^{-1}
8	0.75 mgl^{-1}	0.1 mgl^{-1}
9	1.00 mgl^{-1}	0.1 mgl^{-1}
10	1.00 mgl^{-1}	0.1 mgl^{-1}
11	0.25 mgl^{-1}	0.1 mgl^{-1}
12	1.00 mgl^{-1}	0.1 mgl^{-1}
13	1.00 mgl^{-1}	0.1 mgl^{-1}

3.2.2.1.3 Optimization of various levels of PGR's in shoot initiation stage

The optimization of various and optimized levels of PGRs obtained were subjected to multiple levels of analysis by giving numerical optimization independent variables (PGRs) was carried out using D optimal response surface methodology. The most desirable command for expected goals during initiation stage of micropropagation in spine gourd for nodal and shoot tip explants to find out the best level of various independent factors in order to get optimum results in respect of dependent response variables. The main aim of the optimization was to reduce the number of days required for shoot initiation and to get the maximum number of shoots per explant with maximum average shoot length during initiation stage of micropropagation of spine gourd. After setting the goals by giving the most desirable commands to the software, the diagnostic *and* influence plots available in the software, were used for the optimization of the design. The RSM optimization approach tests an optimum response by changing levels of several variables at one time using special experimental designs. The optimization of the variable levels was achieved by desirable maximization of the initiation stage responses along the fitted polynomial model by numerical optimization procedure of design expert software. The best solution exerted through the software and their predicted score were then validated by comparing with the actual observations recorded for number of days required for shoot initiation, number of shoots per explants and average length of shoots during initiation stage of micropropagation in spine gourd for both node and shoot tip explants.

Table 16: Optimization of media supplements on MS media at initiation stage of nodal explant

Sr. No.	Suggested combination	
	Factor 1	**Factor 2**
1.	BAP	
2.	BAP	NAA
3.	BAP	CH
4.	BAP	Kn
5.	BAP	Kn
6.	BAP	IAA

Table 17: Optimization of media supplements on MS media at initiation stage of shoot tip explant

Sr. No.	Suggested combination	
	Factor 1	**Factor 2**
1.	BAP	NAA
2.	BAP	CH
3.	BAP	Kn
4.	BAP	Kn
5.	BAP	IAA

3.2.2.2 Multiplication stage

The best suggested optimized combination of media supplements i.e. PGR's from each experiment were selected for further multiplication of the nodal as well as shoot tip explants. The Murashige and Skoog's (1962) was used as a basal media for all the treatments. is kept constant. The levels of 2.0 mgl^{-1} TDZ and 2.0 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP were not run in RSM, however, their means were considered for comparison with the others. The well-developed plants were cut below the node and inoculated in optimized level of media supplement observed from each separate experiment.

3.2.2.3 Rooting Stage

For rooting of micro propagated shoots, experiment was designed using RSM for optimizing various combination of PGRs and to study their effect on the various responses like average number of days required for root initiation, average number of roots per shoot and average length of root in both the node and shoot tip explants.

Explants	Treatment details
Node : -	1) NAA
	2) IBA
Shoot tip :-	1) NAA
	2) IBA

3.2.2.3.1 Experimental Design for optimizing various levels of PGRs using D Optimal Response Surface Methodology for rooting of micro propagated shoots

The multiplied shoots having average length of 5-6 cm were aseptically separated and sub-cultured on rooting medium i.e. MS medium supplemented with different concentrations of PGR's as suggested by D-Optimal RSM. The experimental design was constructed using optimal customization command of response surface methodology where construction of the design of response surface methodology for only one factor was possible. For all treatments Murashige and Skoog's (1962) basal media is kept constant.

Table 18: Experimental design for optimizing various levels of NAA on nodal explants

RUN	Factor 1 A: NAA (mgl^{-1})
1	1.0 mgl^{-1}
2	1.5 mgl^{-1}
3	1.5 mgl^{-1}
4	1.0 mgl^{-1}
5	1.5 mgl^{-1}
6	2.0 mgl^{-1}
7	1.0 mgl^{-1}
8	2.0 mgl^{-1}
9	1.0 mgl^{-1}
10	1.0 mgl^{-1}
11	2.0 mgl^{-1}
12	2.0 mgl^{-1}
13	1.5 mgl^{-1}

Table 19: Experimental design for optimizing various levels of IBA on nodal explants

RUN	Factor 1 A: IBA (mgl^{-1})
1	1.5 mgl^{-1}
2	1.5 mgl^{-1}
3	1.5 mgl^{-1}
4	2.0 mgl^{-1}
5	2.0 mgl^{-1}
6	1.0 mgl^{-1}
7	2.0 mgl^{-1}
8	1.0 mgl^{-1}
9	1.5 mgl^{-1}
10	2.0 mgl^{-1}
11	1.0 mgl^{-1}
12	1.0 mgl^{-1}
13	2.0 mgl^{-1}

Table 20: Experimental design for optimizing various levels of NAA on shoot tip explants

RUN	Factor 1 A: NAA (mgl^{-1})
1	1.5 mgl^{-1}
2	1.5 mgl^{-1}
3	1.0 mgl^{-1}
4	1.0 mgl^{-1}
5	1.5 mgl^{-1}
6	1.5 mgl^{-1}
7	1.0 mgl^{-1}
8	2.0 mgl^{-1}
9	1.5 mgl^{-1}
10	2.0 mgl^{-1}
11	1.0 mgl^{-1}
12	2.0 mgl^{-1}
13	2.0 mgl^{-1}

Table 21: Experimental design for optimizing various levels of IBA on shoot tip explants

RUN	Factor 1 A: IBA (mgl^{-1})
1	1.0 mgl^{-1}
2	1.0 mgl^{-1}
3	2.0 mgl^{-1}
4	2.0 mgl^{-1}
5	2.0 mgl^{-1}
6	1.0 mgl^{-1}
7	2.0 mgl^{-1}
8	1.0 mgl^{-1}
9	2.0 mgl^{-1}
10	1.5 mgl^{-1}
11	1.5 mgl^{-1}
12	1.5 mgl^{-1}
13	1.5 mgl^{-1}

3.2.2.4 Hardening Stage

Hardening plays an important role in successful establishment of plant material in outer field condition. The well rooted *In-vitro* plants were taken out from the culture vessels and washed thoroughly with sterile water in order to remove the adhered nutrient agar media on root zone. Initially, plants were planted in small pots with potting media containing cocopeat and soil in 1:1 ratio. Hardening media was firmly pressed after keeping plants in pots. A staking was given by long thin sticks to give mechanical support to the vines. After that the pots were irrigated to maintain the moisture and finally covered the whole plant with the polythene cover to create high relative humidity for plants. Next day after transplanting, two small holes were created on polythene cover to provide aeration. The nutrients were applied at an interval of 3-4 days. The liquid form of NPK (19:19:19) was applied to supply nutrients to the plants. The plants were initially maintained at 75% Shade net with 80% relative humidity. After 10 days, these plants were shifted to the 50% Shade net with 75% relative humidity.

3.2.2.5 Observations recorded

3.2.2.5.1 Average number of days required for callus initiation

The number of days required for callus initiation were recorded when different explants showed the callus initiation. Average number of days were calculated by considering the mean of 10 test tubes for each treatment.

3.2.2.5.2 Amount of callus

In each case average amount of callus was calculated by using visual method and treatments which showed less than average were categorized as very low and low amount of callus whereas treatments which produced more than average were categorized as medium and high amount of callus (Table). The explants was did not respond to the PGRs for callus initiation were considered as no growth. Various symbols used to denote the responses are listed below.

a) -C = No growth
b) +C = Very low callus
c) ++C = Low Callus
d) +++C = Medium callus
e) ++++C = High Callus

3.2.2.5.3 Morphology of callus

Based on the morphological character of a callus, the calluses were categorized as give below by using visual method,

a) BF = Brown Fragile
b) LPF = Light pink fragile
c) WF = White fragile

3.2.2.5.4 Average number of days required for shoot initiation

From the day of inoculation to the day of explants growth initiation is considered as the days required to shoot initiation. Average number of days required for shoot initiation were taken from the mean of treatments.

3.2.2.5.5 Average number of shoots per explants

Total number of shoots per explant were taken after 30 days from the day of inoculation.

$$\text{Average no. of shoots} = \frac{\text{Total number of shoots}}{\text{Number of explants}}$$

3.2.2.5.6 Average length of shoot (cm)

Length of shoot is calculated after 30 days of inoculation of explant and the average was calculated by considering the mean of all explants in single treatment.

3.2.2.5.7 Average number of days to root initiation

Mean of the days required between sub-culturing of shoots on rooting media to visible appearance of roots initiation were taken as average number of days to root initiation.

3.2.2.5.8 Average number of roots per explants

Total number of roots per sub-cultured explant was recorded after 30 days of inoculation

$$\text{Average no. of roots} = \frac{\text{Total number of roots}}{\text{Number of shoots}}$$

3.2.2.5.9 Average root length (cm)

The length of the root of an explant was measured by considering the longest root and was taken after 30 days of inoculation. The mean of the treatment was taken to record the average root length in cm.

3.2.2.5.10 Per cent survival (%)

It is expressed in per cent. It is the ratio of the number of seedlings survived to the total number of seedlings kept for hardening.

3.2.2.6 Statistical analysis

The experiment was conducted under controlled conditions. The experiments were designed using RSM. The statistical analysis for the data was performed with Design-Expert version 11.0.5.0 software. (www.statease.com) using Response Surface Methodology and CRD for determining significant differences among treatments at $p< 0.05$ level.

Table 22: Various terms used in RSM (Design Expert 11.0.5.0 statistical package)

1)	**Prob> F**	This is the probability value that is associated with the F value for this term. Term that has a probability value less than 0.05 would be considered a significant effect. Probability value greater than 0.1 is generally regarded as not significant.
2)	**Adequate Precision**	It is a measure of the range in the predicted response relative to its associated error, in other words a signal to noise ratio. Its desired value is 4 or more.
3)	**Linear**	Sequential sum of squares for the linear terms. A small P- value (prob>F) indicates that adding linear terms has improved the model.
4)	**2F1**	Sequential sum of squares for the two factor interaction (AB) terms. A small P value (prob>F) indicates that adding interaction terms has improved the model.
5)	**Coefficient Estimate**	Regression coefficient representing the expected change in response 'Y' per unit change is 'X' when all remaining factors held constant.

4. RESULT AND DISCUSSION

The present investigation was conducted to study the effect of different growth regulators during various stages of micropropagation, such as initiation, multiplication, rooting and hardening of Spine gourd (*Momordica dioica*) and to optimize micropropagation protocol using axillary and apical bud as explants with the help of Response Surface Methodology (RSM). Various experiments were conducted using RSM, each with two independent factors and 13 treatments prepared by using different combinations of the factors as per Design Expert version 11.0.5.0 software. (www.statease.com). The data generated from all experiments were analyzed using Design-Expert Software is presented and discussed here under suitable headings and for all the responses a generalized polynomial equation was obtained.

Explants such as leaf, petiole and internode were used to know their capacity to produce callus for its use in further organogenesis and somatic embryogenesis on various combinations of growth regulators.

4.1 Effect of Plant growth regulators (media supplementations) on callusing of different explants

4.1.1 Leaf

In-vitro cultured leaf explants started to produce the callus of various amounts on a media supplemented with various plant growth regulators and their combinations. The differences in responses of callus production by use of different PGR concentrations are presented in Table (23). The results revealed that, among the various PGR's combinations tried, the earliest callus initiation on leaf explants was registered in media supplemented with NAA concentrations *viz.*, 1.0, 2.0, 3.0 and 4.0 mgl^{-1} after 6.0, 6.0, 6.75 and 6.5 days respectively, whereas maximum number of days required for callus initiation was recorded in a media supplemented with 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. *i.e.,* 18.5 days. However, media supplemented with various concentrations of BAP+CH did not show callus induction except 2.0 mgl^{-1} BAP + 200 mgl^{-1} CH. Where the initiation was recorded on 19^{th} day.

The observations recorded for the amount of callus (Table 23) revealed that high percentage of callus was noticed in the media with combinations 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D, 2.0 mgl^{-1} NAA and 1.5 mg l^{-1} BAP + 0.5 mgl^{-1} Kn, whereas medium of callus was reported in media added with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. However, very less amount of callus production on leaf explants was recorded in the media supplemented with rest of plant growth regulators combinations. The swelling of the leaf explants was also reported before callus initiation in all the treatment combinations.

As regards, the type of callus produced on leaf explant, almost all the treatments produced white fragile callus. However, the treatments 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D and 1.5 mgl^{-1} BAP +1.0 mgl^{-1} Kn produced the brown and light pink fragile callus respectively.

Table 23: Response of leaf explant of spine gourd to MS media supplemented with various concentration and combinations of plant growth regulators

Sr. No.	Treatments (mgl^{-1})	Av. No. of days for callus initiation	Growth of callus	Callus morphology
1.	1.0 mgl^{-1} NAA	06.00	+C	WF
2.	2.0 mgl^{-1} NAA	06.00	++++C	WF
3.	3.0 mgl^{-1} NAA	06.75	+C	WF
4.	4.0 mgl^{-1} NAA	06.50	++++C	WF
5.	2.0 mgl^{-1} TDZ	12.00	++C	WF
6.	2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D	10.10	++++C	BF
7.	2.0 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP	07.67	++C	WF
8.	1.0 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
9.	1.5 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
10.	2.0 mgl^{-1} BAP + 200 mgl^{-1} CH	19.00	+C	WF
11.	2.5 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
12.	3.0 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
13.	1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	18.00	+C	WF
14.	2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	17.50	+C	WF
15.	2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	18.50	+C	WF
16.	1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	17.50	++C	LPF
17.	2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	18.30	++C	WF
18.	2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	17.70	+C	WF
19.	0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	08.50	+C	WF
20.	0.50 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	08.33	+C	WF
21.	0.75 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	08.67	+C	WF
22.	1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	07.30	+++C	WF

*Where

-C	=	No growth	BF	=	Brown Fragile
+C	=	Very low callus	LPF	=	Light pink fragile
++C	=	Low Callus	WF	=	White fragile
+++C	=	Medium callus			
++++C	=	High Callus			

The plant growth regulators are important factors that can selectively influence the genes to trigger differentiation of cells in culture (Thorpe, 1993). Both TDZ and BAP have been used often to induce shoot organogenesis in various plant species (Nugent, *et al.* 1991, Arene, *et al.* 1993 and Isac, *et al.* 1994). In the present experiment, the initiation of callus started with the swelling of the leaf explant of spine gourd. The similar results were reported by Nabi *et al.* (2002[a]) in Teasle gourd. In the present investigation, callus induction from leaf explant was observed and was accelerated when media is supplemented with NAA as well as BAP as reported by Nabi *et al.* 2002[b], Karim and Ahmed (2010), Karim and Ullah (2011) and Swamy, *et al.* (2015) in spine gourd.

No callus formation was observed in the leaf explant placed on a media fortified with BAP + CH. This might be inhibitory effect of the Casein hydrolysate as reported by Rai, *et al*. (2105) in Spine gourd and Prakash *et al.* 2003 in male explants of jojoba.

4.1.2 Petiole

In the present experiment, among the various treatment combinations of growth regulators tried as a media supplement, an internode explant placed in BAP plus CH supplemented MS media showed no callus initiation. The response of internode explant was variable with the MS media fortifications.

Table 24: Response of Petiole explant of spine gourd to MS media supplemented with various concentration and combinations of plant growth regulators

Sr. No.	Treatments (mgl^{-1})	Av. No. of days for callus initiation	Growth of callus	Callus morphology
1.	1.0 mgl^{-1} NAA	7.00	+C	WF
2.	2.0 mgl^{-1} NAA	-	-C	-
3.	3.0 mgl^{-1} NAA	6.40	++C	WF
4.	4.0 mgl^{-1} NAA	6.33	++C	WF
5.	2.0 mgl^{-1} TDZ	12.3	++C	WF
6.	2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D	8.87	+++C	BF
7.	2.0 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP	9.50	+C	WF
8.	1.0 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
9.	1.5 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
10.	2.0 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
11.	2.5 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
12.	3.0 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
13.	1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	17.0	+C	WF
14.	2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	18.0	+C	WF
15.	2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	-	-C	-
16.	1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	-	-C	-
17.	2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	-	-C	-
18.	2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	17.0	+C	WF
19.	0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	-	-C	-
20.	0.50 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	8.0	+C	WF
21.	0.75 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	9.5	+C	WF
22.	1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	8.8	+C	WF

*Where

-C	=	No growth	BF	=	Brown Fragile
+C	=	Very low callus	LPF	=	Light pink fragile
++C	=	Low Callus	WF	=	White fragile
+++C	=	Medium callus			
++++C	=	High Callus			

The observations recorded regarding response of petiole explants to MS media supplemented with various growth regulators showed the significant differences among the

treatments, Table (24). In experiment, among the various treatment combinations of growth regulators tried as a media supplement, an petiole explant placed on BAP+CH and BAP + 1.0 mgl^{-1} Kn reported for no callus initiation, if showed also the late response was recorded. The MS media fortified with various concentrations of NAA, reported the minimum number of (6.33 to 7.0) days for callus initiation in petiole explant of spine gourd, whereas it was maximum (18.0 days) in media supplemented with the combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn.

In respect to the amount of callus, the petiole explant placed on MS media supplemented with the combination of 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D was recorded the highest amount of callusing among all the tried treatments. The other treatments had reported to be low and very low percentage of callus induction on MS media supplemented with various treatment combinations.

In all the treatments the morphology of callus was reported to be similar *i.e.,* White fragile type of callus. Whereas only in case of petiole explants placed on MS media supplemented with 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D had registered the brown type of callus.

Considerably minimum response was shown by petiole explants on amount of callus initiation and in some combinations there was no callus initiation was reported. The results of the present investigation are in similar with the earlier findings of Punja, *et al.* (1990), Seo, *et al.* (2000) in cucumber cultivars using petiole and leaf explants and Thiruvengadam, *et al.* (2010) in *Momordica charantia.*

4.1.3 Internodes

In the present experiment, among the various treatment combinations of growth regulators tried as a media supplement, an internode explant placed in BAP plus CH supplemented MS media showed no callus initiation. The response of internode explant was variable with the MS media fortifications. The minimum number of days *i.e.* 6.0 days required for callus initiation was reported in media supplemented with the concentration of 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D. While maximum number of days for callus initiation *i.e.* 17.5 to 18.5 days were reported in a media fortified with the combination of BAP and Kinetin Table (25).

The highest percentage of callus induction was reported in a media fortified with 2.0 mgl^{-1} NAA, whereas medium callus was initiated in a media supplemented with 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D. However, rest of the treatments showed the low and very low percentage of callus. In all the treatments under study, white fragile type of callus was observed except in 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2, 4-D and 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn fortifications, which produced brown and light pink fragile callus respectively.

The similar results were reported by Karim and Ahmed (2010) in Teasle gourd internode, leaf, petiole, shoot tip and nodal explants which produced friable light green coloured callus. However in the present investigation, the callus initiated were of fragile in nature with

white, brown and light pink coloured, depending on the media supplementations as mentioned above.

Table 25: Response of Internode explant of spine gourd to MS media supplemented with various concentration and combinations of plant growth regulators.

Sr. No.	Treatments (mgl^{-1})	Av. No. of days for callus initiation	Growth of callus	Callus morphology
1.	1.0 mgl^{-1} NAA	10.00	+C	WF
2.	2.0 mgl^{-1} NAA	7.10	++++C	WF
3.	3.0 mgl^{-1} NAA	7.20	+C	WF
4.	4.0 mgl^{-1} NAA	10.6	+++C	WF
5.	2.0 mgl^{-1} TDZ	11.00	+C	WF
6.	2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} 2,4-D	6.00	+++C	BF
7.	2.0 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP	11.40	++C	WF
8.	1.0 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
9.	1.5 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
10.	2.0 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
11.	2.5 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
12.	3.0 mgl^{-1} BAP + 200 mgl^{-1} CH	-	-C	-
13.	1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	-	-	-
14.	2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	-C	-C	-C
15.	2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn	-	-	-
16.	1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	18.5	18.5	18.5
17.	2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	+C	+C	+C
18.	2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn	WF	WF	WF
19.	0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	-	-	-
20.	0.50 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	-C	-C	-C
21.	0.75 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	-	-	-
22.	1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA	18.5	18.5	18.5

4.2 Optimization of micropropagation techniques in spine gourd using RSM

The experiments related with optimization of micropropagation techniques in spine gourd were carried out using nodal and shoot tip explants only. The other explants such as leaf, petiole and internode were discarded as they have shown the tendency to produce callus. Further this optimization experiment was carried out in three part *viz.,* initiation, multiplication and rooting. The hardening of the plantlets obtained was also studied.

4.2.1 Shoot initiation

4.2.1.1 Node

The different media supplementations *i.e.* growth regulators such as BAP, BAP+NAA, BAP+CH, BAP+0.5 mgl^{-1} Kn, BAP+1.0 mgl^{-1}Kn and BAP+IAA were used for the optimization of micropropagation techniques in spine gourd using nodal explant. Each media supplementation is treated as a separate part of the research work. The range of combinations generated using D-Optimal RSM is presented and discussed as a separate experiment.

4.2.1.1.1 Response of spine gourd nodal explant to various levels of BAP using D-Optimal RSM

The various levels of BAP as generated by D-optimal response surface methodology were tried in MS media to study the response of nodal explant of spine gourd. The results pertaining to various responses such as shoot initiation, average number and length of shoots is presented in Table (26) and discussed hereunder suitable headings.

Table 26: Effect of different levels of BAP on the morphogenic responses of nodal explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: BAP (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	3.0	08.9	1.3	2.50
2	1.0	08.0	1.5	2.85
3	2.5	09.3	1.3	2.50
4	2.0	11.5	1.1	3.10
5	2.0	11.7	1.1	3.15
6	1.5	12.7	1.2	3.80
7	1.0	09.0	1.4	2.90
8	2.0	11.3	1.2	3.00
9	3.0	09.1	1.0	2.40
10	2.5	09.5	1.1	2.60
11	3.0	08.8	1.1	2.55
12	2.0	11.4	1.0	3.13
13	1.5	12.5	1.3	3.85
Coefficient of quadratic polynomial model of BAP for responses of initiation stage				
Factor		**Initiation (Av. days)**	**Av. number of shoots/ explant**	**Av. length of shoot**
Intercept		11.56	1.14	3.18
BAP(A)		-0.3764	-0.1413*	-0.3859*
A^2		-2.70*	0.1617*	-0.4416
F- Value		9.62	6.53	7.15
P-Value		0.0047	0.0154	0.0118
Mean		10.28	1.20	2.95
SD		1.02	0.1102	0.3291
Adequate precision		6.2793	6.2944	5.7563
R^2		0.6581	0.5662	0.5886

*P-Value = <0.05

4.2.1.1.1.1 Number of days for initiation

The number of days required for shoot initiation from the nodal explant of spine gourd placed on MS media fortified with various level of BAP ranged from 8.0 to 12.7 days (Table. 26 and Fig. 2). The minimum number of days *i.e.* 8.0 days required for shoot initiation was

observed in the nodal explant placed on MS media supplemented with 1.0 mgl^{-1} of BAP, however the maximum 12.7 days were reported in 1.5 mgl^{-1} of BAP supplementation for shoot initiation.

