STUDIES ON TISSUE CULTURE IN GERBERA (*Gerbera jamesonii* L.)

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MASTER OF SCIENCE IN HORTICULTURE

(FLORICULTURE AND LANDSCAPE ARCHITECTURE)

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(*Gerbera jamesonii* L.)

BY

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July, 2016

CERTIFICATE

Ms. T. NAVYA SWETHA has satisfactorily prosecuted the course of research and that the thesis entitled **"STUDIES ON TISSUE CULTURE IN GERBERA (*Gerbera jamesonii* L.)"** submitted, is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination.

I certify that neither the thesis nor its part thereof has been previously submitted by him for a degree of any University.

Date : **(Dr. A. GIRWANI)**

Place: Rajendranagar, Hyderabad. **Chairperson**

CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON TISSUE CULTURE IN GERBERA (*Gerbera jamesonii* L.)''** submitted in partial fulfillment of the requirements for the degree of Master of Science in Horticulture (Floriculture and Landscaping architecture) of SRI KONDA LAXMAN TELANGANA STATE HORTICULTURAL UNIVERSITY, RAJENDRANAGAR, HYDERABAD - 500 030 is a record of the bonafide research work carried out by **Ms. T. NAVYA SWETHA** under our guidance and supervision.

No part of the thesis has been submitted by the student for any other degree or diploma. The published part and all assistance received during the course of the investigations have been duly acknowledged by the author of the thesis.

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Date :

Place : Hyderabad ***(T.NAVYA SWETHA)***

DECLARATION

I, **Ms. TEKULA NAVYA SWETHA** hereby declare that the thesis entitled **"STUDIES ON TISSUE CULTURE GERBERA (*Gerbera jamesonii* L.)"** submitted to the S.K.L Telangana State Horticultural University, Rajendranagar, Hyderabad for the degree of Master of Science in Horticulture (Floriculture and Landscape Architecture) is the result of original research work done by me. I declare that no material contained in the thesis has been published earlier in any manner.

Date: **Name:** Tekula Navya Swetha

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LIST OF SYMBOLS AND ABBREVIATIONS

BA	6-Benzyl adenine
BAP	6-Benzyl purine adenine
IAA	3-Indole acetic acid
IBA	3-Indole butyric acid
NAA	α-Napthalene acetic acid
HCL	Hydrochloric acid
$Hgcl_2$	Mercuric acid
MS	Murashige and Skoog
µg	Microgram
CD (P = 0.05%)	Critical Difference at 5 per cent levl
cm	Centimeter
CRD	Completely randomized design
KCL	Potassium chloride
TDZ	Thidiazuron
NaOH	Sodium hydroxide
$Ca(ClO_2)$	Calcium hypochlorite
2,4-D	2,4- Dichorophenoxy aectic acid
Cvs	Cultivars
Kg	Kilogram
RH	Relative humidity
Inch	Inches
Ml	Milli litre
$K_2Cr_2O_7$	Potassium dichromate

KN	Kinetin
U.V	Ultra violet
2,4-D	2,4- Dichlorophenoxy acetic acid
FYM	Farm Yard Manure
dia	Diameter
H_2SO_4	Sulphuric acid
L	Litre
EDTA	Ethylene diamine tetra acetic acid
et al.	and others
pH	Potential of Hydrogen
mg	Milligram
mm	Millimeter
N	Normality
C:N	Carbon nitrogen
ABA	Abscisic acid
GA_3	Gibberilic acid
^oC	Degree Celsius
µ mol/ µ M	Micromoles
ppm	Parts per million
SE±m	Standard error of mean
v/v	volume/volume
w/v	Weight/volume

Chapter-I

Introduction

Chapter I

INTRODUCTION

Gerbera (*Gerbera jamesonii* L.) commonly known as Transvaal Daisy, Barberton Daisy or African Daisy belongs to the family Asteraceae, is an important commercial flower grown throughout the world in a wide range of climatic conditions. Gerbera is one of the leading cut flowers and ranks among the top ten cut flowers of the world (Pathasarathy and Nagaraju, 1999).

The plant is reported to be widely distributed in South Africa, Europe, Asia and Indonesia. In India, it is distributed from Kashmir to Kanyakumari, growing best at altitudes ranging between 1300 and 3200 meters above mean sea level. Assam is having the largest area under cultivation (600 ha) and Uttarakhand being the major cut flower producer (7.2 lakhs) (Ministry of Agriculture, Govt. of India, 2013-14). The genus *Gerbera* was named in the honor of a German naturalist, Traugott Gerber. It is considered to be native to South African and Asiatic regions. The genus *Gerbera* consists of about 40 species comprising half-hardy and perennial flowering plants. Among the different species, *Gerbera jamesonii* is the only species under cultivation.

Gerbera is generally propagated by division of suckers or clumps. Propagation through seeds is not preferred as the plants exhibit heterozygosity, non-uniformity and also, the improved semi-double and double cultivars do not set seeds. Propagation by division of suckers or clumps gives true to type plants, but the multiplication rate is very low. Many new varieties are being introduced every year. To popularize these varieties and also to meet the demand for quality planting material of elite varieties, there is a need to develop technology for rapid multiplication. This could be accomplished through micro propagation and is the preferred method.

Tissue culture finds its tremendous application in ornamental crops, especially in areas of propagation and crop improvement. Plant propagation either through meristem or non-meristem culture enables production of large number of plants within a short span of time in limited space. Propagation through meristem has an added advantage of virus elimination.

In vitro multiplication has been defined in three steps, initiating culture, increasing propagules and preparation of soil transfer (Murashige, 1974). These steps have been widely adopted by both research and commercial tissue culture laboratories because they not only described procedural steps in the micro propagation process but also usually represent points at which cultural envinorments need to be changed (George, 1993).

Two major developments which made shoot culture feasible were the development of improved media for plant tissue culture (Murashige and Skoog, 1962) and discovery of the cytokinins as a class of growth regulators (Miller, 1961) and Skoog *et al.* (1965) with an ability to release lateral buds from dormancy (Sachs and Thimann, 1964). These developments were not immediately applied to shoot culture and some years elapsed before it was appreciated that multiple shoots could be induced to form by appropriate growth regulator treatments (George, 1993).

Micropropagation work in Gerbera was initiated by shoot tip culture for rapid multiplication of elite varieties as early as 1974 by Murashige *et al.* and in 1985 by Huang and Chu.

The capitulum explants are also being used for micro propagation with the advantage of easier sterile isolation *in vitro* and non-destructive nature as only inflorescences are used and no shoots are lost from the plant (Pierik *et al* 1982). The results of clonal propagation *in vitro* using either capitulum explants or subcultured shoots depended on the cultivar and auxins and cytokinin concentration in the medium. With capitulum explants shoot formation of some cultivars are very low and almost independent of the BA.

Keeping in view of all the above facts, the investigation was planned to optimize the culture conditions for micropropagation of Gerbera using capitulum as an explant, the present study on Tissue culture in Gerbera is proposed with following objectives.

1. To study the response of capitulum explant for culture establishment at different concentrations of BAP and BAP in combination with NAA and IAA.

2. To standardize the optimum concentration of BAP in combination with NAA for achieving high frequency of multiple shoot regeneration under *in vitro* conditions.

3. To standardize the optimum concentration of auxins (NAA and IBA) for better rooting of shoots under *in vitro* conditions.

4. To standardize a suitable hardening medium for acclimatization of well rooted plantlets.

Chapter-II

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

The available and relevant literature pertaining to the present investigation entitled “STUDIES ON TISSUE CULTURE IN GERBERA (*Gerbera jamesonii*L.)” has been reviewed. The literature related to present study on gerbera and on related crops also have been reviewed and presented in this chapter under the following headings.

The success of plant tissue culture as a means of plant propagation depends on multiplication and establishment of plantlets which in turn are greatly influenced by number of factors.

2.1 Factors influencing the multiplication of plantlets

2.2 Factors affecting establishment of plantlets

2.1 FACTORS INFLUENCING MULTIPLICATION OF PLANTLETS

2.1.1 Explant

The type of explant, its size, position, age, physiological state and the manner in which it is cultured can all determine, whether the cultures could be successfully maintained and the morphogenesis could be induced. Shoot tip, flower bud, capitulum, leaf blade, peduncle, petiole are the commonly used explants for *in vitro* gerbera multiplication.