The regression analysis of the data presented in (Table 26) revealed that the coefficient of determination (R^2) is 0.6581. The adequate precision was found to be 6.2793, which is appreciably higher than the minimum desirable i.e. 4.0 for high prediction ability. The Model F-value of 9.62 implies that the model is significant. There was only a 0.47% chance that an F-value of such large could occur due to noise. The P-value observed here under the experiment is 0.0047, indicating the significance of model terms (P=<0.05). The significant results were obtained in respect of various levels of BAP used as a supplementation on MS media in respect of number of days required for shoot initiation.

As regards the coefficient for number of days required for shoot initiation, model showed that the levels of different combinations had significant ($p<0.05$) effect. The positive sign indicates increase in response with the increase in level of factors, whereas negative sign indicates the reciprocal effect of response. The BAP levels used in the present experiment at linear terms had non-significant effect on the number of days for initiation in negative manner, which is highly accepted and appreciated in case of number of days required for shoot initiation as a desirable outcome. However, the quadratic levels of BAP had significant effect on days required for shoot initiation in negative manner as expected.

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. However, this equation should not be used to determine the relative impact of each factor. The response surface equation derived for predicting number of days required for shoot initiation could be given as:

Initiation (Days) = +1.50254 + 10.43485 * BAP - 2.70280 * BAP^2

In present investigation results showed that earliest shoot initiation (8days) was registered in MS media supplemented with 1.0 mgl^{-1} BAP followed by 3.0 and 2.5 mgl^{-1} BAP. The maximum days required for shoot initiation *i.e.* 12.7 days were noticed in nodal explant placed on MS media fortified with 1.5 mgl^{-1} BAP. The major focus of the researchers working in tissue culture of crops was concentrated on the percentage of shoot induction. However, the number of days required for shoot initiation also plays an important role in rapid multiplication from the various explants. The nodal shoot segments proved to be most suitable explants material for regeneration of shoots in *Momordica* species in short period of time as reported by Agarwal and Kamal (2004) and Sultana, *et al.* (2005). In the present investigation, MS media supplementation with BAP alone was found to be most suitable for bud breakage of single shoot from each node rather than in combinations as reported by Jamatia (2016) in *Momordica dioica*. Similar results were also reported by Rahaman, *et al.* (2012) and Thakur, *et al.* (2011) in *Cucumis melo* and *Momordica balsamia* respectively using nodal explant at the concentration of 1.0 mgl^{-1} BAP. The

use of lower concentration of the BAP for Shoot induction was also reported by Jamatia (2016) and Rai, *et al.* (2012). The incorporation of higher levels of BAP in media induces the callus formation which in turn delays the shoot regeneration. The similar trend was observed in present experiment as reported by Jamatia (2016) and Rai, *et al.* (2012) in Spine gourd.

4.2.1.1.1.2 Number of shoots per explant

The observations pertaining to the number of shoots as influenced by various levels of BAP concentrations is depicted in Table 26 and Fig 2. The number of shoots produced per explant were significantly influenced by the levels of BAP under study. The number of shoots produced per explant placed on MS medium supplemented with various concentrations of BAP varied from 1.0 to 1.5. In the present investigation, the minimum number of shoots per explant *i.e.,* 1.0 shoot/ explant was reported in the MS media supplemented with 2.0 and 3.0 mgl^{-1} of BAP. However, the maximum number of shoots per explant i.e. 1.5 shoots/explant were observed in 1.0 mgl^{-1} BAP fortified MS medium.

For number of shoots per explant, the regression analysis of a data presented in (Table 26) showed that the coefficient of determination (R^2) was 0.5662, which is near to the normal expected value. The adequate precision was recorded to be 6.2944, which is more than the minimum desirable *i.e.* 4 for high prediction ability and to navigate the design effectively.

As regards the coefficient for number of shoots per explant, model showed that the different levels of BAP had significant ($p<0.05$) effect on response of spine gourd. The different concentrations of BAP at linear and quadratic terms had significant effect on the number of shoots per explant in negative and positive manner respectively**.**

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = + 2.06562 - 0.788051 * BAP + 0.161682 * BAP^2

Among the various BAP concentrations tested for number of shoots per explant in Spine gourd using nodal explant, the highest number of shoots *i.e.* 1.5 shoots per explant were recorded in MS medium supplemented with 1.0 mgl^{-1} of BAP while minimum number of shoots *i.e.* 1.0 per explant was registered in the MS medium fortified with 2.0 and 3.0 mgl^{-1} BAP. The results are in conformity with those obtained by Jamatia *et al.* (2016) in *Momordica dioica*. This might be due to the regulatory role of BAP which might have stimulated proliferation of meristems as reported by Thakur *et al.* (2001) in *Momordica balsamia.* The role of BAP alone in high frequency of shoot formation as in the current experiment was also supported by Karim and Ullah (2011) in cotyledon explants of *M. dioica* Roxb. Karim and Ullah used direct organogenesis from cotyledon whereas in the current experiment nodal explants were used for direct regeneration.

4.2.1.1.1.3 Length of shoots

The data given in Table (26) and graphically represented in Fig (2) revealed that the length of the shoots produced per explant is significantly influenced by the various concentration levels of BAP on MS media and ranged from 2.4 to 3.85 cm. The minimum average length of shoot per explant (2.4 cm) was recorded in an explant placed on a MS media supplemented with 3.0 mgl^{-1} of BAP. However, maximum average shoot length *i.e.* 3.85 cm was reported in the nodal explant on a MS medium fortified with 1.5 mgl^{-1} BAP.

Regression analysis in respect of length of shoots as effected by the different levels of BAP, discloses the coefficient of determination (R^2) as 0.5886. For average length of shoots, model showed the adequate precision (5.7563) which is noticeably higher than the minimum desirable (4) for high prediction ability of the model terms. The Model F-value (7.15) implied that the model was significant with P-value 0.0118.

The coefficient for average length of shoot model of spine gourd nodal explants showed that the different levels of BAP concentrations had significant ($p<0.05$) effect on response of spine gourd. The positive sign indicates that there was increase in response with the increase in level of factors, whereas negative sign indicates the reciprocal effect of response. The used concentrations of BAP at linear terms of had significant effect on the length of shoot in negative manner. Whereas, the media supplements at quadratic levels of BAP had non-significant effect on length of shoots in negative terms.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 2.18739 + 1.38046 * BAP - 0.441589 * BAP^2

A range of concentrations of BAP given by D-Optimal RSM were used to study the response of nodal explant of spine gourd in terms of number of days required for shoot initiation, number of shoots per explant and length of shoots. In the present study, highest average length of shoot (3.85 cm) was registered in 1.5 mgl^{-1} BAP fortified MS media while lowest average length of shoot was in an explant placed on MS media supplemented with 3.0 mgl^{-1} BAP. The similar results were reported by Jamatia (2016) in Spine gourd where he got average length of 1.06 cm using 2.0 mgl^{-1} BAP on MS media. The significant results obtained in present study were also in conformity with the earlier reports of Karim and Ullah (2001) in cotyledonary explants of *Momordica dioica* with 1.0 mgl^{-1} BAP alone.

4.2.1.1.2 Response of spine gourd nodal explants to various levels of BAP and NAA using D-Optimal RSM

D-optimal response surface methodology were used to generate experimental designs for nodal explants of Spine gourd at various levels of BAP+NAA combinations with MS culture media as a basal media. The results reported to various responses such as shoot initiation,

average number and length of shoots is presented in Table 27 and discussed hereunder with the suitable headings.

Table 27: Effect of different levels of BAP and NAA supplementations on the morphogenic responses of nodal explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: NAA (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots / explant	Response 3 Av. length of shoot (cm)
1	2.5	0.5	7.00	1.00	3.10
2	3.0	0.5	9.00	1.10	2.58
3	1.0	0.5	7.33	1.00	2.50
4	2.0	0.5	6.40	1.20	3.80
5	2.0	0.5	6.30	1.20	3.70
6	1.5	0.5	7.00	1.00	4.00
7	3.0	0.5	9.10	1.10	2.60
8	1.5	0.5	7.10	1.10	4.20
9	3.0	0.5	8.90	1.20	2.20
10	2.5	0.5	7.20	1.10	3.00
11	1.0	0.5	7.34	1.10	2.60
12	1.0	0.5	7.32	0.90	2.70
13	2.0	0.5	6.50	1.30	3.60

Coefficient of quadratic polynomial model of BAP and NAA for responses of initiation stage

Factor	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot
Intercept	6.60	1.10	3.88
BAP(A)	0.7229*	0.0571*	-0.2100*
NAA(B)	-0.0491	-0.0177	-0.0423
AB	-0.0225	0.0500	-0.0700
A^2	1.64*	-0.0907*	-1.26*
B^2	-0.0993	0.0808*	-0.1245
F- Value	23.65	4.46	6.64
P-Value	0.0003	0.0381	0.0137
Mean	7.42	1.10	3.12
SD	0.2987	0.0691	0.3604
Adequate precision	12.9829	7.1237	6.9840
R^2	0.9441	0.7610	0.8259

*P-Value = <0.05

4.2.1.1.2.1 Number of days required for shoot initiation

The observations concerned to number of days required for shoot initiation as affected by using various combinations and levels of BAP and NAA on a MS media are presented in Table 27 and Fig. 3. It is evident from the Table No. 27 that number of days required for shoot initiation from the nodal explant of spine gourd ranged from 6.3 to 9.1 days after inoculation. The nodal explants inoculated on MS media supplemented with 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA recorded the average minimum number of days i.e. 6.3 for shoot initiation. The average maximum

number of days i.e. 9.1 days for shoot initiation were recorded by the nodal explants placed on a MS media fortified with the combinations of 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA.

The data obtained are subjected to regression analysis using D-Optimal RSM software. The data revealed that the coefficient of determination (R^2) was 0.9441. Model under study reported the adequate precision of 12.98, which is considerably higher than the minimum desirable (4) for high prediction ability of model to navigate the design space. It is evident from the Model F-value (23.65) that the model is significant with the P-values of 0.0003.

It is seen from the coefficient for number of days required for shoot initiation that the levels of different combinations of media supplements like BAP and NAA had significant ($p<0.05$) effect on response. The linear terms of BAP had positive significant effect, whereas NAA had non-significant in negative terms, which is most desirable as it indicates the early initiation of shoots in explants. The interaction of BAP and NAA levels also had a non-significant effect on number of days required for shoot initiation in negative manner. The quadratic levels of BAP and NAA had showed that positive significant effect and negative non-significant respectively on days required for shoot initiation.

The response surface equation derived for predicting average number of days for shoot initiation could be given as:

Initiation (Days) = - 236.27675 - 4.72800 * BAP + 992.43088 * NAA - 2.25000 * BAP * NAA + 1.64396 * BAP^2 - 992.84141* NAA^2

In the present investigation, which was conducted with the different levels of media supplements of BAP+NAA showed significant differences for days taken for shoot initiation. The minimum number of days (6.3) required for shoot initiation was observed in the explants placed on the MS media supplanted with combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. Whereas, highest number of days required for shoot initiation (9.1) were recorded in the explants inoculated on MS media supplemented with PGR's combination of 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The earlier shoot initiation may be attributed to the effect of Auxins and cytokinins as reported by Kapadia, *et al.* (2018), Hoque, *et al.* (1995) Hoque, *et al.* (2000), Nabi, *et al.* (2002[a]), Mohammad and Sharif (2010), Rai, *et al.* (2012), Patel, *et al.* (2015) and Jadhav, *et al.* (2015) in micropropagation studies of Spine gourd.

4.2.1.1.2.2. Number of shoots per explant

The data in respect of the number of shoots produced per nodal explant of spine gourd as effected by the various levels of BAP+NAA supplementations on MS media is presented in Table 27 and graphically depicted in Fig. 3. It is clear from the Table 27 that average number of shoots produced per explant were ranged from 0.9 to 1.3. The minimum number of shoots per explant (0.9) was observed in the nodal explants placed on MS media supplemented with 1.0

mgl^{-1} BAP + 0.5 mgl^{-1} NAA while maximum number of shoots per explant (1.3) were recorded in a MS media supplemented with combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA.

The Coefficient of determination (R^2) in respect of the number of shoots per explant for the present model under study is 0.7610. For the same analysis, adequate precision was found to be 7.1237, which is significantly higher than the minimum desirable (4) for high prediction ability to navigate the design. The Model F-value (4.46) implied that the model was significant (P-value 3.81%).

In respect of coefficients for number of shoots per explant, present study model showed that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of Spine gourd nodal explants. The non-significant effects of BAP, NAA alone and in combination in respect of number of shoots per explants were observed at linear, interaction and quadratic levels. However, linear levels of NAA had non-significant effect in negative terms. The quadratic levels of BAP also had negative non-significant effect whereas NAA had positive significant effect on number of shoots produced per explant.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = + 208.59823 - 2.07986 * BAP - 820.13781* NAA + 5.00000 * BAP * NAA - 0.090749 * BAP^2 + 808.37004 * NAA

The effect of various combinations of auxin and cytokinins were examined and varied results were found in number of shoots per explants of *Momordica dioica* using nodal explants. The present investigation showed that highest number of shoots per explants (1.3) were noticed in 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, however lowest number of shoots per explants (0.9) were obtained from the PGR's combination of 1.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The number of shoots produced per explant was low i.e. 0.9 to 1.3. This might be attributed to the increased callus formation with use of increased concentration of BAP. The high rate of callus formation might have hindered the growth of shoots and lead to the formation of somaclonal variants. The similar trend was reported by Rai, *et al.* (2012) in Spine gourd. However, the contradictory results have been reported by Kapadia, *et al.*, (2018), Patel, *et al.* (2015) in Spine gourd, and Karim and Ahmed (2010) in *M. dioica* using somatic embryogenesis. The desirable effect of the nodal explant for more number of shoots is also reported by Mohammad and Shorif (2010) in spine gourd.

4.2.1.1.2.3 Length of shoots

The average length of shoots per explant indicated significant differences among the various media supplementations with BAP and NAA used for the nodal explants of Spine gourd (Table 27 and Fig. 3). The minimum length (2.2 cm) was recorded in the nodal explants placed on MS media supplemented with 3.0 mgl^{-1} BAP plus 0.5 mgl^{-1} of NAA. The maximum

length of shoot (4.2 cm) was registered in the nodal explant on MS media supplemented with 1.5 mgl^{-1} of BAP + 0.5 mgl^{-1} NAA.

The regression analysis of average length of shoots for nodal explants of spine gourd revealed that the coefficient of determination (R^2) is 0.8259. The adequate precision was found to be 6.9840 for average shoot length which is substantially higher than the minimum desirable (4) for high prediction ability. The Model F-value (6.64) suggested that the model was significant along with the support of P-value (0.0137).

It is evident from the table that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of nodal explants. The linear and interaction effects of different levels of BAP and NAA had non-significant effect on the length of shoot in negative manner. The quadratic levels of both BAP and NAA had significant and non-significant effect on length of shoots respectively but NAA had negative non-significant effect.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = - 316.87234 + 8.34339 * BAP + 1254.70169 * NAA - 7.00000 * BAP * NAA - 1.26335 * BAP^2 - 1244.93392 * NAA^2

Different degrees of lengths and growth of shoots produced were achieved from the nodal explants placed on a MS media supplemented with the various combinations of BAP and NAA in Spine gourd. In the present study, maximum length of shoots per explants (4.2 cm) was obtained from the combination of 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, which is followed by use of media supplement of 2 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The minimum length of shoots (2.2 cm) was recorded in the MS media supplemented with the higher concentration of BAP and NAA i.e., 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. In the present investigation, lower levels of the BAP and NAA were found to be the best in respect of initiation and length of shoot. The higher levels of cytokinin and Auxin might have induced the callus formation which in turn reduced the length of shoots of plants. The present findings are in conformity with those reported by Rai *et al.,* (2012), Patel *et al.* (2015), Jamatia *et al.,* (2016) in Spine gourd.

4.2.1.1.3 Response of spine gourd nodal explant to various levels of BAP and 0.5 mgl^{-1} Kn using D-Optimal RSM.

The experiment was conducted with various levels of BAP and 0.5 mgl^{-1} Kn as generated by D-optimal response surface methodology. The nodal explants were placed on MS media to study their response to the various levels of BAP and 0.5 mgl^{-1} Kn. The results pertaining to various responses such as shoot initiation, average number and length of shoots are presented in Table 28 and graphically represented in Fig. 4 and discussed hereunder suitable headings.

Table 28: Effect of different levels of BAP and 0.5 mgl^{-1} Kn on the morphogenic responses of nodal explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: Kn (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	1.5	0.5	3.00	1.0	2.15
2	1.5	0.5	3.10	1.1	2.16
3	2.0	0.5	4.00	1.5	6.86
4	2.0	0.5	4.10	1.4	6.87
5	2.5	0.5	3.16	1.5	1.30
6	2.0	0.5	4.20	1.6	6.88
7	1.5	0.5	3.20	1.2	2.14
8	2.0	0.5	3.90	1.7	6.86
9	2.5	0.5	3.15	1.4	1.40
10	2.0	0.5	4.00	1.5	6.87
11	2.5	0.5	3.17	1.3	1.50
12	1.5	0.5	3.00	1.1	2.15
13	2.5	0.5	3.15	1.4	1.30

Coefficient of quadratic polynomial model of BAP and 0.5 mgl^{-1} Kn for responses of initiation stage

Factor	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot
Intercept	4.02	1.52	6.87
BAP(A)	0.0412	0.1500*	-0.3875*
Kn (B)	0.0491	0.0302	-0.0095
AB	-0.0225	0.0250	-0.0275
A^2	-0.9156*	-0.2838*	-5.11*
B^2	0.0272	0.0206	-0.0018
F- Value	69.85	7.97	4706.76
P-Value	<0.0001	0.0083	<0.0001
Mean	3.47	1.36	3.73
SD	0.0874	0.1065	0.0588
Adequate precision	18.95	7.1578	138.70
R^2	0.9804	0.8505	0.9997

*P-Value = <0.05

4.2.1.1.3.1 Number of days required for shoot initiation

The observations regarding number of days required for shoot initiation as affected by using various combination and concentration of BAP and 0.5 mgl^{-1} of Kn on MS media are summarized in the table 28 and graphically represented in the Fig. 4. It is evident from the Table No 28 that number of days required for shoot initiation from the nodal explant of spine gourd were ranged from 3.0 to 4.2 days after inoculation. The nodal explants inoculated on MS media supplemented with 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn recorded the average minimum number of days

i.e. 3.0 for shoot initiation. The average maximum number of days i.e. 4.2 days for shoot initiation were recorded by the nodal explants placed on a MS media fortified with the combination of 2.0 mgl^{-1} BAP and 0.5 mgl^{-1} Kn.

The data obtained are subjected to regression analysis using D-Optimal RSM. The data revealed that the coefficient of determination (R^2) was 0.9441 for days to shoot initiation. Model under study reported the adequate precision of 12.98, which is considerably higher than the minimum desirable (4) for high prediction ability of model to navigate the design space. It is evident from the Model F-value (23.65) that the model is significant with the P-values of 0.0003.

As regards the coefficient for number of days required for shoot initiation model showed that the levels of different combinations of media supplements like BAP and 0.5 mgl^{-1} Kn had significant ($p<0.05$) effect on response. The linear levels of BAP and Kn and quadratic levels of Kn had non-significant effect on the number of days required for shoot initiation. The interaction and quadratic levels of BAP had non-significant and significant effect respectively on number of days required for shoot initiation in negative terms, whereas negative effect were expected most desirable in case of number of days required for shoot initiation because less number indicates the earliest shoot initiation.

The response surface equation derived for predicting average number of days for shoot initiation could be given as:

Initiation (Days) = + 50.26326 + 16.98191 * BAP - 258.14829 * Kn - 4.50000 * BAP * Kn-3.66235 * BAP^2 + 272.05882 * Kn^2

Research results confirmed that the different levels of media supplements of BAP + 0.5 mgl^{-1} Kn had significant differences for days taken for shoot initiation. The minimum number of days (3.0) required for shoot initiation was observed in the explants placed on the MS media supplanted with lower concentration of combination 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn, whereas, maximum number of days required for shoot initiation (4.2) were recorded in the explants inoculated on MS media supplemented with plant growth regulators combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. The combination of BAP and Kn showed very little shoot regeneration response as compare to the BAP alone and in combination with the NAA as reported by Jamatia (2015), Hoque, *et al.* (1995) and Paula, *et al.* (1990).

4.2.1.1.3.2 Number of shoots per explant

The data in respect of the number of shoots produced per nodal explant of *Momordica dioica* as effected by the various levels of BAP+0.5 mgl^{-1} Kn supplementations on MS media is presented in Table 28 and graphically depicted in Fig. 4. It is evident from the Table 28 that average number of shoots produced per explant were ranged from 1.0 to 1.7 shoots. The minimum number of shoots per explant (1.0) was reported in the nodal explants placed on MS media supplemented with 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn, while maximum number of shoots per

explant (1.7) were recorded in a MS media supplemented with PGRs combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn.

Data presented in Table 28 revealed that the coefficient of determination (R^2) was 0.8505 for number of shoots per explant of spine gourd. The adequate precision was found to be 7.1578, which is significantly higher than the minimum desirable (4) for high prediction ability to navigate the design. The Model F-value (7.97) implied that the model was significant (P-value 0.0083).

In respect of coefficient for number of shoots per explant, present study model showed that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of Spine gourd nodal explants. The linear and quadratic levels of BAP had significant effect on the number of shoots per explant**,** whereas interactions, linear and quadratic terms of Kn levels had non-significant effect on number of shoots per explant.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = + 51.34406 + 2.34118 * BAP - 212.86459 * Kn - 5.85786 * BAP * Kn - 1.13529 * BAP^2 + 205.88235 * Kn^2

Different combinations of PGR's were examined and varied results were found in number of shoots per explants of *Momordica dioica* using nodal explants. The present findings showed that highest number of shoots per explants (1.7) were noticed at the combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn, whereas lowest number of shoots per explants (1.5) were recorded at the PGRs combination of 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. Most of the Cucurbitaceae members produced shoots from the combination of auxin and cytokinins as reported by Sultana and Bari Miah (2003) in *M. charantia.* These results are in conformity with the earlier results of Devendra, *et al.* (2009), Nabi, *et al.* (2002^b), Jamatia, *et al.* (2016) in *Momordica dioica* from organogenesis.

4.2.1.1.3.3 Length of shoots

Data in respect to average length of shoots per explant as effected by various levels of media supplementations with BAP and 0.5 mgl^{-1} Kn indicated significant differences for the nodal explants of Spine gourd (Table 28 and Fig. 4). The minimum length (1.3 cm) of shoot was recorded in the nodal explants placed on MS media supplemented with 2.5 mgl^{-1} BAP plus 0.5 mgl^{-1} of Kn. The maximum length of shoot (6.88 cm) was registered in the nodal explant on MS media supplemented with 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA.

Data of regression analysis were presented in Table 28 revealed that the coefficient of determination (R^2) was 0.9997 for average length of shoot per explant. The Model F-value (4706.76) and P-value (<0.0001) suggested that the model was significant with the adequate precision of 138.70, which outstandingly higher than the minimum desirable (4) for high prediction ability for average length of shoot

It is evident from the table 28 that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of nodal explants. The BAP and Kn level at linear terms, their interactions and quadratic levels had non-significant effect on the length of shoot in negative manner, out of these only linear and quadratic levels of BAP had significant effect.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = - 82.71471 + 83.67147 * BAP + 27.69884 * Kn - 5.50000 * BAP * Kn - 20.42412 * BAP^2 - 17.64706 * Kn^2

Diverse range of length and growth of shoots produced were achieved from the nodal explants placed on a MS media supplemented with the various combinations of BAP and 0.5 mgl^{-1} Kn in Spine gourd. In the present study, maximum length of shoots per explants (6.88 cm) was obtained from the combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn which is followed by 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. The minimum length of shoots (1.3 cm) was recorded in the MS media fortified with the combination of 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The higher levels of cytokinine and auxin might have induced the callus formation which in turn reduced the length of shoots of plants. Similar results were reported by the Paula, *et al.* (1992) while investigating somatic embryogenesis in *Cucurbita pepo* using cotyledonary and shoot tip explants.

4.2.1.1.4 Response of spine gourd nodal explants to various levels of BAP and 1.0 mgl^{-1} Kn using D-Optimal RSM.

Experimental design was generated using Response surface methodology of design expert software for the two factors i.e. BAP and Kn to study the relative effect on the responses *viz.,* days to shoot initiation, number of shoots and length of shoot per explant. The data generated are presented in Table 28 and graphically presented in Fig. 5 and discussed here under the suitable headings.

4.2.1.1.4.1 Number of days required for shoot initiation

The observations regarding number of days required for shoot initiation were affected by various levels of BAP and 1.0 mgl^{-1} Kn supplementations on MS culture media are summarized in Table 29 and graphically represented in Fig. 5. It is clear from the Table 29 that the number of days required for shoot initiation were raged from 3.0 to 6.1 days. The minimum 3.0 days were required for shoot initiation from nodal explants placed on MS media supplemented with 1.5 and 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. Whereas, combinations of 2.5 mgl^{-1} BAP + 0.1 mgl^{-1} Kn supplementation registered maximum number of days (6.1) for shoot initiation.

Data collected in respect the days required for shoot initiation were subjected to the regression analysis. It is revealed from the data that the coefficient of determination (R^2) was 0.7426 for the model. Further, model reported the adequate precision of 5.5049, which is

substantially higher than the minimum desirable (4) for high prediction ability of design. The model F-value of 4.04 implied that the model was significant with P-value of 0.0481.

Table 29: Effect of different levels of BAP and 1.0 mgl^{-1} Kn supplementations on the morphogenic responses of nodal explant of spine gourd using D-Optimal Response Surface Methodology.

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: Kn (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	1.5	1.0	3.0	1.5	5.05
2	2.0	1.0	3.0	1.0	5.93
3	2.0	1.0	3.2	0.9	5.92
4	2.5	1.0	6.0	1.5	3.30
5	2.0	1.0	3.0	1.1	5.94
6	1.5	1.0	3.1	1.6	5.07
7	2.5	1.0	6.1	1.4	3.40
8	2.0	1.0	3.3	1.0	5.94
9	2.5	1.0	5.9	1.6	3.30
10	1.5	1.0	3.2	1.7	5.06
11	2.0	1.0	3.0	0.9	5.92
12	2.0	1.0	3.1	1.1	5.93
13	2.0	1.0	3.2	1.0	5.91

Coefficient of quadratic polynomial model of BAP and 1.0 mgl^{-1} Kn for responses of initiation stage

Factors	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot
Intercept	3.13	0.9880	5.93
BAP(A)	1.51*	-0.0937	-0.8353*
Kn (B)	-0.0930	-0.0561	0.1213*
AB	0.1041	-0.0721	0.0462*
A^2	1.43*	0.5366*	-1.71*
B^2	-0.1282	-0.0154	0.0824*
F- Value	265.27	20.19	15935.94
P-Value	<0.0001	0.0005	<0.0001
Mean	3.78	1.25	5.13
SD	0.1206	0.997	0.0133
Adequate precision	37.45	9.309	290.5252
R^2	0.9947	0.9352	0.99

*P-Value = <0.05

As regards the coefficient for number of days required for shoot initiation model, it is evident that the levels of different combinations of media supplementations had significant ($p<0.05$) effect on responses by nodal explants of spine gourd. The BAP level at linear terms had positive significant effect on the number of days for initiation. Whereas Kn had negative non-significant effect on days required for shoot initiation. The interactions of BAP and Kn had non-significant effect on number of days required for shoot initiation. The quadratic levels of BAP had positive significant effect and Kn had non-significant effect in negative terms on days required for shoot initiation.

The response surface equation derived for predicting average number of days for shoot initiation on using BAP and 1.0 mgl^{-1} Kn in combination could be given as:

Initiation (Days) = - 16.60224 - 23.83377 * BAP + 79.28608 * Kn + 3.78641 * BAP * Kn + 5.72580 * BAP^2 - 42.36849 * Kn^2

The present investigation on micropropagation of nodal explants of Spine gourd revealed that there are significant differences among the responses of nodal explants due to various concentrations of BAP + 1.0 mgl^{-1} Kn supplementations on MS basal media. The minimum number of days required for shoot initiation *i.e.,* 3.0 days were recorded in treatment combination of 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn and 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn for the nodal explants of the spine gourd. However maximum number of days required for shoot initiation (6.1) were noticed in the combination of 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. In the present model, early and higher percentage of regeneration frequency was achieved by use of BAP + Kn combinations as reported by Kawale and Chaudhary (2009) in *Trichosanthes cucumerina* using cotyledonary explants for organogenesis.

4.2.1.1.4.2 Number of Shoots per explant

Experimental results revealed that two different kinds of cytokinin's combination had significant effect on number of shoots per explant *Momordica dioica*. The data in respect of number of shoots per explant as influenced by various levels of BAP and Kinetin are presented in Table 29 and graphically represented in Fig. 5. Results of average number of shoots per explants were ranged from 0.9 to 1.6 shoots. The minimum number of shoots per explant (0.9) was reported in treatment combinations of 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} of Kn for nodal explants of Spine gourd. However, the maximum number of shoots per explant (1.6) were registered in the MS media fortified with combination of 2.5 mgl^{-1} BAP +1.0 mgl^{-1} Kn.

Regression analysis of a data presented in Table 29 revealed that the coefficient of determination (R^2) is 0.9352 for number of shoots per explant. The model reported the adequate precision of 9.309 which is appreciably higher than the minimum desirable (4) for high prediction ability of design. The F-value of model is 20.19 which implied that the model was significant for number of shoots per explants with P-value of 0.0005.

As regards the coefficient for number of Shoots per explant, model showed that the levels of different combinations of cytokinins had significant ($p<0.05$) effect on response of number of shoots per explant of spine gourd. The linear and quadratic terms of BAP as well as linear terms of Kn and interaction effect were non-significant as regards, the number of shoots per explant in negative terms. However, quadratic levels of Kn had positive significant effect on number of shoots per explant.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = - 0.023979 - 6.03334 * BAP +14.86294 * Kn - 2.62136 * BAP * Kn + 2.14632 * BAP^2 - 5.09126 * Kn^2

From the results it is conformed that there are significant differences in the number of shoots per explant as influenced by the various levels of BAP + 1.0 mgl^{-1} Kn. The highest number of shoots per explant (1.6) were reported in nodal explants of spine gourd placed on the MS media supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. Whereas, minimum number of shoots per explants of spine gourd (0.9) were recorded in the nodal explants placed on MS media supplemented with 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. Similar results were reported by Jamatia (2016) in *Momordica dioica* and Kawale and Choudhary (2009) in *Cucurbita pepo.*

4.2.1.1.4.3 Length of Shoots

The average length of shoot per explant recorded the significant differences as affected by the various levels of media supplementation with BAP+1.0 mgl^{-1} Kn (Table 29 and Fig. 5). It is seen from the Table 29 that the average length of shoot per explant were ranged from 3.3 to 5.94 cm. The minimum length of the shoot (3.3 cm) was recorded in the nodal explant placed on a MS media supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. The maximum length of shoot per explant (5.94 cm) was registered in respect of MS supplemented with combination of 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn.

The regression analysis of average length of shoots for nodal explants of spine gourd revealed that the coefficient of determination (R^2) was 0.99. The adequate precision was found to be 290.5252, for average length of shoots, which is noticeably higher than the minimum desirable (4) for high prediction ability. The studied model for the average length of shoots is found to be significant with F-value of 15935.94 and P-value of <0.0001.

It is revealed from coefficient for average length of shoot that the model is significant at the different levels of media supplementation ($p<0.05$) and had significant effect on responses. The linear, interaction and quadratic terms of BAP and Kn had positive significant effect on the average length of shoot. Furthermore, linear and quadratic terms of BAP had shown significant effect in negative terms.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 12.87488 + 23.93524 * BAP - 58.10093 * Kn + 1.67961 * BAP * Kn - 6.84028 * BAP^2 +27.24776 * Kn^2

The length of the shoots per nodal explants of *Momordica dioica* was significantly influenced by the various combinations and levels of the cytokinins. Results of investigation reported that minimum average shoot length of 3.3 cm was achieved on the MS media fortified with the combination of 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn, whereas maximum average length of shoot 5.94 cm was reported in case of nodal explant placed on MS media supplemented with 2.0 mgl^{-1}

BAP + 1.0 mgl^{-1} Kn, followed by 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn (5.07 cm). The present findings are in conformity with those of Paula (1992) in Cotyledon explants of *Cucurbita pepo* and Kiełkowska and Havey (2011) in stem fragments of *Cucumis sativus.*

4.2.1.1.5 Response of spine gourd nodal explants to various levels of BAP and CH using D-Optimal RSM

Response surface methodology of design expert software was used to generate the experimental design for the two factors i.e. BAP and casein hydrolysate to study the relative effect on the responses *viz.,* days to shoot initiation, number of shoots and length of shoot per explant. The data generated are presented in Table 30 and graphically presented in Fig. 6 and discussed here under the suitable headings.

Table 30: Effect of different levels of BAP and CH supplementations on the morphogenic responses of nodal explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B : CH (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots / explant	Response 3 Av. length of shoot (cm)
1	1.0	200	6.34	1.00	3.94
2	2.0	200	6.17	1.47	4.90
3	3.0	200	4.80	1.00	2.10
4	2.0	200	6.16	1.40	4.91
5	2.0	200	6.19	1.45	4.92
6	1.0	200	6.33	1.10	3.95
7	1.5	200	5.60	1.28	4.00
8	1.0	200	6.36	1.20	3.93
9	1.5	200	5.60	1.15	4.10
10	3.0	200	4.80	1.10	1.30
11	2.5	200	6.05	1.50	4.04
12	2.5	200	6.00	1.51	4.05
13	3.0	200	4.90	1.20	1.40

Coefficient of quadratic polynomial model of BAP and CH for responses of initiation stage			
Factors	**Initiation (Av. days)**	**Av. number of shoots/ explant**	**Av. length of shoot**
Intercept	6.09	1.46	4.73
BAP (A)	-0.6101*	0.0333	-1.038*
CH (B)	0.1239	0.0120	0.3035
AB	-0.0435	-0.0470	0.1447
A^2	-0.3989	-0.3116*	-1.90*
B^2	-0.1859	-0.0786	-0.1132
F- Value	4.04	4.99	20.70
P-Value	0.0481	0.0289	0.0005
Mean	5.79	1.26	3.66
SD	0.3975	0.1155	0.4107
Adequate precision	5.5049	6.1488	13.1967
R^2	0.7426	0.7809	0.9367

*P-Value = <0.05

4.2.1.1.5.1 Number of days required for shoot initiation

The observations regarding number of days required for shoot initiation as affected by various combination and concentrations of BAP plus CH supplementations on MS culture media are summarized in Table 30 and graphically represented in Fig. 6. It is evident from the Table 30 that the number of days required for shoot initiation were ranged from 4.8 to 6.36 days. The minimum days (4.8) for shoot initiation was recorded for nodal explant placed on MS media supplemented with 3.0 mgl^{-1} BAP and 200 mgl^{-1} CH. Whereas, combinations of 1.0 mgl^{-1} BAP and 200 mgl^{-1} CH supplementation registered maximum number of days (6.36) for shoot initiation.

The recorded data in respect of days required for shoot initiation were subjected to the regression analysis. It is revealed from the data that the coefficient of determination (R^2) was 0.7426 for the model. Further the model reported the adequate precision of 5.5049, which is substantially higher than the minimum desirable (4) for high prediction ability of design. The Model F-value of 4.04 implies that the model was significant with P-value of 0.0481.

As regards the coefficient for number of days required for shoot initiation model, it is evident that the levels of different combinations of media supplementations had significant ($p<0.05$) effect on responses by nodal explants. The BAP level at linear terms had positive significant effect on the number of days for initiation in negative terms. This is desirable as it indicated the earliest shoot initiation. Whereas, CH had non-significant effect on days required for shoot initiation. The interaction effect and quadratic levels of BAP and CH, both had non-significant effect on number of days required for shoot initiation in negative terms.

The response surface equation derived for predicting average number of days for shoot initiation on using BAP and CH in combination could be given as:

Initiation (Days) = - 7.44093E+05 + 88.05659 * BAP + 7438.87820 * CH - 0.435356 * BAP * CH - 0.398851* BAP^2 - 18.591922 * CH^2

The present research findings showed the significant differences for days required for shoot initiation on the use of different levels of BAP+CH on nodal explants of spine gourd. The minimum number of days required for shoot initiation i.e., 4.8 days were recorded from the combination of 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH for the nodal explants of the spine gourd. However maximum number of days required for shoot initiation (6.36) were noticed in the combination of 1.0 mgl^{-1} BAP + 200 mgl^{-1} CH. In the present study, BA along with the CH induced vigorous regeneration of shoots but promoted axillary buds to develop whitish fragile callus at the higher concentrations of BAP and CH in turn which inhibit the further development of shoots. This might be attributed to the role of CH in inducing vigorous shoots, promoting callus formation. Also the inhibitory effect for shoot differentiation might have played the role. The results are in conformity with those reported by Rai *et al.*, (2012) in spine gourd., Ahmad and Anis (2005) in *Cucumis sativus* L., Prakash *et al.* (2003) in *Jojoba*.

4.2.1.1.5.2 Number of shoots per explant

The data in respect of number of shoots per nodal explant of spine gourd as effected by the different levels of BAP+CH supplementations on MS media are presented in Table 30 and graphically represented in Fig. 6. It is clear from the data that the number of shoots per nodal explants were ranged from 1.0 to 1.51 shoots. The minimum number of shoot per explant (1.0) was recorded in a media supplemented with 1.0 mgl^{-1} BAP + 200 mgl^{-1} CH and 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH. However, the maximum number of shoots per explant (1.51 shoots) were registered for the MS media supplemented with 2.5 mgl^{-1} BAP + 200 mgl^{-1} CH.

Regression analysis of a data presented in Table 30 revealed that the coefficient of determination (R^2) is 0.7809 for number of shoots per explant. The model reported the adequate precision of 6.1488 which is appreciably higher than the minimum desirable (4) for high prediction ability of design. The F-value of model is 4.99 which implied that the model was significant for number of shoots per explants with P-value of 0.0289.

As regards the coefficient for number of shoots per explant, model showed that the levels of different combinations had significant ($p<0.05$) effect on response. The linear terms of BAP and CH levels had non-significant effect on the Number of Shoots per explant**.** The interaction and quadratic levels of BAP and NAA had negative non-significant effect on number of shoots per explant, whereas only quadratic levels of BAP had significant effect in negative manner.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = - 3.14615E+05 + 95.20351 * BAP + 3145.08733 * CH - 0.469620 * BAP * CH- 0.311553 * BAP^2 - 7.86007 * CH^2

The results revealed that highest number of shoot per explant (1.51) had reported on the MS media supplemented at 2.5 mgl^{-1} BAP + 200 mgl^{-1} CH, whereas lowest number of shoots per explants (1.0) were obtained on the MS media supplemented with 1.0 mgl^{-1} BAP + 200 mgl^{-1} CH and 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH. The similar results were reported by Rai *et al.* (2012) in spine gourd, Ahmad and Anis (2005) in *Cucumis sativus* L. Agrawal *et al.* (1999) and Prakash *et al.* (2003) in male and female *Jojoba*.

4.2.1.1.5.3 Length of shoots

The average length of shoot per explant recorded the significant differences as affected by the various levels of media supplementation with BAP+CH (Table 30 and Fig. 6). It is seen from the Table 30 that the average length of shoot per explant were ranged from 1.3 to 4.92 cm. The minimum length of the shoot (1.3 cm) was recorded in the nodal explant placed on a MS media supplemented with 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH. The maximum length of shoot per

explant (4.92 cm) was registered in respect of MS supplemented with combination of 2.0 mgl^{-1} BAP + 200 mgl^{-1} CH.

The regression analysis of average length of shoots for nodal explants of spine gourd revealed that the coefficient of determination (R^2) was 0.9367. The adequate precision was found to be 13.1967 for average length of shoots, which is noticeably higher than the minimum desirable (4) for high prediction ability. The studied model for the average length of shoots model is significant with F-value of 20.70 and P-value of 0.0005.

It is revealed from the coefficient for length of shoot that the model is significant at the different levels of media supplementation ($p<0.05$) and had significant effect on responses. The BAP level at linear terms had significant effect on the length of shoot in negative manner. Whereas linear terms of CH as well as interaction of BAP and CH had non-significant effect. The quadratic levels of BAP and CH had significant and non-significant effect respectively on length of shoots but in negative terms.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = -4.53003E+05 - 282.90825 * BAP + 4529.88240 * CH +1.44744 * BAP * CH - 1.90215 * BAP^2 -11.32436 * CH^2

The present study revealed the highest average length of shoots (4.92 cm) were recorded in nodal explants on MS media fortified with 2.0 mgl^{-1} BAP + 200 mgl^{-1} CH. However lowest average length of shoots (1.3 cm) were recorded in case of nodal explants placed on MS culture media supplemented with 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH. The similar results reported Rai *et al.* (2012) in spine gourd and Ahmad and Anis (2005) in *Cucumis sativus* L.

4.2.1.1.6 Response of spine gourd nodal explant to various levels of BAP and IAA using D-Optimal RSM

The various combinations of BAP and IAA as generated by D-optimal response surface methodology were tried in MS media to study the response of nodal explant of spine gourd. The results pertaining to various responses such as shoot initiation, average number and length of shoots are presented in Table 31 and discussed hereunder suitable headings.

4.2.1.1.6.1 Number of days required for shoot initiation

Results pertaining to the number of days required for shoot initiation from nodal explants of spine gourd placed on MS media fortified with various level of BAP and IAA ranged from 5 to 7.1 days (Table 31 and Fig.7). The minimum number of days required for shoot initiation (5.0) was recorded in nodal explant placed on MS media supplemented with the combinations of 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. The maximum number of days i.e., 7.1 for shoot initiation were recorded for the combinations of 0.75 mgl^{-1} of BAP + 0.1 mgl^{-1} IAA.

Table 31: Effect of different levels of BAP and IAA on the morphogenic responses of nodal explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A BAP (mgl^{-1})	Factor 2 B : IAA (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	1.00	0.10	6.0	1.80	5.90
2	1.00	0.10	6.2	2.00	6.00
3	0.50	0.10	6.3	1.29	8.00
4	1.00	0.10	6.1	1.90	6.10
5	0.50	0.10	6.1	1.28	8.20
6	0.25	0.10	7.0	1.00	7.76
7	1.00	0.10	6.0	1.80	6.20
8	0.25	0.10	7.1	1.10	7.80
9	0.50	0.10	6.2	1.27	8.40
10	0.75	0.10	5.0	1.20	5.10
11	0.25	0.10	6.9	1.20	7.72
12	0.75	0.10	5.1	1.30	5.90
13	0.75	0.10	5.2	1.10	6.00

Coefficient of quadratic polynomial model of BAP and IAA for responses of initiation stage

Factor	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot (cm)
Intercept	5.43	1.15	6.66
BAP (A)	-0.5913*	0.3461*	-1.17*
IAA (B)	-0.0396	0.1989	-1.36
AB	0.1365	0.0482	0.2531
A^2	0.9825*	0.2421*	0.0859
B^2	0.2927	-0.0264	1.69
F- Value	11.99	15.09	4.10
P-Value	0.0025	0.0012	0.0465
Mean	6.09	1.40	6.84
SD	0.2879	0.1304	0.7503
Adequate precision	9.0919	10.300	5.5356
R^2	0.8954	0.9151	0.7453

*P-Value = <0.05

The regression analysis of a data (Table 31) revealed that the coefficient of determination (R^2) was 0.8954. The adequate precision was found to be 9.0919 for number of days required for shoot initiation model, which is appreciably higher than the minimum desirable (4) for high prediction ability to further navigate the design. The results revealed that the model was significant with the F-value of 11.99 and P-value of 0.0025.

As regards the coefficient for number of days required for shoot initiation, model showed that the levels of different combinations had significant ($p<0.05$) effect on responses. The BAP and IAA levels at linear terms had positive significant and non-significant effect respectively on the number of days required for shoot initiation. The interaction of BAP and NAA had non-significant effect on number of days required for shoot initiation. The quadratic levels of BAP and

NAA had positive significant and non-significant effect respectively on days required for shoot initiation.

The response surface equation derived for predicting average number of days for shoot initiation on using BAP and IAA in combination could be given as:

Initiation (Days) = + 9.70315 - 10.67437 * BAP - 17.04721 * IAA + 7.27833 * BAP * IAA + 6.98702 * BAP^2 + 117.06576 * IAA^2

Investigation conducted on *In-vitro* regeneration of *Momordica dioica* using nodal explants with the various combination of both the auxin and cytokinine to know their effect on the number of days required for shoot initiation showed the significant differences among the various treatments. Research findings were revealed that maximum number of days required for shoot initiation i.e., 7.1 days were registered in treatment with 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. However, the minimum number of days required for shoot initiation (5.0) was reported on MS media supplemented with PGRs combination of 0.75 mgl^{-1} BAP + 0.1 mgl^{-1} IAA which is followed by 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA (6 days). The nodal shoot segments reported to be most suitable explant material for regeneration of shoots in *Momordica* spp. The results are inconformity with those reported by Agarwal and Kamal (2004) and Sultana, *et al.* (2005) in Spine gourd. Earlier bud breaking was recorded by the use of same combinations of auxin and cytokinins in nodal explants of *Momordica dioica* by Chaudhary *et al.* (2017).