Pierik and Segers (1973) reported that when gerbera inflorescence was cut into six instead of four explants, the total number of shoots per flower was the same, but it decreased when the inflorescence was cut into eight, ten or tweleve explants. A slightly higher number of shoots per explant was obtained from the inflorescence collected in the month of June and July compared to those of other months. They concluded that there was a very small probability that these shoots were of

adventitious origin. They insisted that shoot primordia in a quiescent state existed in the axils of the involucral bracts.

Working on the explant sources for stem segement culture (Miyazaki and Tashiro 1978) reported that explants from young plants (9 week old) produced adventitious shoots more readily than those from older plants (19 weeks) in chrysanthemum cultivar Kayo – no – Sakura. Segements from different internodes responded differently to the concentrations of applied growth regulator.

Sreelatha *et al.* (1998) compared the effect of basal and apical portions of leaf explants on callus initiation in anthurium species. They noticed that callus initiation was better in the basal portions of leaf explants. The difference in response between the basal and apical portions was attributed to the difference in the physiological state as well as the number of cells undergoing de-differentiation. Physiological changes may account for the changes in the contents of endogenous hormones, nutrients and metabolites.

Babu and Chawla (2000) used corm slices of gladiolus and obtained better shoot induction (89%) from top slice of cormel (dia. 1.0 to 1.5 cm) with an average of 2.4 shoots per explant in response to MS medium supplemented with KIN (4 mg/L) rather than BAP. Regarding bottom section of cormels, most of the cultures exhibited mortality where the physiological base of the bottom section was on the nutrient medium and the cut surface on upper side.

Zhao *et al.* (2002) reported that the contamination rate lowered greatly when the receptacle was used as explant for gerbera. Maximum per cent contamination was observed in shoot tip and flower (fully developed) explants.

Martin *et al*. (2003) cultured brown and green lamina explants of anthurium cultivars Senator and Tinora Red . Results found that the explants from brown young lamina of Senator and Tinora Red showed 60% and 70% callus formation and green lamina explants showed 40% and 45% callus formation respectively.

Nhut *et al*. (2004) depicted highest shoot formation from basal plates (30 mm diameter) as compared to shoot tip (5 mm in length), longitudinal corm section (20 mm in width) and daughter corm (15-20 mm diameter) in the medium containing BA (1 mg/L) in gladiolus .

Kumar and Kanwar (2006) studied on regeneration ability of petiole, leaf and petal explants in gerbera and reported that maximum callus induction and growth in petal explant on MS medium supplemented with 1, 1.5 and 2 mg/L 2,4-D in petiole and in leaf with 1.5 and 2 mg l/L 2,4-D. On other hand, petiole explants did not respond to any of treatments tired.

Ali *et al.* (2008) stated that apical meristems showed more pronounced effect for shoot formation than nodal meristems, and reported that best response of shoot formation after 6 days inoculation from apical meristem and after 7 days inoculation from nodal meristem on MS medium medium supplemented with 4.0 mg/L BAP in carnation.

Akter *et al.* (2012) handled various explants of gerbera, namely flower bud, flower stalk, leaf tip, leaf with midrib, leaf blade, petiole for callus induction and *in vitro* development of plantlets. Among the different explants, flower bud of 7-9 old exhibited the best response towards callus formation , shoot regeneration and subsequent development of plantlets.

Shylaja *et al.* (2014) reported that age of flower buds (judged by size) in gerbera was found to be crucial factor for culture establishment and shoot regeneration, the optimum being unopened flower buds with basal diameter of 1.0-1.2 cm, the bigger and smaller buds were not giving response with respect to culture establishment and shoot regeneration.

2.1.2 Surface sterilization of explants

Sterilization of explants is an essential requirement in order to improve the success of micropropagation.

For establishment of plants in culture media, nodal segements (1.0 – 1.5 long), internodal segements (1.0 – 1.5 cm long) and shoot tips (0.5 cm long) of chrysanthemum were used as explants. These explants were washed with running water for 15 minutes and then with 5 per cent aqueous solution of liquid soap, teepol (Bhattacharya *et al.* 1990). The explants were then repeatedly washed with single distilled water and disinfected with 90 percent ethanol for 10 seconds and subsequently surface sterilized with 0.1 per cent mercuric chloride for 2 minutes followed by repeated rinsing with sterile double distilled water.

Singh and Arora (1994) excised shoot tips (5 mm size) from the field grown plants of *Chrysanthemum morifolium* cv. 'Riot'. These were rinsed with teepol (0.5%) for 10 minutes and then thoroughly washed with running water for 30 minutes. They were surface sterilized with mercuric chloride (0.1%) for 2 minutes, washed thoroughly with sterile distilled water then transferred into culture tubes.

The shoot tips of carnation were washed under running tap water for 1 - 1 ½ hours and dipped in 70 per cent alcohol for 45 seconds, they were then disinfected with 0.2 per cent $HgCl_2$ along with two drops of Tween-20 for ten minutes and thoroughly rinsed with sterile distilled water for 3 - 4 times (Jagannatha, 1997).

Kaur *et al.* (1999) treated the shoot buds of gerbera with bavistin (0.2%) for 7 – 8 minutes and then washed thrice with sterile water for 5 minutes followed by treatment with $Hgcl_2$ (0.1%) for 2 – 3 minutes and washed thrice with sterile water for 5 minutes.

The explants of gerbera, size of 5 mm x 5 mm cut transversely to the midrib from mature leaves, were washed thoroughly with water for 5 minutes, disinfected with 70 per cent ethanol for 30 seconds and surface sterilized with filtered sodium hypochlorite (0.1%). After 10 minutes, they were rinsed five times with sterilized distilled water, blotted on sterile filter paper and 15 explants were plated with adoxial surface in contact with the medium (25 ml) in 100 mm petri dishes (Aswath and Choudhary, 2002b).

Mohanty *et al.* (2005) sterilized shoot tips of gerbera explants with 0.1 per cent $Hgcl_2$ for different time durations and then gave one minute dip in 1 per cent KCL to remove Hg^{+2} ions from the surface of the explants. Finally the expalnts were washed thrice with autoclaved distilled water before inoculation.

Srivastava and Sharma (2005) primarily treated leaf segements and petioles of gerbera with carbendazim (0.2%) for 30 minutes and surface sterilized with mercuric chloride (0.1%) for 10 minutes followed by 3-4 times washing with sterile distilled water.

Nhut *et al.* (2007) reported that receptacles (1.5 - 2.0 cm in diameter) of 10 interspecific crosses of young gerbera flowers (7 - 14 days old) were washed thoroughly under tap water for 20 minutes, soaked in My Hao detergent (Vietnam) for 15 minutes, then washed thoroughly under tap water again for 2 hours, rinsed six times with distilled water and then submerged in a 7% (w/v) solution of $Ca(ClO_2)$ for 25 minutes followed by rinsing six times in sterile distilled water in the laminar air flow cabinet.

Hasbullah *et al.* (2008) surface sterilized gerbera seeds, first soaked in distilled water for 30 minutes with addition of 1-2 drops of tween-20 followed by 40 per cent volume by volume (v/v) sodium chloride solution and gently agitated. The seeds were rinsed three times with distilled water and then soaked in 70 per cent volume by volume (v/v) alcohol for 1 minute. Finally, the seeds were rinsed 3 times with sterile distilled water.

Warar *et al.* (2008) surface sterilized yellow flowering variety ‘Sciella’ of gerbera using capitulum as explant with carbendazim (0.1%) for five minutes and after that explants were disinfected with 0.1% per cent $Hgcl_2$ for five minutes. Thereafter, the explants were thoroughly rinsed with sterile distilled water (4-5 minutes).

Cimen atak and Ozge celik (2009) minimize the contamination caused by fungus, endogen and exogen bacteria for which he has taken leaf explants of

anthurium and surface sterilized for 1 minute in 70% (v/v) ethanol and soaked in gentamicin solution for 30 minutes then soaked in 20% (v/v) commercial bleach (commercial bleach contains about 5% (v/v) sodium hypochloride) for 12 minutes. Leaves were rinsed three times in sterile distilled water. Sterile leaf explants were sectioned to about $1cm^2$ pieces.

Shabanpour *et al.* (2011) sterilized capitulum explants (0.5 to 1 cm in diameter) of two gerbera cultivars, by washing under tap water for 20 min and soaked in 1.5% (w/v) $Ca(CIO)_2$ solution for 10 min, then submerged in 0.1% (w/v) solution of $Hgcl_2$ for 10 min and rinsed 3 times in sterile distilled water. Sterilized capitulum were cut into 4 to 6 pieces and cultured on regeneration medium in 100 ml jars.

Son *et al*. (2011) washed the flower bud of gerbera under running tap water with few drops of Teepol for 15 - 20 minutes then immersed in a solution of carbendazim (0.1%) for 5 minutes, later washed thoroughly with distilled water. They were surface sterilized in 0.1% $HgCl_2$ for 7 minutes and finally washed with double glass distilled water for 3 - 4 times to remove any traces of $HgCl_2$ in laminar air flow cabinet.

Nalini (2012) thoroughly washed shoot tips of chrysanthemum using 0.1 per cent teepol followed by washing with sterilized distilled water thrice. The explants were then surface sterilized with 70 per cent ethanol for three minutes, followed by washing with sterilized distilled water, thrice. The outer leaves in each explants were removed and inner most shoot tip, measuring 0.5 to 1.0 cm in length, having 1 or 2 leaf primordial were excised.

Mohamed and Ozzambak (2014) immersed the shoot tips of gerbera in a 10% (v/v) Clorox (1% sodium hypochlorite) to which 1-2 drops of tween 80 were added for 10 min and rinsed three times with sterile distilled water and washed flower stem explants of gerbera thoroughly with tap-water, then soaked in a 70% (v/v) ethanol for a few seconds, then immersed in a 20% (v/v) Clorox (2% sodium

hypochlorite) to which 1-2 drops tween 80 were added for 30 min and rinsed three times with distilled water.

The excised flower buds of gerbera were washed in running tap water and pretreated with 0.1% percent Bavistin and one drop of detergent for 10 minutes followed by washing in sterile distilled water. Further sterilization with 0.1% $Hgcl_2$ for 7 minutes was carried out in Laminar Air Flow Chamber followed by washing thrice in sterile distilled water Shylaja *et al.* (2014).

2.1.3 Culture establishment

Pierik *et al.* (1975) reported that no shoots developed from excised capitulum explants of gerbera on cytokinin free media. The addition of BA or BAP in higher concentration was very essential for culture establishment, the optimum being 10 and 5 mg/L respectively. BAP was found to be slightly more effective than BA. They felt auxin was not essential for shoot formation, although the addition of a low auxin concentration (IAA at 0.1 mg/L or IBA at 0.05 mg l/L) was slightly more stimulative in comparison to no auxin.

Cussio and Bussi (1982) reported that MS medium supplemented with 0.5-1.0 mg/L BA and 0.1 mg/L IAA gave good initial proliferation from nodal cuttings of actively growing shoots of chrysanthemum cultivars, Yellow Galaxy, Turner and Aglow.

Pierik *et al.* (1982) studied effects of cytokinin on shoot formation of *Gerbera jamesonii in vitro* using capitulum as explants. The influence of cytokinin concentrations was examined by comparing 15 cultivars on media with 5, 10 and 20 mg/L BA. The concentration of 10 mg/L BA was found to be the best for 9 cultivars out of 15 cultivars reacted optimally reacted at this concentration.

Dabin and Choiseg (1983) obtained callus formation, when axillary bud explants of chrysanthemum cultivar White Spider was culture on MS medium. However, shoot could be obtained on further subculturing. Direct shoot regeneration

was obtained on MS medium supplemented with Kinetin at 2.5 µM/L in combination with GA_3 at 6 µM/L.

Laliberte *et al.* (1985) carried out an experiment on *in vitro* plantlet production from 'Mardi Grass'capitulum explants of gerbera and reported that explant responded best to MS medium supplemented with 1.0 mg/L BAP and 0.1 mg/L IAA. However, the vigour and number of shoots decreased when the concentration of BAP was raised to 2.0 – 3.0 mg/L.

Kaul *et al*. (1990) regenerated adventitious shoots from leaf and stem explants of 11 chrysanthemum cultivars in MS basal medium supplemented with 5 µM BAP and 5µM NAA. They also noted that stem explants were superior to leaf explants and there were wide variations between cultivars in their shoot regeneration frequency.

Lu *et al.* (1990) obtained direct plant regeneration using fresh *Crysanthemum morifolium* cv. Royal purple stem segements cultured on MS basal medium supplemented with BA 0.5 to 2.0 mg/L in combination with NAA at 0.2 to 2.0 mg/L. However, highest percentage of shoot formation (100%) and greatest number of shoots per plant (14.6) were observed on stem segements taken from the tip of the cuttings.

Reynoird *et al.* (1993) reported the plant regeneration from *in vitro* leaf culture of several gerbera species. Efficient bud regeneration was obtained from a clone of gerbera hybrids leaf explants cultured on MS medium supplemented with 10 µM BA and 2.5 µM NAA. Addition of 0.05 µM or 0.5 µM TDZ to the above medium significantly promoted regeneration from mature leaves.

Barbosa *et al*. (1993) reported that proliferation was best on half strength Murashige and Skoog (MS) medium with BA at 2.27 milligrams per litre (mg/l) for *in vitro* propagation of *Gerbera jamesonii* cultivar 'Appelbloesem' using young capitulum as explants, in which MS medium solidified with 0.7 per cent agar and

supplemented with adenine (80 mg/l)) tyrosine (100 mg/L and different concentrations and combinations of BA with IAA, or IAA with 2, 4-D.

Azad Mandal *et al.* (1998) successfully regenerated plants in chrysanthemum from nodal explants in large flowered cultivars, 'Snow Ball' and 'Sonar Bangla'. Initial cultures were established on MS medium supplemented with NAA (0.2 mg/L) + Kinetin (2mg/L) for enhanced growth of axillary buds. Shoots from axillary bud were further multiplied on NAA (0.2 mg/L) and Kinetin (2.5 mg/L).

Palai *et al.* (1998) reported the efficient plant regeneration through callus culture of gerbera. Callus induction from leaf explants was achieved on MS medium supplemented with 1mg/L Kinetin + 2.5mg/L 2,4-D. The maximum number of shoots buds per callus was reported on MS medium supplemented containing 1 to 1.5mg/L BA + 0.2-0.5 mg/L NAA.

Modh *et al*. (2002) reported the highest proliferation rate of capitula explants of gerbera cultivar 'Atella' on Murashige and Skoog (MS) medium containing 10 milligrams per litre (mg/l) BAP.

Aswath and Survay (2004) studied effect of growth regulators on *in vitro* culture establishment, shoot regeneration and proliferation using shoot tip obtained from *ex vivo* plants of gerbera cultivar 'IIHR Selection GJ-23'. They found MS medium containing 3 mg/L BA and 3mg/L BA + 0.5 mg/L IAA best for *in vitro* culture establishment and shoot multiplication, respectively.

Mohanty *et al.* (2005) concluded that MS medium supplemented with BAP (1 ppm) was best for *in vitro* proliferation of gerbera cultivar 'Alsmeera' using shoot tip as explants. Maximum callus induction was achieved through amendment of 2,4-D (5 ppm) on MS medium from leaf disc during summer season, keeping in diffused light condition.

Priyakumari and Sheela (2005) established an efficient protocol in *Gladiolus grandiflours* L. cv. 'Peach Blossom' using intact cormels as explant and obtained

maximum shoot proliferation on MS medium fortified with BA 4mg/L + NAA 0.5 mg/L.

Surinder and Jitender *et al.* (2006) reported maximum callus induction and growth in petal explant on MS medium supplemented with 1, 1.5 and 2,4-D and in leaf with 1.5 and 2 mg/L 2,4-D for *Gerbera jamesoii*. The calli derived from leaf explant differentiated into roots with 1 mg/L NAA in the medium.

Nafees *et al.* (2009) studied on regeneration in callus of colchicine (0, 10, 20, 30, 40 mg/L) treated seeds of gerbera. The maximum seed germination, callusing and regeneration was observed in control seeds while these parameters decline with increasing doses of colchicine and finally no germination was obsereved at 40 mg/L pre-soaked seeds.

The flower bud of three varieties of gerbera was cut into 4 pieces and each of them was cultured separately on MS medium supplemented with various concentrations of BAP (3, 5 and 10 mg/L) in combination with IAA (0.1 mg/L) for culture establishment. Among the different levels of BAP tried, addition of 3 mg/L of BAP along with 0.1 mg/L IAA to MS medium resulted in more number of explants responding to culture establishment (Son *et al.* 2011).

Ghanati *et al.* (2012) developed an efficient procedure for the development of callus and shoot formation from different explants especially leaf explants of *Eustoma grandiflorum*. MS medium containing 14.4 µM IAA, 0.465 µM Kn, 22.05 µM NAA was the best medium reported for the induction of callus.