4.2.1.1.6.2 Number of shoots per explant

The data regarding number of shoots per nodal explant of spine gourd as effected by the different levels of BAP+IAA supplementations on MS media are presented in Table 31 and graphically represented in Fig. 7. It is clear from the data that the number of shoots per nodal explants were ranged from 1.0 to 1.9 shoots. The minimum number of shoot per explant (1.0) was recorded in a media supplemented with 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. However, the maximum number of shoots per explant (1.9shoots) were registered for the MS media supplemented with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA.

Regression analysis of a data presented in Table 31 revealed that the coefficient of determination (R^2) is 0.9151 for number of shoots per explant. The model reported the adequate precision of 10.30 which is appreciably higher than the minimum desirable (4) for high prediction ability of design. The F-value of model is 15.09 which implied that the model was significant for number of shoots per explants with P-value of 0.0012.

As regards the coefficient for number of shoots per explant, model showed that the levels of different combinations had significant ($p<0.05$) effect on response. Linear and Quadratic terms of BAP level had positive significant effect on the number of shoots per explant**.** Whereas linear and quadratic levels of IAA and interaction effect of BAP and IAA had non-significant

effect on number of shoots per explant. However, quadratic levels of IAA had affected in negative terms.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = + 1.10349 - 1.35780 * BAP + 3.42927 * IAA + 2.56810 * BAP * IAA + 1.72180 * BAP^2 - 10.56847 * IAA^2

A range of PGR's combination examined on the number of shoots per explants on nodal explants of *Momordica dioica* and revealed the significant differences among the treatments. Present findings showed that the maximum number of shoots per explants (1.9) were observed on the MS media supplemented with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA, whereas minimum number of shoots per explant (1.0) were reported in the treatment with 0.25 mgl^{-1} + 0.1 mgl^{-1} IAA. The contradictory results were reported by Chaudhary *et al.* (2017). In present study, it is observed that incorporation of an auxin (IAA) at a lower concentration (0.1 mgl^{-1}) significantly increased the number of shoots by promoting axillary branching. There are several researches indicating that an appropriate auxin-cytokinine combination is mandatory for a better shoot proliferation/growth. Phulwaria, *et al.* (2014), Patel, *et al.* (2016) and Su and Zhang (2014) explained that plant regeneration is not individually regulated by either Auxins or cytokinins alone but it is the product of a complex mechanism of interconnecting hormonal signaling pathways.

4.2.1.1.6.3 Length of shoots

The average length of shoot per explant recorded the significant differences as affected by the various levels of media supplementation with BAP+IAA (Table 31 and Fig.7). It is clear from the Table 31 that the average length of shoot per explant were ranged from 5.1 to 8.4 cm. The minimum length of the shoot (5.1 cm) was recorded in the nodal explant placed on a MS media supplemented with 0.75 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. The maximum length of shoot per explant (8.4 cm) was registered in respect of MS supplemented with combination of 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} IAA.

The regression analysis for average length of shoots per nodal explants of spine gourd revealed that the coefficient of determination (R^2) was 0.7453. The adequate precision was found to be 5.5356 for average length of shoots, which is noticeably higher than the minimum desirable (4) for high prediction ability. The studied model for the average length of shoots model is significant with F-value of 4.10 and P-value of 0.0456.

It is revealed from the coefficient for length of shoot that the model is significant at the different levels of media supplementation ($p<0.05$) and had significant effect on responses. The BAP and IAA level at linear terms had significant and non-significant effect respectively on the length of shoot in negative manner. The interaction and quadratic levels of BAP and IAA had non-significant effect on length of shoots in negative terms.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 12.30795 - 4.54750 * BAP - 103.16486 * IAA + 13.49879 * BAP * IAA + 0.610781 * BAP^2 + 676.09872 * IAA^2

The results of present investigation reported that highest average length of shoots (8.4 cm) were recorded in nodal explants on MS media fortified with 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. However lowest average length of shoots (5.1 cm) were recorded in case of nodal explants placed on MS culture media supplemented with 0.75 mgl^{-1} + 0.1 mgl^{-1} IAA. In the present study, it is observed that BAP concentration more than 0.5 mgl^{-1} produced callus at the base of explants and decreased shoot length. Similar results were reported by Rai *et al.* (2012^{a}) in spine gourd. Present results are also in accordance with Moon *et al.* (2000) in oriental melon cultivar. However contradictory results were reported by Shekhawat *et al.* (2011) on the Spine gourd.

4.2.1.2 Shoot tip

The various kinds of MS basal media fortifications i.e. plant growth regulators such as BAP+NAA, BAP+CH, BAP+0.5 mgl^{-1} Kn, BAP+1.0 mgl^{-1} Kn and BAP+IAA were used for the optimization of micropropagation techniques in spine gourd using shoot tip as an explant. Each media supplementation is treated as a separate part of the research work. The range of combinations generated using D-Optimal RSM is presented and discussed as a separate experiment hereunder.

4.2.1.2.1 Response of spine gourd shoot tip explant to various levels of BAP and NAA using D-Optimal RSM

Experimental designs with various combinations of BAP and NAA as generated by D-optimal response surface methodology were tried in MS media to study the response of shoot tip explant of spine gourd. The results pertaining to various responses such as shoot initiation, average number and length of shoots are presented in Table 32 and discussed hereunder suitable headings.

4.2.1.2.1.1 Number of days required for shoot initiation

The number of days required for shoot initiation from the shoot tip explants of spine gourd placed on MS media fortified with various level of BAP and NAA ranged from 5.0 to 7.2 days (Table 32 and Fig. 8). The minimum number of days i.e. 5.0 days required for shoot initiation were observed in the nodal explants placed on MS media supplemented with 1.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, however the maximum 7.2 days were reported in 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA supplementation for shoot initiation.

The regression analysis of the data presented in (Table 32) revealed that the coefficient of determination (R^2) is 0.9800. The adequate precision was found to be 23.66, which

is appreciably higher than the minimum desirable i.e. 4 for high prediction ability. The Model F-value of 68.75 implied that the model is significant with the P-value of <0.0001.

Table 32: Effect of different levels of BAP and NAA on the morphogenic responses of shoot tip explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: NAA (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	1.5	0.5	5.75	1.5	4.53
2	2.5	0.5	6.50	2.5	5.68
3	3.0	0.5	7.00	1.0	0.25
4	2.0	0.5	6.20	1.9	4.80
5	1.0	0.5	5.00	1.0	4.10
6	3.0	0.5	7.10	1.1	0.50
7	2.5	0.5	6.80	2.8	5.70
8	2.5	0.5	6.80	2.6	5.70
9	1.5	0.5	5.80	1.7	4.60
10	1.0	0.5	5.20	1.1	4.21
11	2.5	0.5	6.70	2.7	5.72
12	2.5	0.5	6.40	2.4	5.60
13	3.0	0.5	7.20	1.2	0.30

Coefficient of quadratic polynomial model of BAP and NAA for responses of initiation stage

Factor	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot
Intercept	6.23	2.43	6.13
BAP(A)	0.9724*	0.2562	-1.20*
NAA (B)	-0.0870	-0.0675	-0.0318
AB	0.0065	-0.0213	0.0191
A^2	-0.0828	-1.52*	-4.40*
B^2	-0.0448	0.1989	0.5826
F- Value	68.75	4.87	5.73
P-Value	<0.0001	0.0307	0.0203
Mean	6.34	1.81	3.98
SD	0.1318	0.4386	1.25
Adequate precision	23.66701	6.3127	6.6009
R^2	0.9800	0.7767	0.8037

*P-Value = <0.05

As regards the coefficient for number of days required for shoot initiation, model showed that the levels of different combinations had significant ($p<0.05$) effect. The positive sign indicates that there was increase in response with the increase in level of factors, whereas negative sign indicates the reciprocal effect of response. The BAP and NAA levels at linear, quadratic terms and their interactions too had non-significant effect on the number of days required for shoot initiation. However, linear terms of BAP had positive significant effect. The linear terms of NAA and quadratic terms of both BAP and NAA affected in a negative manner, which is most desirable as it indicated minimum number of days for shoot initiation.

The response surface equation derived for predicting average number of days for shoot initiation could be given as:

Initiation (Days) = - 103.11897 + 0.978011* BAP + 438.27586 * NAA + 0.650874 * BAP * NAA - 0.082759 * BAP^2 - 448.27586 * NAA^2

Results of micropropagation studies conducted in *Momordica dioica* using shoot tip as explants with the intension to know the effect of various combinations of BAP and NAA on number of days required for shoot initiation had revealed the significant differences among the treatments. The minimum number of days (5.0) required for shoot initiation was observed in the explants placed on the MS media supplanted with combination of 1.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. Whereas, highest number of days required for shoot initiation (7.2) were recorded in the explants inoculated on MS media supplemented with PGR's combination of 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The earlier shoot initiation may be attributed to the effect of Auxins and cytokinins as reported by Kapadia, *et al.* (2018), Devendra *et al.* (2008) and Hoque, *et al.* (1995) in spine gourd. The effects of BAP, NAA and their combinations on the callus induction and regeneration were also studied by Nabi, *et al.* (2002^a), Nabi, *et al.* (2002^b), Hoque *et al.* (2000) in *Momordica dioica* using cotyledonary explants and Ghive, *et al.* (2006^a) in axillary buds of spine gourd.

4.2.1.2.1.2 Number of shoots per explant

The observations regarding the number of shoots produced per shoot tip explant of spine gourd as effected by the various levels of BAP+NAA supplementations on MS media are summarized in Table 32 and graphically represented in Fig. 8. It is seen from the Table 32 that average number of shoots produced per explant were ranged from 1.0 to 2.8. The minimum number of shoots per explant (1.0) was observed in the shoot tip explants placed on MS media supplemented with 3 mgl^{-1} BAP + 0.5 mgl^{-1} NAA while maximum number of shoots per explant (2.8) were recorded in a MS media supplemented with combination of 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA.

The coefficient of determination (R^2) in respect of the number of shoots per explant for the present model under study is 0.7767. For the same analysis, adequate precision was found to be 6.3127, which is significantly higher than the minimum desirable (4) for high prediction ability to navigate the design. The Model F-value (4.87) implied that the model was significant (P-value 0.0307).

In respect of coefficients for number of shoots per explant, present study model showed that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of spine gourd shoot tip explants. Only quadratic levels of BAP had significant effect on number of shoots per explant in negative manner**.** Whereas all other terms like linear, interaction and quadratic levels of BAP and NAA had non-significant effect on number of shoots per explant.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = + 494.24008 + 7.40419 * BAP - 1991.14447 * NAA - 2.12690 * BAP * NAA - 1.52114 * BAP^2 + 1988.64447 * NAA^2

The present investigation revealed that various combinations of BAP and NAA tested for number of shoots per explant in *Momordica dioica* using shoot tip as an explant on MS basal media had a significant effect. The highest number of shoots per explants (2.8) were noticed in 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, which is followed by 1.9 shoots at 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. Whereas lowest number of shoots per explants (1.0) were obtained from the PGR's combination of 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The high rate of callus formation might have hindered the growth of shoots and lead to the formation of somaclonal variants. The similar trend was reported by Rai, *et al.* (2012), Ghive, *et al.* (2006^e) and Devendra *et al.* (2008) in Spine gourd.

4.2.1.2.1.3 Length of shoots

The results of average length of shoots per explant indicated significant differences among the various media supplementations with BAP and NAA used for the shoot tip explants of spine gourd (Table 32 and Fig. 8). The minimum length (0.25 cm) was recorded in the shoot tip explants placed on MS media supplemented with 3.0 mgl^{-1} BAP plus 0.5 mgl^{-1} of NAA. The maximum length of shoot (5.72 cm) was registered in the shoot tip explant on MS media supplemented with 2.5 mgl^{-1} of BAP + 0.5 mgl^{-1} NAA.

The regression analysis of a data presented in Table 32 revealed that the coefficient of determination (R^2) was 0.8037 for average length of shoot. The model reported the adequate precision (6.6009) which is considerably higher than the minimum desirable (4) for high prediction ability. The Model F-value (5.73) and P-value (0.0203) indicated that the model was significant.

It is evident from the table 32 that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of shoot tip explants. The BAP levels at linear and quadratic terms had significant effect on the length of shoot in negative manner, whereas interaction of BAP and NAA and linear terms of NAA were non-significant with linear terms of NAA had negative effect.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 1450.90643 + 15.43724* BAP - 5832.88585 * NAA + 1.90795 * BAP * NAA - 4.39673 * BAP^2 + 5825.88585 * NAA^2

Research conducted for *In-vitro* regeneration of *Momordica dioica* using shoot tip as an explant to know the effect of auxin and cytokinin's combination on the effect of average shoots length revealed that there is a significant difference among the various treatments. In the present study, maximum length of shoots per explants (5.2 cm) was obtained from the combination

of 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, which is followed by use of media supplement of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The minimum length of shoots (0.25 cm) was recorded in the MS media supplemented with the higher concentration of BAP and NAA i.e., 3.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. Finding of present study are in the agreement with Hoque, *et al.* (1995), Hoque, *et al.* (2000), Nabi, *et al.* (2002[a]), Mohammad and Shorif (2010), Rai, *et al.* (2012) and Patel, *et al.* (2015) which suggested that the use of BAP and NAA in combination gave good regeneration in spine gourd. The frequency of callus formation increased with respect to increasing the concentration of BAP and it was well reported that high rate of callus formation hindered the growth and lead to the formation of somaclonal variants therefore suitable concentration of BAP showed the good results. Similar results were reported by Rai, *et al.* (2012) and Ghive, *et al.* (2006[b]).

4.2.1.2.2 Response of spine gourd shoot tip explants to various levels of BAP and CH using D-Optimal RSM

Experimental design was generated using response surface methodology of design expert software for the two factors i.e. BAP and Casein hydrolysate to study the relative effect on the responses *viz.,* days to shoot initiation, number of shoots and length of shoot per explant. The data generated are presented in Table 33 and graphically depicted in Fig. 9 and discussed here under the suitable headings.

4.2.1.2.2.1 Number of days required for shoot initiation

The data regarding number of days required for shoot initiation as affected by various combination and concentrations of BAP and CH supplementations on MS culture media are presented in Table 33 and graphically represented in Fig. 9. It is evident from the Table 33 that the number of days required for shoot initiation were raged from 4.0 to 7.5 days. The minimum days (4.0) for shoot initiation were recorded for shoot tip explant placed on MS media supplemented with 1.0 mgl^{-1} BAP + 200 mgl^{-1} CH. Whereas, combinations of 2.5 mgl^{-1} BAP + 200 mgl^{-1} CH supplementation registered maximum number of days (7.5) for shoot initiation.

The recorded data in respect of days required for shoot initiation were subjected to the regression analysis. It is revealed from the data that the coefficient of determination (R^2) was 0.9238 for the model. Further the model reported the adequate precision of 14.2803, which is substantially higher than the minimum desirable (4) for high prediction ability of design. The Model F-value (16.98) implied that the model was significant with P-value of 0.09%.

As regards the coefficient for number of days required for shoot initiation model, it is evident that the levels of different combinations of media supplementations had significant ($p<0.05$) effect on responses by shoot tip explants. The BAP and CH level at linear terms had positive significant and non-significant effect respectively on the number of days required for shoot initiation. The interactions and quadratic levels BAP and CH had negative effect on number

of days required for shoot initiation. Out of these, quadratic level of BAP was significant and other two had non-significant effect. Here negative values indicating that earliest initiation as aspired.

Table 33: Effect of different levels of BAP and CH supplementations on the morphogenic responses of shoot tip explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: CH (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	2.0	200	6.0	1.00	1.55
2	2.0	200	6.4	1.10	2.00
3	2.0	200	6.6	1.20	1.80
4	2.0	200	6.8	1.30	1.75
5	1.0	200	4.0	0.80	1.25
6	1.5	200	5.8	1.00	1.30
7	2.5	200	7.5	1.30	1.35
8	2.0	200	6.4	1.16	1.65
9	3.0	200	6.2	1.50	1.20
10	2.0	200	6.5	1.15	1.50
11	3.0	200	6.1	1.60	1.40
12	1.0	200	4.2	0.90	1.27
13	2.0	200	6.6	1.25	1.70

Coefficient of quadratic polynomial model of BAP and CH for responses of initiation stage

Factor	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot
Intercept	6.57	1.15	1.59
BAP(A)	1.10*	0.3444*	0.0233
CH (B)	0.1082	0.0354	-0.0667
AB	-0.0250	-0.0500	-0.0550
A^2	-1.38*	0.0239	-0.4793*
B^2	-0.0111	0.0254	0.1518*
F- Value	15.42	16.98	7.42
P-Value	0.0012	0.0009	0.0102
Mean	6.08	1.17	1.52
SD	0.3684	0.0813	0.1297
Adequate precision	11.3032	14.2803	9.3506
R^2	0.9168	0.9238	0.8413

*P-Value = <0.05

The response surface equation derived for predicting average number of days for shoot initiation on using BAP and CH in combination could be given as:

Initiation (Days) = - 1.78836E+05 + 106.59371 * BAP + 1785.62842 * CH - 0.50 * BAP * CH - 1.37968 * BAP^2 - 4.45727 * CH^2

The significant differences for days required for shoot initiation were noted on the use of different levels of BAP+CH on shoot tip explants of spine gourd. The best results were reported on the MS media fortified with the combination of 1.0 mgl^{-1} BAP + 200 mgl^{-1} CH *i.e.,* 4 days of earliest shoot initiation, which is followed by 5.8 days at the combination of 1.5 mgl^{-1} +

200 mgl^{-1} CH. However, maximum number of days required for shoot initiation (7.5) were recorded on media supplemented with combination of 2.5 mgl^{-1} BAP + 200 mgl^{-1} CH. In the present study, CH along with BA induced vigorous and healthy shoots but promoted axillary buds to develop whitish fragile callus at the higher concentrations of BAP which in turn inhibit the further development of shoots. This might be attributed to the role of CH in inducing vigorous shoots, promoting callus formation. Also, the inhibitory effect for shoot differentiation might have played the role as reported by Ahmad and Anis (2005) in *Cucumis sativus* L. and Rai *et al.*, (2012) in spine gourd.

4.2.1.2.2.2 Number of shoots per explant

The observations in respect of number of shoots per shoot tip explant of spine gourd as effected by the different levels of BAP+CH supplementations on MS media are presented in Table 33 and graphically represented in Fig. 9. It is evident from the data that the number of shoots per nodal explants were ranged from 0.8 to 1.6 shoots. The minimum number of shoots per explant (0.8) were recorded in a media supplemented with 1.0 mgl^{-1} BAP + 200 mgl^{-1} CH. The maximum number of shoots per explant (1.6 shoots) were registered for the MS media supplemented with 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH.

Regression analysis of a data presented in Table 33 revealed that the coefficient of determination (R^2) is 0.9238 for number of shoots per explant. The model reported the adequate precision of 14.2803 which is substantially higher than the minimum desirable (4) for high prediction ability of design. The F-value of model (16.98) implied that the model was significant for number of shoots per explants with P-value of 0.09%.

As regards the coefficient for number of shoots per explant, model showed that the levels of different combinations had significant ($p<0.05$) effect on response. Out of all the terms such as linear, interaction and quadratic levels only linear terms of BAP had positive significant effect on the number of shoots per explant, whereas other terms had non-significant effect. Among which the interactions had shown negative effect.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explants (Days) = + 4.05723E+05 + 200.19874 * BAP - 4060.94184 * CH - 1.000000 * BAP * CH + 0.023926 * BAP^2 + 10.16166 * CH^2

The significant differences among the treatments for maximum number of shoots per explants which were placed on the MS culture media supplemented with various combination and concentration of the BAP+CH were observed. The results revealed that the highest number of shoot per explant (1.6) were observed on the MS media supplemented at 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH, whereas lowest number of shoots per explants (0.8) were obtained on the MS media supplemented with 1.0 mgl^{-1} BAP + 200 mgl^{-1} CH. This might be attributed to CH which might

have induced vigorous shoots, promoted callus formation and proved inhibitory for shoot differentiation and shoot length as reported by Rai, *et al.* (2012) in spine gourd and Ahmad and Anis (2005) in *Cucumis sativus* L.

4.2.1.2.2.3 Length of shoots

The observations regarding average length of shoot per explant recorded the significant differences as affected by the various levels of media supplementation with BAP+CH (Table 33 and Fig. 9). It is seen from the Table 33 that the average length of shoot per explant were ranged from 1.2 to 2.0 cm. The minimum length of the shoot (1.2 cm) was recorded in the shoot tip explant placed on a MS media supplemented with 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH. The maximum length of shoot per explant (2.0 cm) was registered in respect of MS supplemented with combination of 2.0 mgl^{-1} BAP + 200 mgl^{-1} CH.

The regression analysis of average length of shoots for shoot tip explants of spine gourd revealed that the coefficient of determination (R^2) was 0.8413. The adequate precision was found to be 9.3506 for average length of shoots, which is noticeably higher than the minimum desirable (4) for high prediction ability. The studied model for the average length of shoots is found to be significant with F-value of 7.42 and P-value of 0.0102.

It is revealed from the coefficient for length of shoot that the model is significant at the different levels of media supplementation ($p<0.05$) and had significant effect on responses. The linear and interaction terms of BAP and CH had non-significant effect on the length of shoot, out of which linear terms of CH and interactions were affecting negatively. The quadratic levels of BAP and CH had significant effect on length of shoots. The positive effect was recorded by while BAP registered negative effect.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 2.42799E+06 + 221.88556 * BAP - 24286.82485 * CH - 1.10 * BAP * CH - 0.479307 * BAP^2 + 60.73441 * CH^2

The highest average length of shoots (2.0 cm) were recorded in shoot tip explants placed on MS media fortified with 2.0 mgl^{-1} BAP + 2 00 mgl^{-1} CH. However lowest average length of shoots (1.2 cm) were recorded in case of nodal explants placed on MS culture media supplemented with 3.0 mgl^{-1} BAP + 200 mgl^{-1} CH. The similar trend was reported by Rai, *et al.* (2102) in spine gourd, Prakash, *et al.* (2003) and Agrawal, *et al.* (1999) in *Jojoba*.

4.2.1.2.3 Response of spine gourd shoot tip explant to various levels of BAP and 0.5 mgl^{-1} Kn using D-Optimal RSM

The investigation was carried out with various levels of BAP and 0.5 mgl^{-1} Kn as generated by D-optimal response surface methodology. The nodal explants were placed on MS media to study their response to the various levels of BAP and 0.5 mgl^{-1} Kn. The results pertaining

to various responses such as shoot initiation, average number and length of shoots are presented in Table 34 and graphically represented in Fig.10 and discussed hereunder suitable headings.

Table 34: Effect of different levels of BAP and 0.5 mgl^{-1} Kn on the morphogenic responses shoot tip explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: Kn (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	2.0	0.5	3.2	2.00	5.19
2	2.0	0.5	3.1	2.10	5.20
3	2.0	0.5	3.3	2.20	5.30
4	1.5	0.5	5.0	1.60	4.89
5	2.0	0.5	3.0	2.00	5.25
6	2.0	0.5	3.2	2.30	5.40
7	2.5	0.5	3.5	1.75	1.20
8	2.0	0.5	3.3	2.10	5.20
9	1.5	0.5	5.1	1.70	4.90
10	2.0	0.5	3.1	2.00	5.21
11	1.5	0.5	4.9	1.50	4.70
12	2.5	0.5	3.6	1.70	1.50
13	2.5	0.5	3.7	1.80	1.60

Coefficient of quadratic polynomial model of BAP and 0.5 mgl^{-1} Kn for responses of initiation stage

Factor	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot
Intercept	3.19	2.09	5.21
BAP(A)	-0.7000*	0.0750	-1.70*
Kn (B)	0.0354	0.0530	0.0153
AB	0.0500	0.0500	0.0725
A^2	1.13*	-0.4264*	-2.12*
B^2	-0.0405	0.0142	0.0657
F- Value	119.96	12.83	474.89
P-Value	<0.0001	0.0021	<0.0001
Mean	3.69	1.90	4.27
SD	0.1087	0.1008	0.1158
Adequate precision	26.5900	8.8334	50.8067
R^2	0.9885	0.9016	0.9971

*P-Value = <0.05

4.2.1.2.3.1 Number of days required for shoot initiation

The observations pertaining to number of days required for shoot initiation as affected by using various combinations of BAP and 0.5 mgl^{-1} of Kn on MS media are presented in the Table 34 and graphically represented in the Fig. 10. It is clear from the data that number of days required for shoot initiation from the shoot tip explant of spine gourd were ranged from 3.0 to 5.1 days after inoculation. The shoot tip explants inoculated on MS media supplemented with 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn reported the average minimum number of days i.e. 3.0 for shoot initiation. The average maximum number of days i.e. 5.1 days for shoot initiation were registered

by the shoot tip explants placed on a MS media fortified with the combination of 1.5 mgl^{-1} BAP and 0.5 mgl^{-1} Kn.

The data obtained are subjected to regression analysis using D-Optimal RSM. The data revealed that the coefficient of determination (R^2) was 0.9885 for days to shoot initiation. Model under study reported the adequate precision of 26.5900, which is considerably higher than the minimum desirable (4) for high prediction ability of model to navigate the design space. It is evident from the Model F-value (119.96) that the model is significant with the P-values of <0.01%.

As regards the coefficient for number of days required for shoot initiation, model showed that the levels of different combinations had significant ($p<0.05$) effect on response. The BAP level at linear terms had significant effect on the number of days required for shoot initiation in negative terms where negative sign indicated the earliest initiation. The levels of Kn had non-significant effect on days required for shoot initiation. The interaction of BAP and Kn had non-significant effect on number of days required for shoot initiation. The quadratic levels of BAP had positive significant effect whereas Kn had negative effect on days required for shoot initiation.

The response surface equation derived for predicting average number of days for shoot initiation could be given as:

Initiation (Days) = - 69.00560 - 24.51892 * BAP + 388.94094 * Kn + 10.00000 * BAP * Kn + 4.52973 * BAP^2 - 405.40541 * Kn^2

The results of plant regeneration studies conducted in *Momordica dioica* using shoot tip as an explant with the intension to know the effect of various combinations of cytokinins alone on the number of days required for shoot initiation had showed that there is a significant difference among the treatments. The findings of the present study confirm that the minimum number of days (3.0) required for shoot initiation were observed in the explants placed on the MS media supplemented with combination 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn which is followed by 3.5 days at 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. However, maximum number of days required for shoot initiation (5.1) were recorded in the explants inoculated on MS media supplemented with plant growth regulators combination of 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. The similar results were reported by Jamatia (2016). The combination of BAP and Kn showed very little shoot regeneration response as compare to the BAP alone and in combination with the NAA as reported by Hoque, *et al.* (1995) and Paula, *et al.* (1990) in spine gourd and *Cucurbita pepo* respectively.

4.2.1.2.3.2 Number of shoots per explant

The observations regarding number of shoots produced per shoot tip explant of *Momordica dioica* as effected by the various levels of BAP+0.5 mgl^{-1} Kn supplementations on MS media are presented in Table 34 and graphically depicted in Fig.10. It is evident from the Table 34 that average number of shoots produced per explant were ranged from 1.5 to 2.3 shoots. The minimum number of shoots per explant (1.5) were registered in the shoot tip explants placed on

MS media supplemented with 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn, while maximum number of shoots per explant (2.3) were recorded in a MS media supplemented with PGRs combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn.

Data presented in Table 34 revealed that the coefficient of determination (R^2) was 0.9016 for number of shoots per explant of spine gourd. The adequate precision was found to be 8.8334, which is significantly higher than the minimum desirable (4) for high prediction ability to navigate the design. The Model F-value (12.83) implied that the model was significant (P-value 0.021 %).

In respect of coefficient for number of shoots per explant, present study model showed that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of spine gourd shoot tip explants. The BAP and Kn levels at linear terms and their interaction had non-significant effect on the number of shoots per explant. The quadratic levels of BAP had significant effect on number of shoots per explant in negative terms, however quadratic levels of Kn were non-significant on number of shoots per explant.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = + 37.79159 + 1.97162 * BAP - 156.58859 * Kn + 10.0 * BAP * Kn - 1.70541* BAP^2 + 141.8918911 * Kn^2

The various combinations of PGR's were examined and a varied range of results were noted in number of shoots per explants of *Momordica dioica* using shoot tip as explants. The present study revealed that highest number of shoots per explants (2.3) were noticed at the combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn, whereas lowest number of shoots per explants (1.5) were recorded at the PGRs combination of 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. These results are in conformity with those reported by Jamatia (2016), Nabi, *et al.* (2002^b) in spine gourd and Sultana and Bari Miah (2003) in *M. charantia.*

4.2.1.2.3.3 Length of shoots

The observations in respect to average length of shoots per explant as affected by various levels of media supplementations with BAP and 0.5 mgl^{-1} Kn recorded significant differences (Table 34 and Fig. 10). The minimum length (1.2 cm) of shoot was recorded in the shoot tip explants placed on MS media supplemented with 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} of Kn. The maximum length of shoot (5.4 cm) was registered in the shoot tip explant on MS media supplemented with 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} NAA.

Data of regression analysis were presented in Table 34 revealed that the coefficient of determination (R^2) was 0.9971 for average length of shoot per explant. The Model F-value (474.89) and P-value (<0.01%) suggested that the model was significant with the adequate

precision of 50.8067, which is excellently higher than the minimum desirable (4) for high prediction ability for average length of shoot.

It is seen from the table 34 that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of shoot tip explants. The BAP level at linear terms had significant effect in negative terms, however Kn had non-significant effect on length of shoot. The interactions and quadratic levels of Kn had non-significant effect on length of shoots. The quadratic levels of BAP had significant effect on length of shoots but in negative terms.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 156.10704 + 23.34685 * BAP - 684.90578 * Kn + 14.50 * BAP * Kn - 8.49838 * BAP^2 + 657.43243 * Kn^2

In the present investigation, maximum length of shoots per explants (5.4 cm) was recorded from the combination of 2.0 mgl^{-1} BAP + 0.5 mgl^{-1} Kn, which is followed by 4.9 cm of average shoot length at 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. The minimum length of shoots (1.2 cm) was recorded in the MS media fortified with the combination of 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. The higher levels of BAP and Kn might have induced the callus formation which in turn reduced the shoot length of plants as reported by Jamatia (2016) in spine gourd.

4.2.1.2.4 Response of spine gourd Shoot tip explants to various levels of BAP and 1.0 mgl^{-1} Kn using D-Optimal RSM.

Response surface methodology of design expert software were used to generate experimental design for the two factors i.e. BAP and 1.0 mgl^{-1} Kn to study the relative effect on the responses *viz.*, days to shoot initiation, number of shoots and length of shoot per explant. The data presented in Table 35 and graphically presented in Fig. 11 are discussed here under the suitable headings.

4.2.1.2.4.1 Number of days required for shoot initiation

The data in respect of number of days required for shoot initiation as affected by various levels of BAP and 1.0 mgl^{-1} Kn supplementations on MS basal media are summarized in Table 35 and graphically represented in Fig.11. It is evident from the Table 35 that the number of days required for shoot initiation were raged from 2.9 to 3.7 days. The minimum 2.9 days were required for shoot initiation from shoot tip explants placed on MS media supplemented with 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. Whereas, combination of 1.5 mgl^{-1} BAP + 0.1 mgl^{-1} Kn supplementation registered maximum number of days (3.7) for shoot initiation from shoot tip explants of spine gourd.

Data collected in respect the days required for shoot initiation were subjected to the regression analysis. It is revealed from the data that the coefficient of determination (R^2) was 0.8276. Further, model reported the adequate precision of 7.9847, which is substantially higher

than the minimum desirable (4) for high prediction ability of design. The model F-value (6.72) implied that the model was significant with P-value of 0.0133.

Table 35: Effect of different levels of BAP and 1.0 mgl^{-1} Kn supplementations on the morphogenic responses of shoo tip explant of spine gourd using D-Optimal Response Surface Methodology.

RUN	Factor 1 A: BAP (mgl^{-1})	Factor 2 B: Kn (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	2.5	1.0	3.1	1.90	6.17
2	2.0	1.0	3.0	1.58	4.10
3	2.0	1.0	3.1	1.60	3.50
4	1.5	1.0	3.3	1.70	2.20
5	2.5	1.0	3.0	2.00	6.50
6	2.0	1.0	2.9	1.65	3.10
7	2.5	1.0	3.2	2.10	6.25
8	2.0	1.0	3.2	1.50	3.60
9	2.0	1.0	3.2	1.55	4.20
10	2.0	1.0	3.0	1.62	3.30
11	2.0	1.0	3.1	1.68	3.80
12	1.5	1.0	3.7	1.80	1.50
13	1.5	1.0	3.5	1.60	2.10

Coefficient of quadratic polynomial model of BAP and 1.0 mgl^{-1} Kn for responses of initiation stage

Factor	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot
Intercept	3.07	1.58	3.67
BAP(A)	-0.2000*	0.1500*	2.19*
Kn (B)	0.0375	0.0109	-0.0993
AB	-0.1250*	0.0000	0.2575
A^2	0.2284*	0.2505*	0.4647
B^2	0.0020	0.0243	-0.0197
F- Value	6.72	8.51	42.63
P-Value	0.0133	0.0069	<0.0001
Mean	3.18	1.71	3.87
SD	0.1198	0.0903	0.3735
Adequate precision	7.9847	7.1057	19.26
R^2	0.8276	0.8587	0.9682

*P-Value = <0.05

As regards the coefficient for number of days required for shoot initiation model, it is evident that the levels of different combinations of media supplementations had significant ($p<0.05$) effect on responses by shoot tip explants of spine gourd. The linear and quadratic terms of BAP and interaction between BAP and Kn had significant effect on the number of days for shoot initiation out of that linear and quadratic terms had affected in negative terms which was desirable indicating the earliest shoot initiation. Whereas Kn had non-significant effect on days required for initiation at both linear and quadratic levels.

The response surface equation derived for predicting average number of days for shoot initiation on using BAP and 1.0 mgl^{-1} Kn in combination could be given as:

Initiation (Days) = - 1.95642 + 0.695946 * BAP + 8.37224 * Kn - 4.54545 * BAP * Kn + 0.913514 * BAP^2 + 0.670092 * Kn^2

It is revealed from the study that there is a significant difference among the treatment combination of various plant growth regulators supplementations on MS media in respect of days required for shoot initiation. The minimum number of days required for shoot initiation (2.9) were recorded in treatment combination of 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn for the shoot tip explants of the spine gourd. However maximum number of days required for shoot initiation (3.7) were noticed in the combination of 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. This research confirm that lower concentration cytokinins combination extended the number of days required for shoot initiation. The similar results were also reported by the Paula, *et al.* (1992) in somatic embryogenesis of *Cucurbita pepo,* Kawale and Chaudhary *et al.* (2009) in *Trichosanthes cucumerina* and Kiełkowska and Havey (2011) in *Cucumis sativus.*

4.2.1.2.4.2 Number of shoots per explant

The results of experimental observations revealed that the two different kinds of cytokinins combination had significant effect on number of shoots per explant *Momordica dioica*. The data in respect of number of shoots per explant as influenced by various levels of BAP and Kinetin are presented in Table 35 and graphically represented in Fig. 11. Results of average number of shoots per explants were ranged from 1.5 to 2.1 shoots. The minimum number of shoots per explant (1.5) was reported in treatment combinations of 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} of Kn for shoot tip explants of Spine gourd. However, the maximum number of shoots per explant (2.1) were registered in the MS media fortified with combination of 2.5 mgl^{-1} BAP +1.0 mgl^{-1} Kn.

Regression analysis of a data presented in Table 35 revealed that the coefficient of determination (R^2) is 0.8587 for number of shoots per explant. The model reported the adequate precision of 7.1057, which is considerably higher than the minimum desirable (4) for high prediction ability of design. The F-value of model is 8.51 which implied that the model was significant for number of shoots per explants with P-value of 0.69%.

As regards the coefficient for number of shoots per explant, model showed that the levels of different combinations of cytokinins had significant ($p<0.05$) effect on response of number of shoots per explant of spine gourd. The BAP levels at linear terms and quadratic terms had positive significant effect on the number of shoots per explant. The interaction effect of BAP and Kn levels was non-significant. The linear and quadratic terms of Kn had non-significant effect. The interaction of BAP and Kn and linear and quadratic terms of Kn had non-significant effect.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explant (Days) = + 13.56667 - 3.70865 * BAP - 16.60848 * Kn + 1.73180E - 13 * BAP * Kn + 1.00216 * BAP^2 + 8.04110 * Kn^2

The results of present experiment confirmed that there are significant differences in the number of shoots per explant as influenced by the various levels of BAP + 1.0 mgl^{-1} Kn. The highest number of shoots per explant (2.1) were reported in shoot tip explants of spine gourd placed on the MS media supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. Whereas, minimum number of shoots per explants of spine gourd (1.5) were recorded in the shoot tip explants placed on MS media supplemented with 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. These results are in conformity with those of Paula, *et al.* (1992) in somatic embryogenesis of *Cucurbita pepo* using shoot tip as explant, Debnath (2013[b]) in *Momordica dioica,* Debnath (2013[a]) in *Momordica cochinchinensis*, Krug, *et al.* (2005) in *Citrullus lanatus* and Komal (2011[c]) in *Trichosanthes dioica*.

4.2.1.2.4.3 Length of shoots

The results of average length of shoot per explant recorded the significant differences as affected by the various levels of media supplementation with BAP+1.0 mgl^{-1} Kn (Table 35 and Fig. 11). It is evident from the Table 35 that the average length of shoot per explant were ranged from 1.5 to 6.5 cm. The minimum length of the shoot (1.5 cm) was recorded in the shoot tip explant placed on a MS media supplemented with 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. The maximum length of shoot per explant (6.25 cm) was registered in respect of MS supplemented with combination of 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn.

The regression analysis of average length of shoots for nodal explants of spine gourd revealed that the coefficient of determination (R^2) was 0.9682. The adequate precision was found to be 9.65, for average length of shoots, which is considerably higher than the minimum desirable (4) for high prediction ability. The studied model for the average length of shoots is found to be significant with F-value of 42.63 and P-value of <0.01%.

It is revealed from coefficient for average length of shoot that the model is significant at the different levels of media supplementation ($p<0.05$) and had significant effect on responses. Out of all various terms only BAP level at linear terms had positive significant effect on the length of shoot. Whereas others like linear terms, interactions and quadratic levels of BAP and Kn had non-significant effect, whereas linear and quadratic terms of Kn had negative effect on length of shoot.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 16.71572 - 12.84734 * BAP - 6.94765 + 132.06350 * Kn + 9.36364 * BAP * Kn + 1.85892 * BAP^2 - 6.49989 * Kn^2

The length of the shoots per shoot tip explants of *Momordica dioica* was significantly influenced by the various combinations and levels of the cytokinins. Results of

investigation reported that the minimum average shoot length of 1.5 cm was achieved on the MS media fortified with the combination of 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn, whereas maximum average length of shoot (6.5 cm) was reported in case of shoot tip explant placed on MS media supplemented with 2.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. The similar trends were reported by Jamatia (2016) in *Momordica dioica* and Kawale and Choudhary (2009) in *Trichosanthes cucumerina* L.

4.2.1.2.5 Response of spine gourd shoot tip explant to various levels of BAP and IAA using D-Optimal RSM

Several combinations of BAP and IAA as generated by D-optimal response surface methodology were tried in MS media to study the response of shoot tip explant of spine gourd. The results pertaining to various responses such as shoot initiation, average number and length of shoots are presented in Table 36 and discussed hereunder suitable headings.

4.2.1.2.5.1 Number of days required for shoot initiation

The data pertaining to the number of days required for shoot initiation from shoot tip explants of spine gourd placed on MS media fortified with various level of BAP and IAA ranged from 6.0 to 9.1 days (Table 36 and Fig. 12). The minimum number of days required for shoot initiation (6.0) were recorded in shoot tip explant placed on MS media supplemented with the combinations of 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. The maximum number of days i.e., 9.1 for shoot initiation were recorded for the combinations of 0.25 mgl^{-1} of BAP + 0.1 mgl^{-1} IAA.

The regression analysis of a data (Table 36), revealed that the coefficient of determination (R^2) was 0.8125. The adequate precision was found to be 6.7140, for number of days required for shoot initiation model, which is significantly higher than the minimum desirable (4) for high prediction ability to further navigate the design. The results revealed that the model was significant with the F-value of 6.07 and P-value of 0.0375.

As regards the coefficient for number of days required for shoot initiation, model showed that the levels of different combinations had significant ($p<0.05$) effect on responses. The linear terms of only BAP level had significant effect on number of days required for shoot initiation whereas all other terms like interactions and quadratic levels of both BAP and IAA had non-significant effect in negative manner except quadratic levels of IAA.

The response surface equation derived for predicting average number of days for shoot initiation on using BAP and IAA in combination could be given as:

Initiation (Days) = + 8.70479 + 1.04305 * BAP - 22.86224 * IAA - 15.03619 * BAP * IAA - 2.44343 * BAP^2 + 288.81492 * IAA^2

The maximum number of days required for shoot initiation i.e., 9.1 days were registered in treatment with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. However, the minimum number of days required for shoot initiation (6.0) were reported on MS media supplemented with PGRs combination of 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. The early initiation of shoot might be attributed

to the BAP+IAA combination which might have effect on bud breaking as reported by Shekhawat, *et al.* (2011) in spine gourd. However, the contradictory results are reported by Moon, *et al.* (2000) in oriental melon.

Table 36: Effect of different levels of BAP and IAA on the morphogenic responses of shoot tip explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A BAP (mgl^{-1})	Factor 2 B : IAA (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of shoots/ explant	Response 3 Av. length of shoot (cm)
1	0.25	0.1	9.10	1.10	7.35
2	0.25	0.1	8.90	1.00	7.32
3	0.50	0.1	7.10	1.25	5.29
4	0.50	0.1	7.50	1.30	5.30
5	0.50	0.1	7.30	1.29	5.32
6	0.75	0.1	8.12	1.63	3.43
7	0.75	0.1	8.20	1.65	3.45
8	0.75	0.1	8.15	1.70	3.46
9	1.00	0.1	6.00	2.15	5.65
10	1.00	0.1	6.10	2.00	5.69
11	0.25	0.1	8.80	0.90	7.31
12	1.00	0.1	6.25	2.10	5.70
13	1.00	0.1	6.20	2.20	5.75

Coefficient of quadratic polynomial model of BAP and IAA for responses of initiation stage

Factor	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot (cm)
Intercept	7.51	1.45	4.26
BAP(A)	-1.04*	0.5410*	-1.12*
IAA (B)	-0.1689	0.0679	0.2598
AB	-0.2819	0.0306	0.2780
A^2	-0.3436	0.0954	2.49*
B^2	0.7220	-0.0529	-0.6054
F- Value	6.07	78.40	19.10
P-Value	0.0175	<0.0001	0.0006
Mean	7.52	1.56	5.46
SD	0.6374	0.0787	0.4729
Adequate precision	6.7140	21.3751	13.7697
R^2	0.8125	0.9825	0.9317

*P-Value = <0.05

4.2.1.2.5.2 Number of shoots per explant

The observations with respect to number of shoots per shoot tip explant of spine gourd as effected by the various levels of BAP+IAA supplementations on MS media are presented in Table 36 and graphically represented in Fig. 12. It is clear from the data that the number of shoots per shoot tip explants were ranged from 0.9 to 2.15 shoots. The minimum number of shoot per explant (0.9) was recorded in a media supplemented with 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA.

However, the maximum number of shoots per explant (2.15 shoots) were registered for the MS media supplemented with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA.

The regression analysis of a data presented in Table 36 revealed that the coefficient of determination (R^2) is 0.9825 for number of shoots per explant. The model reported the adequate precision of 21.3751 which is appreciably higher than the minimum desirable (4) for high prediction ability of design. The F-value of model is 78.40 which implied that the model was significant for number of shoots per explants with P-value of <0.01 %.

As regards the coefficient for number of shoots per explant, model showed that the levels of different combinations had significant ($p<0.05$) effect on response. Only BAP level at linear terms had positive significant effect on the number of shoots per explant**.** Whereas Linear terms of IAA, interactions of BAP and IAA, and quadratic levels of BAP and IAA had non-significant effect on number of shoots per explant, whereas quadratic levels of IAA affected in negative manner.

The response surface equation derived for predicting number of shoots per explant could be given as:

Number of Shoots per explants (Days) = + 0.747927 + 0.512847 * BAP + 2.45647 * IAA + 1.63217 * BAP * IAA + 0.678603 * BAP^2 - 21.17648 * IAA^2

A varied range of PGR's combinations were examined on the number of shoots per explants on shoot tip explants of *Momordica dioica* and results obtained revealed the significant differences among the treatments. Present findings showed that the maximum number of shoots per explants (2.15) were observed on the MS media supplemented with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA which is followed by 1.65 shoots at 0.75 mgl^{-1} + 0.1 mgl^{-1} IAA. , whereas minimum number of shoots per explant (0.9) were reported in the treatment with 0.25 mgl^{-1} + 0.1 mgl^{-1} IAA. In this study, we observed that incorporation of an auxin (IAA) at a lower concentration (0.1 mgl^{-1}) significantly increased the number of shoots by promoting axillary branching as reported by Chaudhary, *et al.* (2017) in spine gourd. This indicated that an appropriate auxin(s)-cytokinin(s) combination was mandatory for a better shoot proliferation/growth as reported by Phulwaria, *et al.* (2014). Auxins–cytokinins crosstalk mechanism may be also one the reason which depicted that cytokinins promote stem cell proliferation in shoot meristem and inhibit its differentiation, whereas auxin triggers organ primordium formation through suppressing cytokinine biosynthesis, and these in turn regulates shoot meristem development and shoot branching. The similar results were reported by Shimizu-Sato, *et al.* (2009) and Su, *et al.* (2011).