Shagufta *et al.* (2012) studied on shoot induction, multiplication of induced shoot and their rooting in gerbera. Best culture establishment and shoot induction was obtained after 8 days of inoculation from apical meristem and after 12 days of inoculation from vegetative buds on MS medium supplemented with 10 mg/L BAP and 8.8 µM BAP + 2.87 µM IAA respectively. Maximum number of multiple shoots was obtained on MS medium containing 10 mg/L BAP.

Bhargava *et al.* (2013) studied on effect of different concentrations of BA (1-5mg/L), kinetin (1-5 mg/L) in combination with IAA (0.5 mg/L) respectively in capitulum explant of gerbera and observed that medium containing 4 mg/L BA + 0.5 mg/L IAA showed better potential for intial culture establishment.

Rahman *et al.* (2013) achieved *in vitro* propagation of 'Red Gerbera' by culturing flower bud, leaf segements and flower stalk segements on MS medium supplemented with different concentration (1.0-6.0 mg/L) of BA in combination with single concentration of NAA (1.0 mg/L). Lower concentration of BA with NAA induced the explants to form callus. On the other hand, higher concentration of BA (5.0) produced shoots and 5.0 mg/L BA with 1.0 mg/L NAA was to be best for shoot proliferation of the three explants optimum response was obtained from flower buds.

Kadu (2013) studied the effect of different concentration of cytokinin and auxin on establishment of *in vitro* culture using axillary bud as explant in gerbera and reported that optimum concentration of cytokinin and auxin for culture initiation was on MS medium supplemented with BAP 2.0 + NAA 0.5 mg/l, which gave early bud initiation, with higher initiation per cent along with longer shoot length.

Mohamed and Ozzambak (2014) studied the effect of different media on the mean number of capitulum explants forming shoots in the gerbera cultivars Ameretto, Red Bull, Ruby Red and Yanara after 9 weeks of culture and reported that cultivar Ameretto resulted in higher number of explants forming shoots in the liquid medium P4c (10 mg/L BA and supported with cotton).

Prasad (2014) studied the response of various kind of growth regulators such as IAA, 6-BAP, Kinetin, 2,4 D on callus induction, shoot initiation, shoot multiplication and formation of roots using gerbera seeds. Callus were obtained from leaf and flower stalk explants cultured on the MS medium supplemented with 1.5 mg/L 2,4D. Shoot regeneration and multiplication was observed in MS medium which was supplemented with 0.3 mg/L IAA.

Shylaja *et al.* (2014) inoculated each segement of flower bud in MS medium supplemented with BAP – NAA and BAP – IAA combinations for culture establishment in gerbera and found that MS medium supplemented with BAP 3 mg/L + NAA 0.1 mg/L resulted better for culture establishment.

2.1.4 Shoot multiplication

Gertsson and Anderson (1985) worked on apical meristem and petiole explants in chrysanthemum cultivars 'Pincamino', 'Prince Anne', 'Super Yellow'and 'Spider'. Multiple shoots were produced in all the cultivars except from petiole explants of cv, Princess Anne when cultured on MS medium supplemented with BA at 1mg/L in combination with IAA at 0.1mg/L.

Cockrel *et al.* (1986) studied the *in vitro* propagation of florists Senecio cruentus cv. Hanso from shoot tips derived from selected seedlings germinated in *in vitro* shoot multiplication was best on MS Zerbera medium supplemented with 2.8 µM IAA.

Sangwan *et al.* (1987) worked on shoot tip explant of *Chrysanthemum morifolium* and reported that the culture medium supplemented with NAA at 0.2 mg/L in combination with Kinetin at 2mg/L was optimum for multiple shooting.

Parthasarathy and Nagaraju (1995) confirmed that low concentration of BAP (0.75 - 1.0 mg/L) is sufficient for obtaining more number of better sized shoots in 'Gold Dust' (17.83) and 'Popular' (14.6) cultivars of gerbera. Though maximum number of shoots was recorded at 2.0 mg/L BAP, it was not significantly different from that obtained with 1.0 mg/L BAP.

Brar *et al.* (1996) studied the effect of Thidiazuron and Benzylaminopurine on multiple shoot proliferation from axillary buds in different cultivars of carnation. Number of multiple shoots produced was influenced by the type and concentration of cytokinin. Barlo IINora cultivar of carnation produced the highest shoot number on MS medium supplemented with 8.8 µM BAP.

Aswath and Chaudhary (2001) studied the effect of cytokinins on proliferation of multiple shoots of gerbera cultivar 'AV 101' by culturing shoot tip as explants and obtained highest rate of shoot proliferation on MS medium supplemented with 1.5 mg/L BAP.

Ray *et al.* (2005) developed an efficient protocol for large scale propagation using young capitulum as explant in gerbera on a medium containing 7 mg/L BA and 0.1 mg/L IAA, which initiated multiple shoots (10 per explants) when placed in continuous dark after 30 days in culture.

Hussein *et al.* (2008) evaluated two different shoot formation media containing 2 mg/L NAA and different concentrations of BAP (2.0 and 4 mg/L) for gerbera. The highest shoot regeneration frequency of 36.6% was obtained on induction medium containing 2 mg/L BAP and 0.25 mg/L ABA and shoot formation medium containing 2 mg/L each of BAP and NAA. Regenerated shoots were successfully rooted on MS medium containing 40 µg/L NAA.

Warar *et al.* (2008) studied the effect of cytikinins and auxins on proliferation of multiple shoots in gerbera variety 'Sciella' using capitulum as explant and reported that the Murashige and Skoog (MS) medium supplemented with 3.0 mg/L BAP + 0.5 mg/L IAA was effective for induction of more number of shoots.

Shabanpour *et al.* (2011) evaluated the organogenic response of orange and pink cultivars of gerbera under *in vitro* culture using capitulum as explants. For proliferation, regenerated shoots were subcultured in medium supplemented with 0.2, 0.4 and 0.6 mg/L of thidiazuron (TDZ) and 2, 4 and 6 mg/L of N^6 – benzyladenine (BA). Maximum shoot induction, (88% and 44.4 % for orange and pink cultivars, respectively) with 4mg/L BA and 0.1mg/L IAA.

Son *et al.* (2011) studied on shoot multiplication, using MS medium supplemented with different concentrations of BAP (1, 2 and 3 mg/L) in combination with NAA (0.1 mg/L) using flower buds in gerbera. Among the different concentrations of BAP in combination with NAA used, the MS medium

supplemented with 3 mg/L BAP and NAA 0.1 mg/L showed significant superiority compared to others, because it recorded less number of days for shoot intiation; also this treatment gave highest number of shoots and shoot fresh and dry weight.

Waseem *et al.* (2011) carried out an experiment to achieve shoot multiplication of chrysanthemum (*Dendranthema morifolium* L.) through shoot tip culture using different plant growth regulators and obtained maximum shoot initiation, shoots per explant, average length of shoots, number of leaves per plant, number of nodes per shoot on MS medium supplemented with NAA (0.5 and 1.0mg/L) and BAP (1.0 and 1.5mg/L).

Akter *et al.* (2012) studied the effect of subculturing MS medium with different concentrations of BA using flower bud and flower stalk as explant for multiple shoot regeneration in Red variety of gerbera and obtained highest number of multiple shoots, shoot length on MS medium supplemented with 2.0 mg/L.

Mishra *et al.* (2014) studied the influence of some phytohormones based culture medium on *in vitro* multiplication of gerbera using nodal stem segements and capitulum as explants and reported that MS medium supplemented with BAP 2.0 mg/l + IAA 1.0 mg/l gave the highest culture regeneration, number of multiple shoots, number of leaves per plant, shoot length and took least days to bud break in both shoot bud and capitulum bud culture.

Nazari *et al.* (2014) investigated the effects of different concentrations of BA (1, 2, and 3 mg/L) solely and or in combinations with (NAA) (0.2 and 0.4 mg/L) on the induction, multiplication and proliferation of shoots in *Gerbera gamesonii* Bolus cv. Royal Soft Pink using shoot tip as explants which were germinated aseptically on MS basal medium and reported highest number of shoots after two sub cultures with 2 mg/L BA and 0.2 mg/L NAA (10 shoots per explant).

2.2 FACTORS AFFECTING ESTABLISHMENT OF PLANTLETS

2.2.1 Rooting

Pierik and Sprenkel (1984) studied the improvement of the rooting of gerbera *in vitro* by propagating shoot tips in amended MS medium containing either IAA at 3-10 mg/L or NAA at 1 or 3 mg/L.They found 100 per cent rooting and maximum roots with NAA (3 mg/L).

Highest root multiplication rates (9.5 - 11.2) were obtained on modified MS medium supplemented with 1 mg/L BAP, independently of the IAA concentration used in a study on *in vitro* propagation of gerbera cultivar 'Applebloesem' using three-leaf-bud explants (Barbosa *et al.*, 1993).

The effect of auxins (NAA or 2,4-D, each at 0.5, 1, 2 and 5 mg/L) on root formation was studied by Aswath and Choudhary (2002a) of *in vitro* shoots in gerbera obtained from shoot tip explant. The number and length of roots were best in half MS medium supplemented with 0.5 mg/L 2,4-D and 0.5 mg/ L NAA and the root formation rate was 100 per cent.

Ali *et al.* (2008) obtained best response for rooting in carnation by culturing *in vitro* shoots of apical and nodal meristems explant on MS medium supplemented with 1.0 mg/L of NAA. By increasing the concentration of NAA, root induction response was decreased and very poor results were obtained at 2.5 mg/L NAA.

Asit *et al.* (2010) attempted to induce and optimize rooting of *in vitro* shoots in gerbera of rhizome explant on MS basal supplemented with 12 different concentrations and combinations of IBA, IAA, NAA and $AgNO_3$. Cent percent rooting of the shootlets and maximum number of roots/plant were observed on MS supplemented with 0.5 mg/l NAA. Addition of 10 µM $AgNO_3$ in combination with 0.5 mg/L NAA and IBA, respectively, showed development of fewer roots but displayed excellent plant health.

Son *et al.* (2011) separated individual shoots of gerbera from a multiple shoot complex obtained from flower bud explant and cultured on MS medium containing various level of NAA (0.5, 1 and 2 mg/L) and reported that per cent rooting, root intiation and number of roots were best in medium supplemented with 2 mg/L NAA.

Shagufta *et al.* (2012) shifted the gerbera shoots of apical meristem and vegetative bud explant in to the MS medium supplemented with NAA ranging from 1.0 to 10 mg/L. The best response for rooting was obtained with 10 mg/L NAA. By decreasing the concentration of NAA, root induction response was decreased and very poor results were reported in 1.0 mg/L of NAA.

Bhargava *et al.* (2013) separated micro-shoots of gerbera capitulum explant from the multiplication medium and transferred individual shoots on different concentrations of auxins, reported that earliest root induction with maximum rooting, longest roots, more number of roots with good root growth were recorded in 2 mg/L IBA.

Kabir *et al.* (2014) concluded that gladiolus shoots of corm explant rooted well when they are excised individually and implanted on half – strength MS medium supplemented with 2.5 mg/L IBA, which resulted 90% root induction after culture of 30 days. The average number of roots per shoot and average root length was observed in this medium.

2.2.2 Hardening medium

The micropropagated plantlets should be hardened before transferring them to open conditions. The medium for hardening should have good water holding capacity, drainage and aeration.

Agar was carefully washed from the regenerated plantlets with well-established root system before transfer to pots (15 cm) filled with a mixture of coco peat and compost (1:1, v/v). Gerbera Plantlets were maintained under high relative humidity (90%) for 3 weeks. Acclimatized plants were kept under a natural photoperiod condition at a temperature of 25 ± 2^0 C. Survival rate of plantlets was almost 100 per cent when plantlets after root development were transferred to plastic pots filled with coco peat, red soil, and sand in a 3:1:1 ratio (Aswath and Choudhary, 2002b).

The regenerated gerbera plantlets were transferred to poly-bags containing soil : sand : FYM (1:1:1) mixture and kept under high humidity for 3 days. The humidity was gradually reduced to normal by using polythene covers up to 7 days. They were further allowed to grow for 4 weeks and transferred to field (Modh *et al.* 2002).

Nga *et al.* (2005) investigated the effect of hardening media, *viz.* soil, sand, rice husk, humus, soil + humus (1:1, v/v), sand + rice husk (1:1, v/v) and humus + rice husk (1:1, v/v) on survival percentage and root growth of gerbera plantlets. The best plant vigour and maximum survival of plants (93.25%) was observed in humus + rice husk (1:1, v/v) mixture followed by sand medium.

Agar-based medium was removed from the roots of plantlets (approximately 25 mm in length) by washing them under running tap water. The plantlets were transferred to plastic pots containing a mixture of sand and compost (2:1 ratio) and maintained in a growth room (18 ± 2^0C, 16 hours photoperiod with 25 μmol m^{-2} s^{-1} illumination). Well-established plants of carnation were transferred into pots containing garden soil after 4 weeks for further acclimatization in greenhouse where they were kept for 2 weeks (Karami *et al.* 2006).

Negi *et al.* (2006) reported that the rooted plants of gerbera were taken out from the containers and washed thoroughly with tap water to remove adhering agar. These plantlets were dipped in 0.1% carbendazim solution for two minutes and transplanted in tray pots containing sterilized peat moss. The potted plants were kept inside a small glasshouse and watered regularly with sprinkler for a week. Further, they were watered twice a day with the help of a water cane. A temperature of 26 ± 2 ^{0}C and humidity of 85 - 90% were maintained inside the glasshouse. These plants were hardened well within a period of 12 - 14 days.

Ali *et al.* (2008) hardened the well rooted *in vitro* plantlets of carantion in glass house after two weeks of culturing. Best response for hardening was obtained

in mixture containing sand + peat + soil (1:1:1) at 95% humidity level under natural light conditions.

Kashyap and Dhiman (2011) carried out an experiment on effect of media on hardening of *in vitro* multiplied plantlets of gloxinia and saintpaulia under low cost polytunnels using the combinations of cocopeat, perlite, soil and sand, reported that maximum plant height and more number of leaves in cocopeat + perlite (3:1).

Kharrazi *et al.* (2011) surveyed on different concentrations of NAA and IBA on root induction of regenerated *in vitro* shoots of carnation using shoot explants and reported highest root percentage for Eskimo Mogr (83%) on MS medium supplemented with 0.5 mg/L NAA, while for Innove Orange Bogr (98%) on MS medium supplemented with 1.5 mg/L IBA.

Bhargava *et al.* (2013) transferred the rooted plants of gerbera to different hardening media namely coco peat, cocopeat: perlite (1:1, v/v) and cocopeat: perlite: vermicompost (1:1:1, v/v) and reported maximum survival of plantlets (90.00%) and newly formed leaves after 4 weeks was observed in cocopeat, whereas, only 80.00% of plants survived when hardening was done in coco peat, perlite and vermi-compost mixture.

Kadu (2013) used various carriers substrate on acclimatization of micropropagated plants of gerbera and reported higher survival rate, longer plants, more number of leaves/ plant with more number of roots/plant with Sand + Soil + FYM + leaf mould as combination.

Kabir *et al.* (2014) transferred healthy rooted plantlets of gladiolus to the earthen pot containing a mixture of soil and compost (2:1) and covered with transparent polyethylene lid to maintain high humidity. After 1-week, the polyethylene lids were removed, and plantlets were kept in shade net and misted twice a day. About 80% of the plantlets were resumed new growth within 30 days. A total number of 40 plantlets were survived in the field out of 50 *in vitro* regenerants.

Muna (2016) carried out an experiment on standardization of media mixture for hardening of *in vitro* plantlets of *Dendrobium* cv. Sonia-17 and obtained enhanced formation of new leaves/plantlet, number of roots/plantlets, length of the root, percentage of survival of the plantlets and maximum plant height using Cocopeat + 3 pieces of coconut husk as hardening media.

Chapter- III

Materials & Methods

Chapter III

MATERIAL AND METHODS

A lab experiment "**STUDIES ON TISSUE CULTURE IN GERBERA" (*Gerbera jamesonii)*** was conducted during the year 2015-2016. The details of material and methods used and the experimental techniques adopted during the course of investigation are described below.

3.1 Location of experimental site

The experiment was carried out at the "AGRI BIOTECH FOUNDATION" ANGRAU Campus, Rajendranagar, Hyderabad.