4.2.1.2.5.3 Length of shoots

The observations with respect to average length of shoot per explant recorded the significant differences as affected by the various levels of media supplementation with BAP+IAA (Table 36 and Fig. 12). It is evident from the Table 36 that the average length of shoot per explant

were ranged from 3.43 to 7.35 cm. The minimum length of the shoot (3.43 cm) was recorded in the shoot tip explant placed on a MS media supplemented with 0.75 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. The maximum length of shoot per explant (7.35 cm) was registered in respect of MS supplemented with combination of 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA.

The regression analysis for average length of shoots per nodal explants of spine gourd revealed that the coefficient of determination (R^2) was 0.9317. The adequate precision was found to be 13.7697 for average length of shoots, which is noticeably higher than the minimum desirable (4) for high prediction ability. The studied model for the average length of shoots model is significant with F-value of 19.10 and P-value of 0.06%.

It is revealed from the coefficient for length of shoot that the model is significant at the different levels of media supplementation ($p<0.05$) and had significant effect on responses. The BAP level at linear terms had significant effect on the length of shoot in negative terms. Whereas IAA had non-significant effect. The interaction of BAP and IAA had non-significant effect on length of shoots. The quadratic levels of BAP had positive significant effect on length of shoots, whereas IAA had non-significant effect on length of shoots in negative terms.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 12.65371 - 25.90322 * BAP + 20.14441* IAA + 14.82709 * BAP * IAA + 17.73917 * BAP^2 - 242.16274 * IAA^2

The results of present investigation reported that highest average length of shoots (7.35 cm) were recorded in shoot tip explants on MS media fortified with 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA which is followed by 5.75 cm in treatment combination of 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. However, the lowest average length of shoots (3.43 cm) were recorded in case of shoot tip explants placed on MS culture media supplemented with 0.75 mgl^{-1} + 0.1 mgl^{-1} IAA. In the present study, it is observed that higher concentrations of BAP produced callus at the base of explants and intern reduced shoot length. The similar trends were also reported by Chaudhary, *et al.* (2017) in *Momordica dioica* and Satapathy, *et al*. (2014) and Lie, *et al.* (2011) in case of *Cucumis trigonus* and *Cucumis melo* respectively.

4.2.1.3 Optimization for initiation stage

The optimization of various independent variables was carried out using D optimal response surface methodology. The most desirable results obtained through experiment were subjected to multiple levels of analysis by giving numerical optimization command for expected goals during initiation stage of micropropagation in spine gourd for node and shoot tip explants to formulate best combination of various independent factors (media supplement levels) to get optimum results from dependent response variables such as number of days required for shoot initiation, number of shoots/ explant and length of shoot. The aim of the optimization was to

decrease the number of days required for shoot initiation with maximizing the number of shoots per explant having maximum average shoot length of spine gourd plant during initiation stage of micropropagation. After setting the goals by giving the most desirable commands to the software, the diagnostic and influence plots available in the software, were used for the optimization of the design. The RSM optimization approach tests an optimum response by changing levels of several variables at one time using special experimental designs. The optimization of the variable levels was achieved by desirable maximization of the initiation stage responses along the fitted polynomial model by numerical optimization procedure of design expert software. The best solution exerted through the software and their predicted score is presented below in Table 37 and 38. The validation of the prediction values was done by actual observations recorded for number of days required for shoot initiation, number of shoots per explants and average length of shoots during initiation stage of micropropagation in spine gourd (Table 37 and 38).

Table 37: Optimization of media supplements on MS media at initiation stage of nodal explant

Sr. no	Suggested combination		Av. days for initiation		Av. number of shoots/ explant		Av. length of shoot	
	Factor 1	Factor 2	Predicted values (days)	Actual values (days)	Predicted values	Actual values	Predicted values (cm)	Actual values (cm)
1.	1.05 mgl^{-1} BAP		9.28	9.0	1.43	1.5	3.13	3.3
2.	1.85 mgl^{-1} BAP	0.49 mgl^{-1} NAA	6.51	6.1	1.08	1.0	3.85	4.1
3.	2.20 mgl^{-1} BAP	199.9 mgl^{-1} CH	5.69	5.0	1.37	1.5	4.10	4.4
4.	1.80 mgl^{-1} BAP	0.50 mgl^{-1} Kn	3.89	4.1	1.43	1.7	6.41	6.5
5.	2.00 mgl^{-1} BAP	1.00 mgl^{-1} Kn	3.29	3.3	0.98	1.0	5.83	5.8
6.	0.46 mgl^{-1} BAP	0.10 mgl^{-1} IAA	6.08	6.0	1.19	1.2	7.41	7.5

Table 38: Optimization of media supplements on MS media at initiation stage of shoot tip explant

Sr. no	Suggested combination		Av. days for initiation		Av. number of shoots/ explant		Av. length of shoot	
	Factor 1	Factor 2	Predicted values (days)	Actual values (days)	Predicted values	Actual values	Predicted values (cm)	Actual values (cm)
1.	1.7 mgl^{-1} BAP	0.50 mgl^{-1} NAA	5.93	5.8	2.21	2.5	6.10	6.5
2.	1.9 mgl^{-1} BAP	200 mgl^{-1} CH	6.44	6.1	1.12	1.3	1.58	1.9
3.	2.4 mgl^{-1} BAP	0.51 mgl^{-1} Kn	3.19	3.4	2.09	2.0	5.21	5.5
4.	2.5 mgl^{-1} BAP	1.10 mgl^{-1} Kn	3.01	2.9	2.01	2.0	6.45	6.6
5.	1.0 mgl^{-1} BAP	0.10 mgl^{-1} IAA	6.10	5.7	2.13	2.3	5.71	5.9

The results from the process of optimization of various responses of initiation stage in micropropagation of spine gourd confirmed that there is no significant difference between predicted values and actual experiment performed from desirable combinations suggested by Design expert software. The actual and predicted values for each response are at par with each other. The observations were showing best results during all stages of initiation, number of shoots per explant and average length of shoot.

4.2.1.3.1 ANOVA for optimized combinations of media supplements on MS media used for initiation stage using nodal and shoot tip explant

Best optimized combinations of media supplements resulted from various experiments conducted through RSM were subjected to analysis of variance in completely randomized design to compare among themselves (Table 39 and 40). While analyzing the best optimized treatments, the MS media supplementation with 2.0 mgl^{-1} TDZ and 2.0 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP were also included, however both were not run in RSM. The values in the given Table 39 and 40 are the average mean values of observations for number of days required for shoot initiation, number of shoots per explant and average length of shoot for initiation stage of micropropagation in spine gourd.

Table 39: ANOVA for optimized combinations of media supplements on MS media used for initiation stage using nodal explant

Sr. No.	Optimized treatments	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot (cm)
1	2.00 mgl^{-1} TDZ*	7.50	1.00	2.50
2	2.10 mgl^{-1} TDZ + 0.50 mgl^{-1} BAP*	6.10	1.70	5.30
3	1.01 mgl^{-1} BAP	9.00	1.50	3.30
4	1.90 mgl^{-1} BAP + 0.50 mgl^{-1} NAA	6.10	1.00	4.10
5	2.14 mgl^{-1} BAP + 200 mgl^{-1} CH	5.00	1.50	4.40
6	1.80 mgl^{-1} BAP + 0.50 mgl^{-1} Kn	4.10	1.70	6.50
7	2.00 mgl^{-1} BAP + 1.00 mgl^{-1} Kn	3.30	1.00	5.80
8	0.46 mgl^{-1} BAP + 0.10 mgl^{-1} IAA	6.00	1.20	7.50
	Mean	**5.88**	**1.325**	**4.92**
	F – ratio	**183.94**	**8.27**	**151.91**
	SE	**0.1385**	**0.10807**	**0.1354**
	CD@1%	**0.55**	**0.45**	**0.56**

* Not optimised using RSM

The comparative analysis of various media supplementations used for nodal explants revealed that among all the combinations the significant minimum days required for shoot initiation (3.30) were reported in the nodal explant placed on MS media supplemented with 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn, which was followed by 1.8 mgl^{-1} BAP + 0.5 mgl^{-1} Kn (4.1 days) and 1.8 mgl^{-1} BAP + 200 mgl^{-1} CH (5.0 days). The maximum number of days required for shoot initiation (9.0) were reported in the MS media supplemented with 1.01 mgl^{-1} BAP, which is followed by the treatment of 2.0 mgl^{-1} TDZ (7.5 days).

The Maximum number of shoots per explants (1.70) were recorded in nodal explants placed on MS media fortified with 2.0 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP and 1.8 mgl^{-1} BAP + 0.5 mgl^{-1} Kn. The minimum number of shoots (1.0) per explants were reported in the MS media supplemented with 1.9 mgl^{-1} BAP + 0.5 mgl^{-1} NAA and 2.0 mgl^{-1} TDZ.

The highest length of shoot (7.5 cm) was noticed in the nodal explants placed on the MS media fortified with 0.46 mgl^{-1} BAP + 0.1 mgl^{-1} IAA which is followed by supplementation

with 1.8 mgl^{-1} BAP + 0.5 mgl^{-1} Kn (6.5 cm). The lowest length of shoot (2.5 cm) was reported in media supplementation with 2.0 mgl^{-1} TDZ which was followed by 1.01 mgl^{-1} BAP (3.30 cm).

The nodal shoot segments proved to be most suitable explants material for regeneration of shoots in *Momordica* species in short period of time as reported by Agarwal (2004) and Sultana, *et al.* (2005). The earlier shoot initiation may be attributed to the effect of auxin and cytokinins as reported by Kapadia, *et al.* (2018), Hoque, *et al.* (1995) Hoque, *et al.* (2000), Nabi, *et al.* (2002[a]), Mohammad and Sharif (2010), Rai, *et al.* (2012), Patel, *et al.* (2015) and Jadhav, *et al.* (2016) in micropropagation studies of Spine gourd. The incorporation of higher levels of BAP in media induced the callus formation which in turn delayed the shoot regeneration. The similar trend was observed in present experiment as reported by Jamatia (2016) and Rai, *et al.* (2012) in Spine gourd. The higher number of shoots per explant might be due to the regulatory role of BAP which might have stimulated proliferation of meristems as reported by Thakur *et al.* (2001) in *Momordica balsamia.* The higher levels of cytokinins and auxins might have induced the callus formation which in turn reduced the length of shoots of plants. Similar results were reported by the Paula, *et al.* (1900) while investigating somatic embryogenesis in *Cucurbita pepo* using cotyledonary and shoot tip explants.

Table 40: ANOVA for optimized combinations of media supplements on MS media used for initiation stage using shoot tip explant

Sr. No.	Optimized treatments	Initiation (Av. days)	Av. number of shoots/ explant	Av. length of shoot (cm)
1	1.99 mgl^{-1} TDZ*	6.0	1.1	0.9
2	2.00 mgl^{-1} TDZ + 0.49 mgl^{-1} BAP*	6.5	1.0	4.3
3	1.70 mgl^{-1} BAP + 0.50 mgl^{-1} NAA	5.8	2.5	6.5
4	1.90 mgl^{-1} BAP + 200 mgl^{-1} CH	6.1	1.3	1.9
5	2.40 mgl^{-1} BAP + 0.51 mgl^{-1} Kn	3.4	2.0	5.5
6	2.50 mgl^{-1} BAP + 1.10 mgl^{-1} Kn	2.9	2.0	6.6
7	1.00 mgl^{-1} BAP + 0.10 mgl^{-1} IAA	5.7	2.3	5.9
	Mean	**5.2**	**1.74**	**4.51**
	F – ratio	**179.08**	**31.75**	**294.45**
	SE	**0.1069**	**0.1069**	**0.1327**
	CD@1%	**0.45008**	**0.45008**	**0.55**

* Not optimised using RSM

The analysis of variance performed for comparing the best optimized combinations of media supplements resulted from various micropropagation experiments revealed that minimum number of days required for shoot initiation (2.9) were reported on the MS media fortified with combination of 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn which is followed by PGRs combination of 2.4 mgl^{-1} BAP + 0.51 mgl^{-1} Kn (3.4 days). Whereas maximum days required for shoot initiation (6.5 days) were registered on the MS media fortified with the combination of 2.0 mgl^{-1} TDZ + 0.49 mgl^{-1} BAP, which is followed by PGRs combinations of 1.9 mgl^{-1} BAP + 200 mgl^{-1} CH (6.1 days).

The shoot tip explants of spine gourd recorded maximum number of shoots (2.5) per explants on MS media supplemented with the PGRs combination of 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA which is followed by combination of 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA (2.3 shoots), whereas minimum number of shoots (1.0) were recorded on MS media supplemented with the combination of 2.0 mgl^{-1} TDZ + 0.49 mgl^{-1} BAP followed by combination of 1.99 mgl^{-1} TDZ (1.1 shoots).

The results of average length of shoots reported that highest length of shoots (6.6 cm) were reported on the MS media supplemented with the combination of 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn, which is followed by the PGRs combination of 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA (6.5 cm). The lowest length of shoots (0.9 cm) was registered on the MS media fortified with the combination of 1.99 mgl^{-1} TDZ which is followed by combinations of 1.9 mgl^{-1} BAP + 200 mgl^{-1} CH (1.9 cm).

The earlier shoot initiation may be attributed to the effect of auxins and cytokinins as reported by Kapadia, *et al.* (2018), Devendra, *et al.* (2008) and Hoque, *et al.* (1995) in spine gourd. In the present study, CH along with BAP induced vigorous and healthy shoots but promoted apical buds to develop whitish fragile callus at the higher concentrations of BAP which in turn inhibit the further development of shoots. This might be attributed to the role of CH in inducing vigorous shoots, promoting callus formation. Also, the inhibitory effect for shoot differentiation might have played the role as reported by Ahmad and Anis (2005) in *Cucumis sativus* L. and Rai *et al.*, (2012) in spine gourd. However, the contradictory results are reported by Hoque, *et al.* (1995) and Paula, *et al.* (1990) where they reported that the combination of BAP and Kn showed very little shoot regeneration response.

Incorporation of an auxin (IAA) at a lower concentration (0.1 mgl^{-1}) significantly increased the number of shoots by promoting axillary branching as reported by Chaudhary, *et al.* (2107) in spine gourd. This indicated that an appropriate auxin(s)-cytokinin(s) combination was mandatory for a better shoot proliferation/growth as reported by Phulwaria, *et al.* (2014). Auxins–cytokinins crosstalk mechanism may be also one the reason which depicted that cytokinins promote stem cell proliferation in shoot meristem and inhibit its differentiation, whereas auxin triggers organ primordium formation through suppressing cytokinine biosynthesis, and these in turn regulates shoot meristem development and shoot branching. The similar results were reported by Shimizu-Sato, *et al.* (2009) and Su, *et al.* (2011).

The one of the main reason for reduction in shoot length may be attributed to the higher rate of callus production from CH and BAP at higher concentration, which proved the inhibitory effect on shoot differentiation and shoot length as reported by Rai, *et al.* (2012) in spine gourd and Ahmad and Anis (2005) in *Cucumis sativus* L.

4.2.2 Shoot multiplication in spine gourd

4.2.2.1 Response of spine gourd nodal explants to various optimized levels of media supplements on shoot multiplication

The best optimized combination of media supplements i.e. PGR's from each experiment, were selected for further multiplication of the nodal explants. The levels of 2.0 mgl^{-1} TDZ and 2.0 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP were not run in RSM, however, their means were considered for comparison with the others. The well-developed plants were cut below the node and inoculated in optimized level of media supplement observed from each separate experiment, and the obtained results are presented in Table 41 and graphically represented in Fig. 13 and discussed hereunder suitable headings.

Table 41: Effect of different optimized levels of media supplements on the morphogenic responses of nodal explant of spine gourd

Sr. No.	Optimized Treatments	Total number of plants multiplied	Av. number of shoots/ explant	Av. length of shoot (cm)
1	2.00 mgl^{-1} TDZ*	10.00	1.25	3.10
2	2.10 mgl^{-1} TDZ + 0.50 mgl^{-1} BAP*	19.00	1.95	5.23
3	1.01 mgl^{-1} BAP	12.00	1.10	3.40
4	1.90 mgl^{-1} BAP + 0.50 mgl^{-1} NAA	13.00	1.45	3.53
5	2.14 mgl^{-1} BAP + 200 mgl^{-1} CH	25.00	1.90	5.35
6	1.80 mgl^{-1} BAP + 0.50 mgl^{-1} Kn	27.00	2.12	8.40
7	2.00 mgl^{-1} BAP + 1.00 mgl^{-1} Kn	28.00	1.54	7.40
8	0.46 mgl^{-1} BAP + 0.10 mgl^{-1} IAA	41.00	2.12	8.56
	Mean	**21.875**	**1.6779**	**5.6217**
	F – ratio	**161.52**	**67.90**	**2592.27**
	SE	**0.841**	**0.04803**	**0.04417**
	CD@1%	**3.48**	**0.20**	**0.18**

* Not optimised using RSM

Significant differences were observed among the treatments with regards to the multiplication of shoots from nodal explants of Spine gourd on MS media supplemented with the various PGRs combinations. After few days of inoculation, small watery callus was observed during growth period of multiplication at earlier stage. The present investigation revealed that the highest number of plants (41) were multiplied from nodal explants of spine gourd placed on MS media supplemented with 0.46 mgl^{-1} BAP + 0.10 mgl^{-1} IAA, which is followed by the combination of 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn (28 plants) and 1.8 mgl^{-1} BAP + 0.5 mgl^{-1} Kn (27 plants). However the minimum number of plants (10) were multiplied on MS media fortified with the combination of 2.0 mgl^{-1} TDZ, which is followed by 1.01 mgl^{-1} BAP (12 plants) and 1.9 mgl^{-1} BAP + 0.5 mgl^{-1} NAA (13 plants).

During multiplication of plants, significant differences were observed among the optimized treatments for average number of shoots per explant. It is evident from the study that the maximum number of shoots per explants (2.12) were reported from nodal explants of spine

gourd placed on the MS media supplemented with 1.8 mgl^{-1} BAP + 0.5 mgl^{-1} Kn and 0.46 mgl^{-1} BAP + 0.10 mgl^{-1} IAA, which is followed by use of 2.1 mgl^{-1} TDZ + 0.5 mgl^{-1} BAP (1.95 shoots). The minimum number of shoots per explants (1.10 and 1.25) were recorded in the explants placed on the MS media supplemented with 2.0 mgl^{-1} TDZ and 1.01 mgl^{-1} BAP respectively. The results shows that combination of cytokinins and auxins had good effect on number of shoots per explants when compare to the cytokinins alone.

The shoots with significantly maximum length (8.56 cm,) were reported in case of the explants placed on the MS media fortified with 0.46 mgl^{-1} BAP + 0.10 mgl^{-1} IAA. Whereas the minimum length of shoots (3.10 cm) was registered in the explants placed on the MS media supplemented with 2.0 mgl^{-1} TDZ, which is followed by 1.01 mgl^{-1} BAP alone (3.4 cm). It confirms that cytokinins alone had very little effect on length of shoot when compared to the combination of cytokinins or combination of cytokinins with Auxins.

The increased shoot number, due to repeated transfer of the micro shoots, may be owing to suppression of apical dominance during subculture that induced basal dominant meristamatic cells to form new shoots as reported by Shekhawat and Shekhawat (2011) in spine gourd. The maximum number of shoots per explant may be attributed to the regulatory role of BAP which might have stimulated proliferation of meristems as reported by Thakur *et al.* (2001) in *Momordica balsamia.* The increased callus formation with use of increased concentration of BAP induced the high rate of callus formation which might have hindered the growth of shoots and led to the formation of somaclonal variants. The present findings are in conformity with the findings of those reported by Karim and Ahmed (2010) in somatic embryogenesis, Shekhawat, *et al.* (2011), Rai, *et al.* (2012) and Debnath, *et al.* (2013[b]) using nodal explant and by Raju, *et al.* (2014) in using tendril explants of *Momordica dioica.*

4.2.2.2 Response of spine gourd shoot tip explants to various optimized levels of media supplements on shoot multiplication

The best optimized combination of PGR's suggested by the RSM were selected for further multiplication of the shoot tip explants in each experiment. The levels viz; 2.0 mgl^{-1} TDZ and 2.0 mgl^{-1} TDZ + 0.49 mgl^{-1} BAP were not performed in RSM. However, their mean is considered for each response for comparison of multiplied plants. The well-developed plants were cut below each node and placed on MS media supplemented with optimized levels of PGR from each experiment and the results obtained are presented in Table 42 and graphically represented in Fig. 14 and discussed hereunder suitable headings.

The significant differences were observed among various optimized treatments for multiplication of shoot tip explants of Spine gourd. The present investigation revealed that the maximum number of plants (44) were multiplied from shoot tip explants of spine gourd which were placed on MS media supplemented with the various PGRs combination of 1.0 mgl^{-1} BAP +

0.1 mgl^{-1} IAA. The second highest number of plants (30) were multiplied on the combination of 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. Whereas lowest number of plants were multiplied (6.0) plants on MS media fortified with the combination of 1.99 mgl^{-1} TDZ, which is followed by 1.9 mgl^{-1} BAP + 200 mgl^{-1} CH (11 plants).

Table 42: Effect of different optimized levels of media supplements on the morphogenic responses of shoot tip explant of spine gourd

Sr. No.	Optimized treatments (mgl^{-1})	Total number of plants multiplied	Av. number of shoots/ explant	Av. length of shoot (cm)
1	1.99 mgl^{-1} TDZ*	06.00	1.21	1.75
2	2.00 mgl^{-1} TDZ + 0.49 mgl^{-1} BAP*	13.00	1.23	4.12
3	1.70 mgl^{-1} BAP + 0.50 mgl^{-1} NAA	30.00	2.71	6.75
4	1.90 mgl^{-1} BAP + 200 mgl^{-1} CH	11.00	1.45	2.10
5	2.40 mgl^{-1} BAP + 0.51 mgl^{-1} Kn	21.00	1.82	5.72
6	2.50 mgl^{-1} BAP + 1.10 mgl^{-1} Kn	24.00	1.93	7.20
7	1.00 mgl^{-1} BAP + 0.10 mgl^{-1} IAA	44.00	2.45	6.50
	Mean	**21.28**	**1.82952**	**4.87714**
	F – ratio	**271.231**	**576.93**	**346.934**
	SE	**0.7868**	**0.02433**	**0.12067**
	CD@1%	**3.31**	**0.10244**	**0.50805**

* Not optimised using RSM

The maximum number of shoots per explant (2.71) were recorded in shoot tip explant placed on MS media supplemented with 1.70 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, whereas minimum number of shoots per explants (1.23) were recorded in optimized level of 2.0 mgl^{-1} TDZ + 0.49 mgl^{-1} BAP.