3.2 Plant Material

One of the promising variety of Gerbera named Savannah was used for the present study. The plant material required for the study was collected from farmers field located at Gollapally, Shamshabad mandal. Varietal characters of the savannah are presented below

Character:

Type of the flower - single

Length of stalk (cm) - 65 cm / 25.59 inch

Diameter of flower (cm) - 11 cm- 13 cm

Flower colour

a) Ray florets - Red
b) Disc florets - black

3.3 Explant and their preparation

Capitulum explants of Gerbera were collected at immature stage (1.0 – 1.5 cm) diameter and subjected to the sterilization process. After sterilization, the involucral bracts were removed and segemented into two equal halves under laminar

air flow chamber. The explants were subsequently placed on solid medium under aseptic conditions.

3.4 Culture vessels

Generally, vessels used to culture plant material include borosilicate or pyrex glasswares. The glassware used for experimental work included conical flasks (100ml, 150ml, 250ml, 500ml and 1,000ml), culture bottles (8 × 3 inches), graduated measuring cylinders (100ml and 1,000ml), beakers and a range of pipettes (1ml, 2ml, 5ml and 10ml). Glassware was thoroughly cleaned before use. It was first cleaned with detergent and water and then with hot chromic acid ($K_2Cr_2O_7$ + H_2SO_4 + H_2O) followed by thorough rinsing with tap water. All glassware was then steam sterilized.

3.5 Nutrient media

Murashige and Skoog (1962) (MS) medium was used in the present study.
The Inorganic and Organic constituents of Murashige and Skoog (MS) medium basal medium are listed below.

Sl. No.	Constituents	Amount(mg/L)
I	**Macro nutrients**	
1	Ammonium nitrate	**1650**
2	Potassium nitrate	**1900**
3	Magnesium sulphate	**370**
4	Potassium phosphate	**170**
5	Calcium chloride	**440**
II	**Micro nutrients**	
A	Boric acid	**6.2**
	Cobalt chloride	**0.025**
	Potassium iodate	**0.83**
	Sodium molybdate	**0.25**
B	Manganese sulphate	**22.3**
	Zinc sulphate	**8.6**
	Copper sulphate	**0.025**
C	Sodium EDTA	**37.3**
	Iron sulphate	**27.8**

D	Glycine	**2.0**
	Pyridoxine-HCl	**0.5**
	Nicotinic	**0.5**
	Thiamine-HCl	**0.1**
	Meso- inositol	**100**
	Sucrose	**30000**
	Agar	**8000**

Salts providing micro nutrients were prepared as stock solutions and the salts providing macronutrients were weighed afresh as and when the medium was

prepared. These salts were dissolved in distilled water in proportions required to reach the desired concentrations.

3.5.1 Preparation of stock solutions for micronutrients

(i) Stock solution A:

0.496g Boric acid (H_3BO_3), 0.00664g Potassium iodate (KI), 0.02g Cobalt chloride ($COCl_2$. $2H_2O$), Sodium molybdate (0.0125) were weighed and dissolved in 50ml double distilled water. The solution was then gently heated to dissolve the contents. The final volume was then made upto 200ml with double distilled water.

(ii) Stock solution B:

1.784g Manganese sulphate ($MnSO_4$. $4H_2O$), 0.688g Zinc sulphate ($ZnSO_4$. $7H_2O$) and 0.002g Copper sulphate ($CuSO_4$. $5H_2O$) were weighed and dissolved in 50ml double distilled water. The final volume was made upto 200ml by adding distilled water.

(iii) Stock solution C:

2.984g Ethylene Diamine Tetra Acetic acid disodium salt (Na_2EDTA) and 2.224g Ferrous sulphate ($Feso_4$. $4H_2O$) were weighed and added to 50ml double distilled water separately in two beakers and boiled. To the boiling Na_2EDTA solution was added slowly. The final volume of the solution was made upto 200ml with boiled distilled water.

(iv) Stock solution D:

0.008g Thimiane HCL, 0.04g Nicotinic acid, 0.04g Pyridoxine HCL and 0.016g Glycine were weighed and dissolved in about 100ml double distilled water and then the solution was made upto a final volume of 200ml.

The stock solution of the micronutrients thus prepared were emptied into amber coloured bottles and stored in a refrigerator at 4^0c. None of the stock solutions were stored for more than 15 days.

3.5.2 Preparation of stock solution of (BAP)

The stock solution of N^6 benzyl aminopurine (BAP) was prepared by dissolving 10 mg in small quantity of 1 N NaoH and volume was made to 200 ml by adding sterile distilled water. The required volume of stock solutions were added to the media as per the treatments.

3.5.3 Preparation of stock solution of auxin (NAA)

The stock solution of α-Napthalene acetic acid ($C_2H_{10}0_2$) was prepared by dissolving 10 mg in a small quantity of ethanol and the volume was made upto 200 ml with sterile distilled water. The required quantities were pipetted out to the media as per the treatments.

3.5.4 Preparation of stock solution of (IBA)

The stock solution of IBA (3-indole butyric acid) was prepared by dissolving 10 mg in few drops of ethanol / methanol and the volume was made upto 200 ml by adding sterile distilled water. The required volume of stock solution was added to media as per treatments.

3.5.5 Preparation of stock solution of (IAA)

The stock solution of Indole 3- acetic acid was prepared by dissolving 10 mg in a small quantity of ethanol and the volume was made upto 200 ml with sterile distilled water. The required quantities were pipette out to the media as per the treatments.

3.5.6 Steps followed in preparation of the medium

1. The macronutrients and Calcium chloride which prepared as concentrated stock solutions (A and C) as described earlier and the required final concentrations were arrived at as per Table 1.

2. The micronutrients which prepared as concentrated stock solutions (B) as described earlier and the required final concentrarions were arrived at as Table 1.

3. Sucrose and mtoinositol were added finally.

4. Growth hormones namely (BAP, NAA, IAA and IBA) were prepared as stock solutions and the final concentrations were arrived at as per requirement.

5. The solution was made upto the final volume by adding double distilled water.

6. pH of the medium was adjusted to 5.8 with 0.1N NaoH or 0.1N HCL.

7. Agar-agar at 8g/L were added finally and boiled for 15 minutes.

8. About 50ml of the medium was dispensed in each culture bottles and closed tightly with caps

9. The culture bottles containing medium were autoclaved for about 45 minutes at a temperature of 121^0 C and a pressure of 1.06 Kg/cm^2 for sterilization.

10. The culture bottles containing the medium were stored in incubation room until further use.

3.5.7 Autoclaving

The jar bottles with nutrient media were autoclaved at a pressure of 1.06 kg cm^{-2} (121 ^{0}C) for 20 minutes. The jar bottles were then removed from the autoclave and allowed to cool. They were kept in the culture room at temperature of 25 ± 2^0C. The inoculation was done after 4 - 5 days ensuring that the glass bottles were free from contaminations.

3.6 Experimental conditions

After inoculation of explants into media the bottles were closed with caps tightly and labelled. They were then kept in growth room at a temperature of 25 ± 2^0C and illuminated for 16 hrs of light at 3000 lux and 8 hour dark per day maintaining a relative humidity of 70 percent.

3.7 Culture establishment

3.7.1 Surface sterilization of explants

Explants were first washed thoroughly under running tap water followed by rinsing with distilled water in order to dust and extraneous materials. Then the material was rinsed in 0.1% tween-20 for 15 minutes and through washing with double distilled water for 5-6 times to remove the detergent and later soaked in 0.1% bavistin solution (fungicide) for 20 minutes and thereafter washed thoroughly with sterile distilled water for 2-3 times. The explants were transferred to the laminar air flow chamber and surface sterilized with 0.1% $Hgcl_2$ solution for about 7 minutes then finally rinsed with distilled water for 3-4 times to remove any traces of $Hgcl_2$.

3.7.2 Inoculation

The sterilized explants were inoculated in jar bottles containing the media (50 ml). The cut ends of explants were kept in such a way that they have maximum contact with the medium.

3.7.3 Transfer area and maintenance of aseptic conditions

Aseptic culture conditions like final surface sterilization, preparation and inoculation of explants and their subculture was carried out in the Laminar air flow cabinet. Before the use of Laminar air flow cabinet, the working area was swabbed with 100% ethyl alcohol. The U.V lamp was then switched on for 30 minutes along with laminar air flow. During the course of transfer and between successive transfers, the instruments used were dipped in 70% ethyl alcohol followed by flame sterilization or sterilized the instruments by placing them in steri-pot and cooled before use. After the inoculation the Laminar-air flow was cleaned with 70% ethyl alcohol and kept closed.

3.7.4 Subculture

Micro shoots formed in the jar bottles were taken out after five weeks of inoculation. The regenerated capitulum was separated by dissecting it into 2-4 equal halves under sterile environment of laminar air flow cabinet with sterile dissecting

needle and forceps. They were again placed in the jar bottles containing fresh media.

3.7.5 Rooting

The micro shoots of more than 1.0 cm in height were taken out after five weeks of inoculation and placed in the jar bottles containing media.

3.7.6 Hardening

Agar-based medium was removed from the rooted plantlets by washing them with distilled water. These plantlets were dipped in 0.1% carbendazim solution for about 2-3 minutes and transplanted in poly bags containing different hardening media. The potted plants were kept inside a small glasshouse and watered regularly with sprinkler for a week. Further, they were watered twice a day with the help of a water cane. A temperature of 26 ± 2^{0}C and humidity of 85 - 90% were maintained inside the glasshouse. Then they were transferred to greenhouse after two weeks for further acclimatization.

3.8 Hardening media

3.8.1 Sand

It consists of small rock grains of size ranging from 0.05 - 2.0 mm in diameter, formed as a result of weathering of various rocks. Its mineral composition depends upon the type of parent rock. Quartz sand, generally used for propagation purposes, consists chiefly of silica complex. Sand used in plastering is satisfactory for rooting of cuttings. Sand is the most widely acceptable rooting medium for cuttings; relatively inexpensive and readily available. It does not retain moisture unlike other media, hence needs frequent watering. It is enough to retain some moisture around the cutting, yet coarse enough to allow water to drain through it. It is used mostly in combination with organic material

3.8.2 Cocopeat

Coco peat is a coir fiber pith that is having coconut husk as its base. It is a soil conditioner and growing medium. Its uniqueness is that it can hold 8 times of water of its own weight. It releases nutrients in solution over long intervals. It is best for commercial and home gardening application. It provides breathing space, *i.e.* letting in and letting out of air for roots which helps better growth. Added to above mentioned characters, it encourages favorable micro organisms around the root zone. Since it is having slow degradation level it is universally accepted. It will re-wet easily without the use of chemical wetting as it is hydrophilic by nature. It can easily be mixed with other growing media.

3.8.3 Vermiculite

Vermiculite is common ingredient in growing medium. The origin of vermiculite is from mineral called mica. Mica is heated to 18000F, which causes it to expand like an accordion. Spaces made by the expansion result in good water holding ability and aeration. It is a light weight material and can be easily compressed. Vermiculite is available in different grades, ranging from a fine grade for use with seeds to a coarse grade measuring more than ¼ inch in diameter. Vermiculite has a good cation exchange capacity and a neutral to slightly alkaline pH (6.3 to 7.8). It also provides small amounts of calcium, magnesium, and potassium for plants use.

3.8.4 Vermicompost

Vermicompost is a nutrient-rich, microbiologically-active organic amendment that results from the interactions between earthworms and microorganisms during the breakdown of organic matter. It is a stabilized, finely divided peat-like material with a low C:N ratio, high porosity and high water-holding capacity, in which most nutrients are present in forms that are readily taken up by plants.

3.9 Details of experiment

The responded treatments in one experiment are subjected to the next experiment.

3.9.1 Experiment 1: Effect of different concentrations of BAP in combination with NAA and IAA on culture establishment

Name of the Crop : Gerbera

Botanical name : *Gerbera jamesonii*

Variety : 'Savannah'

Experimental design : CRD

Explant (E) : Capitulum

Number of treatments : 10

Number of replications : 3

Number of explants per each treatment :10

Treatments:-

T_1- Control (MS medium)

T_2 - MS medium + 1mg/L BAP

T_3 - MS medium + 3mg/L BAP

T_4 - MS medium + 5mg/L of BAP

T_5 - MS medium + 1mg/L of BAP + 0.1mg/L NAA

T_6- MS medium + 3mg/L of BAP + 0.1mg/L NAA

T_7 - MS medium + 5mg/L of BAP + 0.1mg/L NAA

T_8 - MS medium + 1mg/L of BAP + 0.1mg/L IAA

T_9- MS medium + 3mg/L of BAP + 0.1mg/L IAA

T_{10} - MS medium + 5mg/L of BAP + 0.1mg/L IAA

Observations:-

1. Number of explants responded
2. Number of days taken for primordial emergence

3.9.2 Experiment 2: Effect of different concentrations of BAP and NAA on shoot multiplication

Experimental design : CRD

Number of treatments : 7

Number of replications : 3

Number of explants per each treatment : 10

Treatments:-

T_1- control (MS medium)

T_2- MS medium + 1mg/L BAP+0.1 mg/L NAA

T_3- MS medium + 2mg/L BAP+0.1 mg/L NAA

T_4- MS medium + 3mg/L BAP+0.1 mg/L NAA

T_5- MS medium + 1mg/L BAP+0.5 mg/L NAA

T_6- MS medium + 2 mg/L BAP+0.5 mg/L NAA

T_7- MS medium + 3mg/L BAP+0.5 mg/L NAA

Observations:-

1. Number of days taken for shoot initiation
2. Number of shoots per culture
3. Shoot length(mm)

3.9.3 Experiment 3: Effect of different concentrations of NAA and IBA on rooting of *in vitro* shoots

Experimental design : CRD

Number of treatments : 7

Number of replications : 3

Number of shoots per each treatment : 10

Treatments:-

T_1- control (MS medium)

T_2- MS medium + 0.5 mg/L IBA

T_3- MS medium + 1.0 mg/L IBA

T_4- MS medium + 2.0 mg/L IBA

T_5- MS medium + 0.5 mg/L NAA

T_6- MS medium + 1.0 mg/L NAA

T_7- MS medium + 2.0 mg/L NAA

Observations:-

1. Number of days taken for root initiation
2. Rooting percentage (%)
3. Number of roots per shoot
4. Root length (mm)

3.9.4 Experiment 4: Standardization of hardening material for acclimatization of plantlets

Experimental design : CRD

Number of treatments : 8

Number of replications : 3

Number of plantlets per each treatment : 10

Hardening material:(M)

Treatments:-

T_1- Sand (control)

T_2- Cocopeat

T_3- Vermiculite

T_4- Vermicompost

T_5- Cocopeat + Vermiculite (1:1 v/v)

T_6- Cocopeat + Vermicompost (1:1 v/v)

T_7- Vermiculite + Vermicompost (1:1 v/v)

T_8- Cocopeat + Vermiculite + Vermicompost (1:1:1 v/v)

Observations:

1. Survival percentage of plantlets (%)
2. Number of leaves per plantlet
3. Plant height (mm)

LOCATION OF WORK: Agri-Biotech foundation, Rajendranagar, HYD

3.10 Collection of data

3.10.1 Number of explants responded

The number of explants responded out of total inoculated was counted and were expressed as mean

3.10.2 Number of days taken for primordial emergence

The number of days taken for emergence of shoot primordium from the date of inoculation of explants was recorded and was expressed as mean number of days.

3.10.3 Number of days taken for shoot initiation

The number of days taken for initiation of shoots was recorded and expressed as mean number of days.

3.10.4 Number of shoots per culture

The number of shoots produced per culture was counted after five weeks of inoculation and expressed as mean.

3.10.5 Shoot length (mm)

The shoot length was measured from base to the top of the shoot with scale during subculture and average was expressed in terms of millimeters.

3.10.6 Number of days taken for root initiation

The number of days taken for root initiation was counted after five weeks of inoculation and was expressed as mean number of days.

3.10.7 Rooting percentage (%)

The number of shoots rooted out of total inoculated was counted after five weeks of inoculation and expressed as in percentage.

3.10.8 Number of roots per shoot

The number of roots produced per shoot was counted after five weeks of inoculation and expressed as mean.

3.10.9 Root length (mm)

The length of the longest root from the collar region to the root tip was measured using a scale and expressed in millimeters.

3.10.10 Survival percentage of plantlets (%)

The number of plantlets survived out of total plantlets subjected to hardening was counted after five weeks and expressed as percentage.

3 10.11 Plant height (mm)

The plant height was measured from the base to top of the plantlet using a scale after five weeks of hardening and average was expressed in millimeters.

3.10.12 Number of leaves per plantlet

The number of leaves produced from single plantlet was counted after five weeks of hardening and expressed as mean.

3.11 Statistical analysis of data

The experiment data were subjected to arcsine or square root transformation where ever required. The data was subjected to analysis of variance test as suggested by Panse and Sukhatme (1967). Critical difference values were tabulated at one per cent level of probability wherever F test was found to be significant.