The observations of present study showed that the maximum length of shoots (6.75 cm) from shoot tip explants of spine gourd was recorded in treatment combination of 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, which is followed by 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA (6.5 cm). The minimum length of shoot (1.75 cm) was recorded in shoot tip explant placed on MS media fortified with 1.99 mgl^{-1} TDZ.

The present study revealed the significant differences among the optimized treatments for various responses such as number of plants per explant, shoot numbers and length of shoots. The significant results reported suggested that an appropriate auxin(s)-cytokinin(s) combination was mandatory for a better shoot proliferation and growth as reported by Phulwaria, *et al.* (2014). Auxins–cytokinins crosstalk mechanism may be also one the reason which depicted that cytokinins promotes stem cell proliferation in shoot meristem and inhibit its differentiation, whereas auxin triggers organ primordium formation through suppressing cytokinine biosynthesis, and these in turn regulates shoot meristem development and shoot branching. The reduction in the shoot length may be attributed to the higher rate of callus production from CH and BAP at higher concentration, due to inhibitory effect on shoot differentiation and shoot length as reported by Rai,

et al. (2012) in spine gourd and Ahmad and Anis (2005) in *Cucumis sativus* L. The present findings of the experiments are similar to those of Hoque, *et al.* (1995) stem and nodal explants, Shekhawat and Shekhawat (2011), Arekar, *et al.* (2017), Mustapha, *et al.* (2013), Swamy, *et al.* (2105) in *Momordica dioica*, Moreno, *et al.* (1985) in *Cucumis melo* using shoot tips explant.

4.2.3 *In-vitro* rooting of regenerated shoots of spine gourd

4.2.3.1 Node

The well grown *In-vitro* multiplied shoots from nodal explants of spine gourd were transplanted on MS media fortified with various levels of PGR's as constructed using optimal customization command of D-Optimal Response surface methodology for rooting.

4.2.3.1.1 Response of spine gourd nodal explants to various levels of NAA on rooting using D-Optimal RSM

D-optimal response surface methodology was used to generate experimental designs for nodal explants of spine gourd. The various levels NAA concentrations as generated by RSM are used as media supplement with MS media. The results obtained in respect of various responses such as average number of days to root initiation, average number and length of root are presented in Table 43 and graphically represented in Fig. 15 and discussed hereunder the suitable headings.

4.2.3.1.1.1 Number of days required for root initiation

The observations with respect to number of days required for root initiation as affected by the MS media supplemented with various levels of NAA are presented in Table 43 and graphically represented in Fig. 15. It is evident from the Table No. 43 that number of days required for root initiation in regenerated nodal explants of spine gourd were ranged from 18 to 24.5 days after inoculation. The regenerated nodal explants inoculated on MS media supplemented with 1.0 mgl^{-1} NAA recorded the average minimum number of days (18) for root initiation. The average maximum number of days (24.5) for rooting were recorded by the nodal explants placed on a MS media fortified with the concentration of 2.0 mgl^{-1} NAA.

The data obtained are subjected to regression analysis using D-Optimal RSM software. The data revealed that the coefficient of determination (R^2) was 0.7999. Model under study reported the adequate precision of 8.58, which is considerably higher than the minimum desirable (4) for high prediction ability of model to navigate the design space. It is evident from the Model F-value (99.99) that the model is significant with the P-values of 0.0003.

It is seen from the coefficient for number of days required for shoot initiation that the levels of different concentration of media supplements like NAA had significant ($p<0.05$) effect on response of root initiation. The NAA levels at linear terms had positive significant effect on the number of days for initiation, whereas quadratic levels of NAA had non-significant effect

on days required for root initiation in negative manner, here the negative sign indicates the earliest root initiation.

Table 43: Effect of different levels of NAA supplementations on regenerated nodal explants in response to rooting using D-Optimal Response Surface Methodology

RUN	Factor 1 A: NAA (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of roots/ explant	Response 3 Av. length of root (cm)
1	1.0	19.20	2.50	1.60
2	1.5	22.16	2.00	1.56
3	1.5	22.50	1.80	1.70
4	1.0	19.28	2.14	1.61
5	1.5	23.90	1.90	1.80
6	2.0	24.50	1.60	2.20
7	1.0	22.10	2.20	1.40
8	2.0	23.90	1.50	1.80
9	1.0	18.00	2.30	1.50
10	1.0	20.10	2.40	1.70
11	2.0	24.00	1.80	2.16
12	2.0	24.30	1.90	2.10
13	1.5	22.10	2.20	1.60

Coefficient of quadratic polynomial model of NAA for responses of root initiation

Factor	Initiation (Av. days)	Av. number of roots/ explant	Av. length of root
Intercept	22.67	1.98	1.67
NAA (A)	2.22*	-0.3040*	0.2515*
A^2	-0.7095	0.0290	0.1485
F- Value	19.99	15.26	16.17
P-Value	0.0003	0.0009	0.0007
Mean	22.00	2.02	1.75
SD	1.08	0.1652	0.136
Adequate precision	8.58	7.66	7.671
R^2	0.7999	0.7532	0.7638

*P-Value = <0.05

The response surface equation derived for predicting average number of days for root initiation could be given as:

Initiation (Days) = + 9.62100 + 12.95300 * NAA - 2.83800 * NAA^2

The significant differences were recorded in the response of regenerated nodal explants of spine gourd to rooting as affected by the different levels of media supplements. The minimum number of days required for root initiation (18) were reported in regenerated nodal explants placed on MS media supplemented with 1.0 mgl^{-1} NAA, which is followed by 1.5 mgl^{-1} NAA (22.1). Whereas maximum number of days required for root initiation (24.5) were registered on MS media supplemented with 2.0 mgl^{-1} NAA. The lower concentrations of NAA were reported to be good for earlier root initiation in shoots of nodal explants of spine gourd. These results are

in conformity with the earlier findings of Jamatia (2016) and Kulkarni (1999) in shoots of nodal explants of spine gourd.

4.2.3.1.1.2 Number of roots per explant

The data in respect of the number of roots produced per regenerated nodal explant of spine gourd as effected by the various levels of NAA supplementations on MS media is summarized in Table 43 and graphically depicted in Fig. 15. It is clear from the Table 43 that average number of roots produced per regenerated nodal explant of spine gourd were ranged from 1.5 to 2.5 roots. The minimum number of roots per explant (1.5) were observed in the regenerated nodal explants placed on MS media supplemented with 2.0 mgl^{-1} NAA while maximum number of roots per regenerated shoot (2.5) were recorded in a MS media supplemented with combination of 1.0 mgl^{-1} NAA.

The coefficient of determination (R^2) in respect of the number of roots per regenerated nodal explant for the present model under study is 0.7532. For the same analysis, adequate precision was found to be 7.66, which is significantly higher than the minimum desirable (4) for high prediction ability to navigate the design. The Model F-value (15.26) implied that the model was significant (P-value 0.09 %).

In respect of coefficients for number of roots per regenerated nodal explant, present study model showed that the different levels of media supplements had significant ($p<0.05$) effect on response of spine gourd nodal explants. The NAA levels at linear terms had positive significant effect on the number of roots per explant in negative manner**.** Whereas quadratic levels of NAA had non-significant effect on number of roots per explant.

The response surface equation derived for predicting number of roots per regenerated nodal explant of spine gourd could be given as:

Number of Shoots per explants (Days) = + 3.14800 - 0.956000 * NAA + 0.116000 * NAA^2

Among various levels of NAA concentrations tested for number of roots per regenerated nodal explants in spine gourd showed the significant differences in all treatments. The highest number of roots (2.5) were recorded in regenerated nodal explants of spine gourd placed on MS media supplemented with 1.0 mgl^{-1} NAA, which is followed by 1.5 mgl^{-1} NAA (2.2 roots per nodal explant). Whereas minimum number roots per shoot (1.5) were recorded in MS media fortified with 2.0 mgl^{-1} NAA. During the investigation of rooting study, initially small quantity of callus was formed. This is also confirmed by earlier reports of Rai, *et al.* (2012). The results were similar with the earlier findings of Jamatia (2016) in spine gourd.

4.2.3.1.1.3 Length of roots

The average length of roots per regenerated nodal explant indicated significant differences among the various level of media supplementations with NAA in spine gourd (Table 43 and Fig. 15). The minimum length of roots (1.4 cm) was recorded in the regenerated nodal

explants placed on MS media fortified with 1.0 mgl^{-1} NAA. The maximum length of roots (2.2 cm) was registered in the regenerated nodal explant placed on MS media supplemented with 2.0 mgl^{-1} NAA.

The regression analysis of average length of roots per regenerated nodal explants of spine gourd revealed that the coefficient of determination (R^2) is 0.7638. The adequate precision was found to be 7.671 for average root length which is substantially higher than the minimum desirable (4) for high prediction ability. The Model F-value (16.17) suggested that the model was significant along with the support of P-value (0.0007).

It is evident from the table 43 that the levels of different combinations of media supplements had significant ($p<0.05$) effect on average length of roots per regenerated nodal explants. The linear terms of NAA had significant effect on the length of root while the quadratic terms of NAA level reported non-significant effect.

The response surface equation derived for predicting average length of roots regenerated nodal explant could be given as:

Length of shoots (cm) = + 2.24700 - 1.27900 * NAA + 0.594000 * NAA^2

Different degrees of root length were achieved from regenerated nodal explants placed on a MS media supplemented with the various levels of NAA in spine gourd. The maximum length of roots (2.2 cm) was recorded in regenerated nodal explants of spine gourd placed on MS media supplemented with 2.0 mgl^{-1} NAA, which is followed by 1.5 mgl^{-1} NAA (1.7 cm). Whereas minimum length of roots per shoot (1.4 cm) was recorded on MS media supplemented with the concentration of 1.0 mgl^{-1} NAA. The higher levels of NAA had good effect on increased length of root. These results are in agreement with the earlier findings of Jamatia (2016) in nodal explants of spine gourd, Hossain, *et al.* (1997) in *Trichosanthes dioica* Roxb and Karim and Ulla (2011) in cotyledonary explants of spine gourd.

4.2.3.1.2 Response of spine gourd nodal explants to various levels of IBA on rooting using D-Optimal RSM

The experimental design for various levels of IBA as a media supplements was generated using response surface methodology of design expert software for regenerated nodal explants of spine gourd. The results of various responses such as average number of days required for root initiation, average number and length of root per regenerated nodal explant are presented in Table 44 and discussed hereunder with the suitable headings.

4.2.3.1.2.1 Number of days required for root initiation

Results pertaining to the number of days required for root initiation as affected by various concentrations of IBA supplementations on MS culture media are summarized in Table 44 and graphically represented in Fig. 16. It is evident from the Table 44 that the number of days required for root initiation in regenerated nodal explants of spine gourd were ranged from 19.2 to

26 days after inoculation. The minimum number of days (19.2) for root initiation in regenerated nodal explant were registered on MS media supplemented with 2.0 mgl^{-1} IBA. Whereas, MS media supplemented with 1.0 mgl^{-1} IBA recorded the maximum number of days (26) for root initiation in regenerated nodal explant.

Table 44: Effect of different levels of IBA supplementations on regenerated nodal explants in response to rooting using D-Optimal Response Surface Methodology

RUN	Factor 1 A: IBA (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of roots/ explant	Response 3 Av. length of root (cm)
1	1.5	23.5	2.10	1.15
2	1.5	22.9	2.30	1.12
3	1.5	22.0	2.00	1.13
4	2.0	20.6	8.51	2.30
5	2.0	19.2	8.60	2.50
6	1.0	25.0	2.70	1.24
7	2.0	20.9	8.54	2.40
8	1.0	25.5	2.50	1.25
9	1.5	23.0	1.90	1.00
10	2.0	21.8	8.40	2.10
11	1.0	26.0	2.30	1.26
12	1.0	24.0	4.00	1.23
13	2.0	20.5	8.59	2.35

Coefficient of quadratic polynomial model of IBA for responses of root initiation

Factor	Initiation (Av. days)	Av. number of roots/ shoot	Av. length of root
Intercept	22.85	2.08	1.10
IBA (A)	-2.26*	2.83*	0.5425*
A^2	0.0125	3.63*	0.6875*
F- Value	33.29	303.09	203.55
P-Value	<0.0001	<0.0001	<0.0001
Mean	22.68	4.80	1.62
SD	0.8281	0.4337	0.1011
Adequate precision	11.3749	30.97	25.31
R^2	0.8694	0.9838	0.9760

*P-Value = <0.05

The recorded data in respect of days required for root initiation were subjected to the regression analysis. It is revealed from the data that the coefficient of determination (R^2) was 0.8694 for the model. Further the model reported the adequate precision of, which is substantially higher than the minimum desirable (4) for high prediction ability of design. The Model F-value of 33.29 implied that the model was significant with P-value of <0.01 %.

As regards the coefficient for number of days required for root initiation model, it is evident that the levels of different concentrations of IBA had significant ($p<0.05$) effect on responses by regenerated nodal explants. The IBA level at linear terms had significant effect on

the number of days for initiation in negative terms, which is desirable. Whereas quadratic levels of IBA had non-significant effect on days required for root initiation.

The response surface equation derived for predicting average number of days required for root initiation using IBA could be given as:

Initiation (Days) = + 29.75000 - 4.67500 * IBA + 0.050000 * IBA^2

The present research findings showed the significant differences for days required for root initiation for different levels of IBA supplementation for regenerated nodal explants of spine gourd. The minimum number of days required for root initiation (19.2) were recorded in the MS media supplemented with 2.0 mgl^{-1} IBA for the regenerated nodal explants. However maximum number of days required for root initiation (26) days were registered on MS media fortified with 1.0 mgl^{-1} IBA. During investigation, IBA showed the lowest amount of callus growth at initial period rooting as compare to the NAA. The higher concentration of IBA was found to be good for root induction in spine gourd. The present results are in conformity with the earlier reports of Arekar, *et al.* (2012) and Kulkarni, *et al.* (2006) in nodal explants of spine gourd.

4.2.3.1.2.2 Number of roots per explant

The data in respect of number of roots per regenerated nodal explant of spine gourd as effected by the different levels of IBA supplementations on MS media are presented in Table 44 and graphically represented in Fig. 16. It is clear from the data that the number of roots per shoot of nodal explants were ranged from 1.9 to 8.6 roots. The minimum number of roots per shoot (1.9) were recorded in a media supplemented with 1.5 mgl^{-1} IBA. However, the maximum number of shoots per explant (8.6 roots) were registered in the MS media supplemented with 2.0 mgl^{-1} IBA.

Regression analysis of a data presented in Table 44 revealed that the coefficient of determination (R^2) is 0.9838 for number of roots per regenerated nodal explant. The model reported the adequate precision of 30.37 which is appreciably higher than the minimum desirable (4) for high prediction ability of design. The F-value of model is 303.09 which implied that the model was significant for number of roots per explants with P-value of <0.0001.

As regards the coefficient for number of roots per shoot of nodal explant, model showed that the levels of different concentrations of IBA had significant ($p<0.05$) effect on response. The IBA level at linear and quadratic terms had positive significant effect on the number of roots per explant.

The response surface equation derived for predicting number of roots per shoot of nodal explant could be given as:

Number of Shoots per explant (Days) = + 26.23400 - 37.86500 * IBA + 14.50600 * IBA^2

The results revealed that highest number of roots per shoot of nodal explant (8.56) had reported on the MS media supplemented at 2.0 mgl^{-1} IBA. Whereas lowest number of roots

per shoot of nodal explants (1.9) were registered on the MS media supplemented with 1.5 mgl^{-1} IBA. Similar results stating IBA as a best supplement for root regeneration of *Momordica dioica* are reported by Kapadia, *et al.* (2018), Hoque, *et al.* (2007), Hoque, *et al.* (1995), Nabi, *et al.* (2002[a]), Karim and Ahmed (2010). The effect of IBA on rooting of many species such as *Centella asiatica* (Tiwari, *et al.*, 2000 and Banerjee, *et al.*, 1999) and *Teucrium fruticans* L. (Frabetti, *et al.*, 2009) is also reported.

4.2.3.1.2.3 Length of roots

The average length of root per shoot of nodal explant recorded the significant differences as affected by the various levels of media supplementation with IBA (Table 44 and Fig 16). It is seen from the Table 44 that the average length of root per shoot of nodal explant was ranged from 1.0 to 2.5 cm. The minimum length of the root (1.0 cm) was recorded in the shoots placed on a MS media supplemented with 1.5 mgl^{-1} IBA. The maximum length of root per shoot (2.5 cm) was registered in respect of MS supplemented with 2.0 mgl^{-1} IBA.

The regression analysis of average length of roots per shoot of nodal explants of spine gourd revealed that the coefficient of determination (R^2) was 0.9760. The adequate precision was found to be 25.31 for average length of roots, which is noticeably higher than the minimum desirable (4) for high prediction ability. The studied model for the average length of shoots is significant with F-value of 203.55 and P-value of <0.01%.

It is revealed from the coefficient for length of root that the model is significant at the different levels of media supplementation ($p<0.05$) and had significant effect on responses. Both linear and quadratic terms of IBA level had positive and significant effect on the length of root.

The response surface equation derived for predicting length of shoots produced per explant could be given as:

Length of shoots (cm) = + 5.66000 - 7.16500 * IBA + 2.75000 * IBA^2

The investigations on the average length of roots per shoot of nodal explants placed on MS media supplemented with the various levels of IBA had showed the significant differences among the various treatments. The present study revealed the highest average length of roots (2.5 cm) were recorded in nodal explants placed on MS media fortified with 2.0 mgl^{-1} IBA. However lowest average length of roots (1.0 cm) were recorded in case of nodal explants placed on MS culture media supplemented with 1.5 mgl^{-1} IBA. The present results are similar with findings of those reported by Ghive, *et al.* (2006[a]) and Nabi, *et al.* (2002[a]) in spine gourd.

4.2.3.2 Shoot tip

The various kinds of MS basal media fortifications i.e. plant growth regulators such as IBA and NAA were considered for the optimization and used to study rooting responses in shoot tip explants of spine gourd. Each media supplementation is treated as a separate part of the research

work. The various levels of PGRs i.e. IBA and NAA were generated using optimal customization command of D-Optimal RSM.

4.2.3.2.1 Response of spine gourd shoot tip explants to various levels of NAA on rooting using D-Optimal RSM

D-optimal response surface methodology was used to generate experimental designs for studying rooting response of shoot tip explants of spine gourd to the various levels NAA concentrations on MS media. The results in respect of various responses such as average number of days to root initiation, average number and length of root are presented in Table 45 and discussed hereunder the suitable headings.

Table 45: Effect of different levels of NAA supplementations on the rooting responses of shoot tip explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: NAA (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of roots/ explant	Response 3 Av. length of root (cm)
1	1.5	10.60	10.50	2.43
2	1.5	10.80	09.40	2.30
3	1.0	09.00	13.20	2.00
4	1.0	09.10	12.50	2.20
5	1.5	10.50	11.40	2.50
6	1.5	10.30	09.80	2.35
7	1.0	08.70	13.90	2.10
8	2.0	11.70	09.80	2.15
9	1.5	10.40	11.20	2.50
10	2.0	11.30	09.71	2.14
11	1.0	09.50	13.50	1.90
12	2.0	10.90	09.60	2.10
13	2.0	11.50	09.90	2.20

Coefficient of quadratic polynomial model of NAA for responses of root initiation			
Factor	**Initiation (Av. days)**	**Av. number of roots/ explant**	**Av. length of root**
Intercept	10.52	10.46	2.42
NAA (A)	1.14*	-1.76*	0.0487
A^2	-0.3075	1.05*	-0.3172*
F- Value	64.46	34.53	18.85
P-Value	<0.0001	<0.0001	0.0004
Mean	10.33	11.11	2.22
SD	0.287	0.639	0.0934
Adequate precision	16.48	11.46	8.159
R2	0.9280	0.8735	0.7903

*P-Value = <0.05

4.2.3.2.1.1 Number of days required for root initiation

The number of days required for root initiation from the regenerated shoot tip explants of spine gourd placed on MS media fortified with various level of NAA ranged from 9 to 11.7 days (Table 45 and Fig. 17). The minimum number of days (9.0) required for root initiation

were reported in the regenerated shoot tip explants placed on MS media supplemented with 1.0 mgl^{-1} NAA, however the maximum days (11.7) were reported in 2.0 mgl^{-1} NAA supplementation for root initiation.

The regression analysis of the data presented in Table 45 revealed that the coefficient of determination (R^2) is 0.9280. The adequate precision was found to be 16.48 which is appreciably higher than the minimum desirable i.e. 4 for high prediction ability. The Model F-value (64.46) implied that the model is significant with the P-value of <0.0001.

As regards the coefficient for number of days required for root initiation, model showed that the levels of different combinations had significant ($p<0.05$) effect. The NAA level at linear terms had positive significant effect on the number of days required for root initiation, whereas quadratic levels of NAA had non-significant effect on days required for root initiation in negative manner. Negative sign indicates the earliest root initiation which is most desirable.

The response surface equation derived for predicting average number of days required for root initiation could be given as:

Initiation (Days) = + 4.34000 + 5.96500 * NAA - 1.23000 * NAA^2

The present study revealed that *In-vitro* multiplied shoots which were inoculated on the various concentration of the NAA for rooting study showed the significant difference among the treatments. The minimum number of days (9.0) required for root initiation were observed in the regenerated shoots tip explants on the MS media supplemented with 1.0 mgl^{-1} NAA. Whereas, maximum number of days required for root initiation (11.7) were recorded in the explants inoculated on MS media supplemented with 2.0 mgl^{-1} NAA. The present results are in agreement with the earlier findings of those reported by Jadhav, *et al.* (2015), Jamatia (2015) and Kulkarni (1999) in spine gourd.

4.2.3.2.1.2 Number of roots per explant

The observations regarding the number of roots produced per regenerated shoot tip explant of spine gourd as effected by the various levels of NAA supplementations on MS media are summarized in Table 45 and graphically represented in Fig. 17. It is seen from the Table 45 that the average number of roots produced per explant were ranged from 9.4 to 13.4 roots. The minimum number of roots per explant (9.4) were observed in the regenerated shoot tip explants placed on MS media supplemented with 1.5 mgl^{-1} NAA, while maximum number of roots per explant (13.4) were recorded in a MS media supplemented with 2.0 mgl^{-1} NAA.

The coefficient of determination (R^2) in respect of the number of roots per regenerated shoot tip explant for the present model under study is 0.8735. For the same analysis, adequate precision was found to be 11.46 which is significantly higher than the minimum desirable (4) for high prediction ability to navigate the design. The Model F-value (34.53) implied that the model was significant (P-value <0.01 %).

In respect of coefficient for number of roots per explant, present study model showed that the levels of different combinations of media supplements had significant ($p<0.05$) effect on response of spine gourd shoot tip explants. The NAA level at linear and quadratic terms had positive significant effect on the number of roots per explant, whereas linear terms of NAA effected in negative manner**.**

The response surface equation derived for predicting number of roots per explant could be given as:

Number of roots per explant (Days) = + 25.22750 - 16.16750 * NAA + 4.21500 * NAA²

Investigation revealed that various concentrations of NAA tested for number of roots per regenerated shoot in *Momordica dioica* using shoot tip as an explant on MS basal media showed a significant difference among the treatments. The highest number of roots (13.9) per regenerated shoot tip explants were noticed on MS media supplemented with 1.0 mgl^{-1} NAA. Whereas the lowest number of roots (9.4) per regenerated shoot tip explants were recorded from in 1.5 mgl^{-1} NAA. The similar results were also recorded by Hoque, *et al.* (2000) in cotyledonary explants and Jamatia (2016) in *in-vitro* nodal explants of *Momordica dioica*.

4.2.3.2.1.3 Length of roots

The results of average length of roots per regenerated shoot tip explant indicated significant differences among the various levels on MS media supplemented with NAA in spine gourd (Table 45 and Fig. 17). The minimum length of root (1.9 cm) was recorded in the regenerated shoot tip explants placed on MS media supplemented with 1.0 mgl^{-1} NAA. The maximum length of root (2.5 cm) was registered in the shoot tip explant on MS media supplemented with 1.5 mgl^{-1} NAA.

The regression analysis of a data presented in Table 45 revealed that the coefficient of determination (R^2) was 0.7903 for average length of root. The model reported the adequate precision (8.159) which is considerably higher than the minimum desirable (4) for high prediction ability. The Model F-value (18.85) and P-value (0.04%) indicated that the model was significant.

It is evident from the table 45 that the levels of different combinations of media supplements had significant ($p<0.05$) effect on rooting response on regenerated shoot tip explants. The linear terms of NAA level had non-significant effect on the length of root. Whereas quadratic terms of NAA had significant effect on length of root in negative manner.

The response surface equation derived for predicting length of roots per regenerated shoot tip explant could be given as:

Length of shoots (cm) = - 0.585500 + 3.90450 * NAA - 1.26900 * NAA²

In the present study, maximum length of roots (2.5 cm) per regenerated shoot was recorded in the regenerated explants on the MS media fortified with 1.5 mgl^{-1} NAA. The minimum length of roots (1.9 cm) was recorded in the MS media supplemented with 1.0 mgl^{-1} NAA. The

results showed that higher concentration had good effect on root length as compared with the lower level of concentrations. The similar trend of results was also reported by Jamatia (2016) in *in-vitro* nodal explants of *Momordica dioica.*

4.2.3.2.2 Response of spine gourd shoot tip explants to various levels of IBA on rooting using D-Optimal RSM

Several levels of IBA as generated by D-optimal response surface methodology were tried as media supplement with MS media to study the rooting response of regenerated shoot tip explant of spine gourd. The results pertaining to various responses such as root initiation, average number and length of roots are presented in Table 46 and graphically presented in Fig. 18 and discussed hereunder suitable headings.

Table 46: Effect of different levels of IBA supplementations on the rooting responses of shoot tip explant of spine gourd using D-Optimal Response Surface Methodology

RUN	Factor 1 A: IBA (mgl^{-1})	Response 1 Initiation (Av. days)	Response2 Av. number of roots/ explant	Response 3 Av. length of root (cm)
1	1.0	23.0	5.10	2.30
2	1.0	24.0	4.33	2.50
3	2.0	18.8	7.10	2.40
4	2.0	17.7	7.00	2.65
5	2.0	18.3	6.90	2.67
6	1.0	25.0	3.50	2.40
7	2.0	19.0	7.30	2.50
8	1.0	24.5	4.50	2.60
9	2.0	17.4	7.20	2.70
10	1.5	20.5	6.50	2.20
11	1.5	21.7	7.10	2.10
12	1.5	22.2	6.10	2.40
13	1.5	22.6	6.66	2.30

Coefficient of quadratic polynomial model of IBA for responses of root initiation			
Factor	**Initiation (Av. days)**	**Av. number of roots/ explant**	**Av. length of root**
Intercept	21.75	6.59	2.25
IBA (A)	-2.94*	1.37*	0.0670
A^2	-0.5675	-0.8612*	0.2670*
F- Value	60.26	47.13	7.48
P-Value	<0.0001	0.0001	0.0103
Mean	21.13	6.10	2.44
SD	0.810	0.4383	0.1289
Adequate precision	15.11	13.02	5.3944
R^2	0.9234	0.9041	0.5993

*P-Value = <0.05

4.2.3.2.2.1 Number of days required for root initiation

The data pertaining to number of days required for root initiation as affected by the MS media supplemented with various levels of IBA are presented in Table 46 and graphically represented in Fig.18. It is evident from the Table 46 that number of days required for root

initiation in regenerated shoot tip explants of spine gourd were ranged from 17.4 to 25.0 days after inoculation. The regenerated shoot tip explants inoculated on MS media supplemented with 1.0 mgl^{-1} IBA recorded the average minimum number of days (25.0) for root initiation. The average maximum number of days (17.4) for rooting were recorded by the shoot tip explants placed on a MS media fortified with 2.0 mgl^{-1} IBA.

The data obtained are subjected to regression analysis using D-Optimal RSM software. The data revealed that the coefficient of determination (R^2) was 0.9234. Model under study reported the adequate precision of 15.11, which is considerably higher than the minimum desirable (4) for high prediction ability of model to navigate the design space. It is evident from the Model F-value (60.26) that the model is significant with the P-values of <0.01 %.

It is seen from the coefficient for number of days required for root initiation that the levels of different concentrations of IBA had significant ($p<0.05$) effect on response of root initiation. The IBA levels at linear terms had significant effect while quadratic terms of IBA registered non-significant effect on the number of days for root initiation in negative terms. The negative value indicates the earliest root initiation which is most desirable.

The response surface equation derived for predicting average number of days for root initiation could be given as:

Initiation (Days) = + 25.47000 + 0.925000 * IBA - 2.27000 * IBA^2

The significant differences were recorded in the response of regenerated shoot tip explants of spine gourd to rooting to the different levels of media supplements. The minimum number of days required for root initiation (17.4) were reported in regenerated shoot tip explants placed on MS media supplemented with 2.0 mgl^{-1} IBA. Whereas maximum number of days required for root initiation (25.0) were registered on MS media supplemented with 1.0 mgl^{-1} IBA. The auxin IBA had better effect on induction of roots in spine gourd. The similar results are reported by Mustafa (2012) in spine gourd, Karim and Ahmad (2010), Karim (2013) in embryo derived plants of Spine gourd and Shekhawat *et al.* (2011) in same plant.

4.2.3.2.2.2 Number of roots per explant

The data in respect of the number of roots produced per regenerated shoot tip explant of spine gourd as effected by the various levels of IBA supplementations on MS media is summarized in Table 46 and graphically depicted in Fig. 18. It is clear from the Table 46 that average number of roots produced per regenerated shoot tip explant of spine gourd were ranged from 3.5 to 7.3 roots. The minimum number of roots per explant (3.5) were observed in the regenerated shoot tip explants on MS media supplemented with 1.0 mgl^{-1} IBA while maximum number of roots per regenerated shoot (7.3) were recorded in a MS media supplemented with combination of 2.0 mgl^{-1} IBA.

The coefficient of determination (R^2) in respect of the number of roots per regenerated shoot tip explant for the present model under study is 0.9041. For the same analysis, adequate precision was found to be 13.02, which is significantly higher than the minimum desirable (4) for high prediction ability to navigate the design. The Model F-value (47.13) implied that the model was significant (P-value <0.01%).

In respect of coefficient for number of roots per regenerated shoot tip explant, present study model showed that the different levels of media supplements had significant ($p<0.05$) effect on response of spine gourd shoot tip explants. The IBA level at linear and quadratic term had significant effect on number of roots per regenerated shoot, whereas quadratic levels of IBA had affected in negative manner.

The response surface equation derived for predicting number of roots per regenerated shoot tip explant of spine gourd could be given as:

Number of Shoots per explant (Days) = - 5.27500 + 13.07750 * IBA - 3.44500 * IBA²

The maximum number of roots (7.3) were recorded in regenerated shoot tip explants of spine gourd placed on MS media supplemented with 2.0 mgl^{-1} IBA. Whereas minimum number roots per shoot (3.5) were recorded in MS media fortified with 1.0 mgl^{-1} IBA. The results of the investigation are in agreement with those reported by Karim and Ahmad (2010) in shoot tip explants of *Momordica dioica.*

4.2.3.2.2.3 Length of Roots

The average length of roots per regenerated shoot tip explant indicated significant differences among the various level of media supplementations with IBA in spine gourd (Table 46 and Fig. 18). The minimum length of roots (2.1 cm) was recorded in the regenerated shoot tip explants placed on MS media fortified with 1.5 mgl^{-1} IBA. The maximum length of roots (2.7 cm) was registered in the regenerated shoot tip explant placed on MS media supplemented with 2.0 mgl^{-1} IBA.

The regression analysis for average length of roots per regenerated shoot tip explants of spine gourd revealed that the coefficient of determination (R^2) is 0.5993. The adequate precision was found to be 5.3944 for average root length which is substantially higher than the minimum desirable (4) for high prediction ability. The Model F-value (7.48) suggested that the model was significant along with the support of P-value (1.03%).

It is evident from the table that different levels of media supplements had significant ($p<0.05$) effect on average length of roots per regenerated shoot tip explants. Both linear and quadratic terms of IBA level had non-significant and positive significant effect respectively on the length of root.

The response surface equation derived for predicting average length of roots regenerated shoot tip explant could be given as:

Length of shoots (cm) = + 4.45200 - 3.07000 * IBA + 1.06800 * IBA^2

Different degrees of root length were achieved from regenerated shoot tip explants placed on a MS media supplemented with the various levels of IBA. The maximum length of roots (2.7 cm) was recorded in regenerated shoot tip explants of spine gourd placed on MS media supplemented with 2.0 mgl^{-1} IBA. Whereas minimum length of roots per shoot (2.1 cm) was recorded on MS media supplemented with the concentration of 1.5 mgl^{-1} IBA. The results are in conformity with the Nabi *et al.* (2012[b]) in spine gourd, Karim and Ahmad (2010) for somatic embryogenesis and Jadhav, *et al.* (2015) in spine gourd.

4.2.3.3 Optimization for rooting stage

The optimization of the various levels of rooting stage using optimal customization command of D-optimal response surface methodology was carried out. The most desirable results obtained from the experiments were subjected to multiple levels of analysis by giving numerical optimization command for expected goals during rooting stage after multiplication in spine gourd for node and shoot tip explants to find out the best combination of various independent factors to get optimum results from dependent response variables. Here the aim of the optimization was to minimize the number of days required for root initiation and maximize the average number of roots and average root length of regenerated node and shoot tip explants of spine gourd. The best solution exerted through the software and their predicted score and actual observations through validation are presented in Table 47 and 48.

Table 47: Optimization of media supplements on MS media at rooting stage of Nodal explant

No.	Factor 1	Av. No. of days for root initiation		Av. No. of roots/ explant		Av. length of root	
		Predicted values (days)	Actual values	Predicted values	Actual values	Predicted values (cm)	Actual values (cm)
1.	1.0 mgl^{-1} NAA	19.73	20.0	2.30	2.10	1.56	1.80
2.	2.0 mgl^{-1} IBA	20.60	21.0	8.52	8.50	2.33	2.40

Table 48: Optimization of media supplements on MS media at rooting stage of shoot tip explant

No.	Factor 1	Av. No. of days for root initiation		Av. No. of roots/ explant		Av. length of root	
		Predicted values	Actual values	Predicted values	Actual values	Predicted values	Actual values
1.	1.2 mgl^{-1} NAA	09.69	10.0	12.61	12.50	2.14	2.40
2.	2.0 mgl^{-1} IBA	18.29	19.0	07.10	07.50	2.58	2.70

However, validation of the optimized results given by the software by performing actual experiment stated that there are no significant differences among the predicted values and actual observations. The optimized observations showed the best results for both the experiments

for various responses such as earliest root initiation, number of root per shoot and length of root in both kind of explants i.e., in node and shoot tip.

4.2.4 Hardening of rooted plants

Hardening plays an important role in successful establishment of plant material in outer field condition. The well rooted *In-vitro* plants were taken out from the culture vessels and washed thoroughly with sterile water in order to remove the adhered nutrient agar media on root zone. Initially, plants were planted in small pots with potting media containing cocopeat and soil in 1:1 ratio. Hardening media was firmly pressed after keeping plants in pots. A staking was given by long thin sticks to give mechanical support to the vines. After that the pots were irrigated to maintain the moisture and finally covered the whole plant with the polythene cover to create high relative humidity for plants. Next day after transplanting, two small holes were created on polythene cover to provide aeration. The nutrients were applied at an interval of 3-4 days. The liquid form of NPK (19:19:19) was applied to supply nutrients to the plants. The plants were initially maintained at 75% Shade net with 80% relative humidity. After 10 days, these plants were shifted to the 50% Shade net with 75% relative humidity. The plants regenerated from the nodal explants had shown 67.85 per cent survival rate within 20.0 days, whereas plants regenerated from shoot tip explants recorded 80.64% survival rate within 18.0 days. During the hardening process plants developed an efficient root system, fully expanded leaves and were found to be photosynthetically active. The addition of soil from its native habitat during hardening to rooting media could increase the survival chances in the field. The similar results were reported by Nabi, *et al.* (2002[a]) in spine gourd. Water logging during hardening showed negative effect on the growth of the plantlets which have been also confirmed by Shekhawat, *et al.* (2011). The findings are in agreement with the earlier reports of (Rai *et al.* 2012) and Arekar *et al.* (2012) in nodal explants of Spine gourd.

5. SUMMARY AND CONCLUSION

The present study was conducted for optimization of protocol for micropropagation of *Momordica dioica.* The optimization was done by assessing the effect of combinations of different plant growth regulators at different stages of regeneration of spine gourd genotype RDMSG-2. Experimental design for various combinations of growth regulators was designed using D optimal Response Surface Methodology. Different growth hormones like BAP alone and in combination with NAA, CH, Kn, IAA were used as suggested by D optimal RSM. In present investigation, different explants like leaf, petiole, node, internode and shoot tip of female genotype, RDMSG-2 of spine gourd were used for callusing and regeneration studies. The parameters studied were callus, shoot initiation, multiplication, rooting and hardening. The results obtained in the various experiments conducted for these parameters have been summarized below.

For the callusing studies, different explants like leaf, petiole and internodes were subjected to various combinations of media supplements on the MS medium. Well matured explants of spine gourd (Genotype RDMSG-2) were taken from field and sterilized with mercuric chloride and double distilled water and were cultured on the MS media supplemented with various levels of PGRs. Among all the treatments under study, leaf explants gave the earliest callus initiation within 6.0 days at 1.0 and 2.0 mgl^{-1} NAA. The highest amount of callus growth was recorded on MS media supplemented with 2.0 mgl^{-1} and 4.0 mgl^{-1} NAA. The white fragile callus was observed in the treatments except 2.0 mgl^{-1} TDZ + 1.5 mgl^{-1} BAP (brown fragile callus) and 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kinetin (pink fragile callus).

Depending on the callusing studies, nodal and shoot tip explants were used for further regeneration studies using RSM. As regards nodal explants, the earliest shoot initiation was observed within 3.0 days in the treatment combinations of 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn, 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kn and 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. The maximum number of shoots per explant (1.9) and maximum length of shoot (8.4 cm) was observed in the MS media supplemented with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA and 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} IAA respectively. In case of shoot tip explants tried, the minimum number of days required for shoot initiation (2.9) were observed in treatment combination 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn. The maximum number of shoots (2.8) per explant were obtained in MS media fortified with 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA, whereas maximum length of shoot (7.35 cm) was observed on the media supplemented with 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA.

The most desirable and statistically proven results obtained from the various experiments conducted for optimization of shoot initiation protocol were subjected to multiple levels of analysis by giving numerical optimization command for expected goals of optimization of best levels of plant growth regulators as a media supplement. The comparison between

combinations predicted by RSM and actual combinations tried for nodal as well as shoot tip explants of spine gourd revealed no significant differences between them. The optimized combination 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn observed earliest shoot initiation (3.3 days) in nodal explants. The maximum number of shoots (1.7) per explant were observed in nodal explants placed on MS media fortified with 1.8 mgl^{-1} BAP + 0.5 mgl^{-1} Kn while maximum length of shoot (7.5 cm) was obtained on media with optimized supplementation of 0.46 mgl^{-1} BAP + 0.1 mgl^{-1}IAA. As regards shoot tip explants, the MS media supplemented with optimized combination of 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn recorded earliest shoot initiation (2.9 days). The maximum number of shoots per explant (2.5) were observed in treatment 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA. Whereas, the maximum length of shoot (6.6 cm) was obtained on MS media fortified with optimized combination of 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn.

The levels of the media supplements optimized by using RSM in each experiment are further used for multiplication of nodal and shoot tip explants. The MS media supplemented with optimized combination of 0.46 mgl^{-1} BAP + 0.10 mgl^{-1} IAA registered maximum plants (41.0) with maximum number of shoots per explant (2.12) and had maximum length shoots (8.56 cm) in the regenerated nodal explant. As regards shoot tip explants, the maximum plants (44.0) were regenerated on MS media supplemented with optimized combination of 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. The maximum number of shoots (2.7) per explant were obtained on 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA and the maximum length of shoot (7.2 cm) was observed in MS media optimized with 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn.

The well regenerated nodal and shoot tip explants using optimized levels of media supplements were inoculated on various concentrations of rooting medium as generated by RSM. The nodal explants responded within 18.0 days for root initiation at 1.0 mgl^{-1} NAA, whereas average maximum number of roots (8.6) and length of roots (2.5 cm) per regenerated nodal explants were observed on MS media supplemented with 2.0 mgl^{-1} IBA. In case of regenerated shoot tip explants, the earliest root initiation (9.0 days) and average maximum number of roots (13.9) were observed in MS medium supplemented with 1.0 mgl^{-1} NAA. The maximum average length of roots (2.7 cm) was registered in the MS medium fortified with 2.0 mgl^{-1} IBA.

Healthy and well rooted plants regenerated using nodal as well as shoot tip explants were transplanted in cocopeat and soil (1:1) media for hardening. The plants regenerated from the nodal explants had shown 67.85 per cent survival rate within 20 days, whereas plants regenerated from shoot tip explants recorded 80.64% survival rate within 18 days.

CONCLUSION

1 The three explants *viz.* leaf, petiole and internode were used for callusing studies. The leaf explant showed good response for early callus initiation on 1.0 and 2.0 mgl^{-1} NAA, as well as high amount of callus. The white fragile callus was observed in the treatments except 2.0 mgl^{-1} TDZ

+ 1.5 mgl^{-1} BAP (brown fragile callus). and 1.5 mgl^{-1} BAP + 1.0 mgl^{-1} Kinetin (pink fragile callus).

2 As regards the shoot initiation in nodal explants, the media supplement treatments 1.5 mgl^{-1} BAP + 0.5 mgl^{-1} Kn and 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn were found best as they recorded earliest shoot initiation. For the maximum number of shoots per nodal explants, media treatment with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA was observed best. The maximum shoot length in the nodal explants was recorded using media supplement combination of 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} IAA. In case of shoot tip explants, the treatment of media supplement 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn was found best for earlier shoot initiation, while treatment with combination of 2.5 mgl^{-1} BAP + 0.5 mgl^{-1} NAA had maximum number of shoots per explant and media fortification with 0.25 mgl^{-1} BAP + 0.1 mgl^{-1} IAA was observed best for the shoot length.

3 As regards the optimization of media supplements for shoot initiation protocol of nodal explant of spine gourd, the optimized combination of 2.0 mgl^{-1} BAP + 1.0 mgl^{-1} Kn could be used for earlier shoot initiation, the treatment 1.8 mgl^{-1} BAP + 0.5 mgl^{-1} Kn was considered best for giving maximum number of shoots per explant while optimized combination, 0.46 mgl^{-1} BAP + 0.1 mgl^{-1}IAA gave maximum shoot length. As regards shoot tip explants, it can be concluded that for the earlier shoot initiation, the optimized treatment of media supplement 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn can be used. However, for getting the maximum number of shoots per explant, the optimized treatment 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA could be used for commercialization while, for the maximum shoot length, optimized media supplement treatment 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn was found best.

4 For the multiplication of regenerated nodal explants the optimized treatment of media supplement 0.46 mgl^{-1} BAP + 0.10 mgl^{-1} IAA was found best as it gave maximum number of plants, with maximum number of shoots per explant and maximum length of shoots. As regards shoot tip explants, media optimized with 1.0 mgl^{-1} BAP + 0.1 mgl^{-1} IAA gave maximum number of plants from the regenerated shoots. However, treatment 1.7 mgl^{-1} BAP + 0.5 mgl^{-1} NAA produced maximum number of shoots per shoot tip explant and optimized treatment combination of 2.5 mgl^{-1} BAP + 1.1 mgl^{-1} Kn can be used for maximum length of shoots in regenerated shoot tip explants.

5 The best rooting in multiplied nodal explants was achieved by using the media supplement 1.0 mgl^{-1} NAA for earliest root initiation while media can be supplemented with 2.0 mgl^{-1} IBA for maximum number of lengthy roots per regenerated shoots. In case of shoot tip regenerated shoots, early rooting with maximum number of roots was achieved by using 1.0 mgl^{-1} NAA. However, for maximum length roots, 2.0 mgl^{-1} IBA could be successfully used for shoot tip regenerated plants.

6 Healthy and well rooted plants regenerated using nodal as well as shoot tip explants were transplanted in cocopeat and soil (1:1) media for hardening. The plants regenerated from the nodal

explants had shown 67.85 per cent survival rate within 20 days, whereas plants regenerated from shoot tip explants recorded 80.64% survival rate within 18 days.

7 Among the tried explants for regeneration, the shoot tip explants were found the best as they gave earliest shoot and root initiation with maximum number of long shoots as well as roots per explant. Also, maximum number of plants were multiplied from the shoot tip explants on the optimized media.

Future line of research work

Plant tissue culture technology is widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques in recent years, become a major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Spine gourd is one of the cucurbits which is having great demand in present days because of its tremendous nutraceutical values. Conventional propagation method restricted its large-scale commercial multiplication and domestication. Micropropagation techniques in Spine gourd are already standardized but there is a need of focused research on optimization of protocols for embryo culture, somatic embryogenesis, organogenesis, genetic transformation, protoplast fusion, haploid production and rooting studies etc. The judicious choice of explant source coupled with some refinements in media composition, may increase the success rate of micropropagation. Improvement in regeneration ability and acclimatization in *ex-vitro* condition are crucial for maximum exploitation of this medicinally important plant for germplasm conservation. When more emphasis is paid to these research areas the future commercial *in-vitro* micropropagation of *Momordica dioica* will be revolutionized.

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* Literatures not seen

VITAE

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MASTER OF SCIENCE (HORTICULTURE)

IN

VEGETABLE SCIENCE

2019

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