Chapter-IV

Results and Discussion

Chapter IV

RESULTS AND DISCUSSION

Improvement of Gerbera (*Gerbera jamesonii* L.*)* had been largely confined to conventional breeding and selection methods. Tissue culture offers advantage like virus elimination (Posnette and Jha, 1960), rapid multiplication of elite genotypes (Boxus, 1974), preservation of germplasm (Millin and Schlegel, 1976), year round production of planting material, overcoming seasonal barriers and for speedy international exchange of planting material.

Micropropagation work in gerbera was initiated by shoot tip culture for rapid multiplication of elite varieties as early as 1974 by Murashige *et al.* and in 1985 by Huang and Chu. The capitulum explants used for micro propagation as they are easier sterile isolation *in vitro* and non-destructive nature as only inflorescences are used and no shoots are lost from the plants (Pierik *et al* 1982). Appropriate tissue culture methods of regeneration have also to be developed if gene transfer through Agrobacterium is attempted to incorporate novel characters.

Although numerous reports of adventitious shoot regeneration from various explants of gerbera are available but in some cases, shoots are also produced from callus intermediary. But, shoots derived from callus may not help to retain clonal fidelity. Hence, in the present investigation, enhanced axillary shoot method was attempted taking one of the elite gerbera varieties namely ‘‘Savannah’’.

The micropropagation of gerbera from capitulum explants was done in five distinct phases namely

1. Disinfection of capitulum explant

2. *In vitro* establishment from cultured explants

3. Shoot multiplication from subcultured explants

4. Rooting of the *in vitro* produced shoots

5. Transferring the *in vitro* raised plantlets to polybags for acclimatization

These culture stages have specific requirements and the success of this technique depends on explant and specific hormonal combinations in the medium that have to be used.

Thus in the present investigation, an attempt has been made to determine the response of capitulum explants (1.0-1.5 cm dia) and hormonal treatments for micropropagation. To achieve this objective, four experiments were formulated and conducted to study on the capitulum explants of variety ‘‘Savannah’’ by culturing on Murashige and Skoog medium supplemented with different hormonal concentrations.

In the first experiment, ten treatments were formulated with MS basal medium (control), MS medium + BAP individually, MS medium + BAP in combination with IAA, MS medium + BAP in combination with NAA at different concentrations. The sterilized explants were inoculated in jar bottles containing MS media (50 ml) for culture establishment. The cut ends of explants were kept in such a way that they have maximum contact with the medium and observations were recorded on number of explants responded and number of days taken for primordial emergence.

In the second experiment, seven treatments were formulated with MS basal medium (control), MS medium + BAP in combination with NAA at different concentrations. Micro shoots formed in the jar bottles of first experiment were taken out after five weeks of inoculation. The regenerated capitulum was separated by dissecting it into 2-4 equal halves under sterile environment of laminar air flow cabinet with sterile dissecting needle and forceps. They were again placed in the jar bottles containing fresh media and observations were recorded on number of days taken for shoot initiation, number of shoots per culture and shoot length.

In the third experiment, seven treatments were formulated with MS basal medium (control), MS medium + IBA, MS medium + NAA at different concentrations. The micro shoots of more than 1.0 cm in height were taken out after five weeks of inoculation and placed in the jar bottles containing media and observations were recorded on number of days taken root initiation, rooting percentage, number of roots per shoot and root length.

In the fourth experiment, plantlets were studied for acclimatization in different hardening media and for this eight treatments were formulated with sand (control), cocopeat, vermiculite and vermicompost individually and in combinations. The rooted plants were washed with distilled water to remove agar media on plantlets and then dipped in 0.1% Bavistin for 2-3 minutes and transplanted in poly bags containing different hardening media. Further observations were recorded on survival percentage of plantlets, plant height and number of leaves per plantlet.

The results obtained in the present investigations entitled “Studies on Tissue culture in Gerbera” are presented and discussed under the following headings in this chapter.

EXPERIMENT-1: 4.1 Effect of different concentrations of BAP in combination with NAA and IAA on culture establishment

The data pertaining to culture eatablishment of ten treatments formulated with MS basal medium with different concentrations and combinations of BAP, IAA and NAA are depicted in table 4.1.1 to 4.1.2 while trends are depicted graphically in Fig 4.1.1 to 4.1.2.

4.1.1 Number of explants responded

The results described here illustrate the response of capitulum explants on different culture media for culture establishment in gerbera. The number of explants responded are recorded after five weeks of inoculation varied significantly among different treatments. The results (Table 4.1.1) revealed that among the ten

treatments, the explants cultured with MS medium supplemented with 3mg/L of BAP + 0.1mg/L IAA (T_9) has recorded significantly highest number of responded explants (6.00) followed by 5mg/L of BAP (T_4) which was on par with 5mg/L BAP + 0.1mg/L IAA (T_{10}) with 5.00 and 4.66 number of responded explants respectively. While no explants have responded in control (T_1) after five weeks of inoculation. It was also observed that responded explants in (T_3) MS medium + 3mg/L BAP with (4.00) was found to be on par with T_7- MS medium + 5mg/L BAP + 0.1mg/L NAA and T_8- MS medium + 1 mg/L BAP + 0.1mg/L IAA with 4.00 and 3.66 number of responded explants respectively.

In the present study, number of explants responded were maximum with optimum concentration of BAP 3 mg/L with 0.1 mg/L IAA might be due to the concentration and combination of auxins and cytokinins in the nutrient medium which was important factor to determine the successful plant regeneration. Cytokinins used in plant cell culture regulate cell division, stimulate axillary and adventitious shoot proliferation.

These results are in agreement with the report of Son *et al.* (2011) in flower bud of gerbera and in contrary to that of Pierik *et al.* (1975) who reported that addition of high concentration of BA is very essential for regeneration of shoots from capitulum of gerbera and optimum concentration is 10mg/L. While, Naz *et al.* (2012) reported that the vegetative buds of gerbera responded best on MS medium supplemented with (8.8 µM BAP + 2.87 µM IAA) i.e., 2mg/L BAP + 0.5 mg/L IAA. However, Laliberte *et al*. (1985) reported that 'Mardi Grass' capitulum explants of gerbera responded best to the MS medium supplemented with 1 mg/L BAP and 0.1 mg/L IAA. The vigour and number of shoots decreased when the concentration of BAP was raised to 2-3 mg/L.

4.1.2 Number of days taken for Primordial emergence

The results described here illustrate the potential of MS medium supplemented with plant regulators in influencing morphogenic response in

capitulum explant of gerbera. The number of days taken for primordial emergence (Table 4.1.2) after five weeks of inoculation varied significantly among different treatments and revealed that 3mg/L of BAP + 0.1mg/L IAA (T_9) has recorded significantly shortest time for primordial emergence (14.66 days) followed by 5mg/L of BAP (T_4) and 5mg/L BAP + 0.1mg/L IAA (T_{10}) with 17.66 and 21.00 days respectively. However, there was no primordial emergence was in T_1 control (MS medium). Number of days taken for primordial emergence on T_2 MS medium + 1mg/L BAP was found to be on par with T_6 MS medium + 3mg/L BAP + 0.1mg/L NAA with 31.33 days respectively. While, the results on (T_3)- MS medium + 3mg/L BAP (24.66 days) was found to be on par with (T_7)- MS medium + 5mg/L BAP + 0.1mg/L NAA and (T_8)- MS medium + 1 mg/L BAP + 0.1mg/L IAA with (24.66 days) and (26.00) days respectively.

In this study, primordial emergence from cultured explants was remarkably influenced by the type and concentration of growth regulator used. The number of days taken for primordial emergence was minimum with optimum concentration of BAP 3 mg/L with 0.1 mg/L IAA which might be due to the enhancement of shoot formation by removal of apical dominance in shoots and also due to supplemention with cytokinin in the media which promoted the shoot differentiation through protein and enzymatic activity. However, Naz *et al.,* (2012) reported earliest shoot induction on MS medium supplemented with (8.8 µM BAP + 2.87 µM IAA) i.e., 2mg/L BAP + 0.5 mg/L IAA using vegetative buds of gerbera.

EXPERIMENT-2: 4.2 Effect of different concentrations of BAP and NAA on shoot multiplication

The data pertaining on shoot multiplication in seven treatments formulated with MS basal medium with different concentrations and combinations of BAP and NAA are depicted in table 4.2.1 to 4.2.3 while trends are depicted graphically in Fig 4.2.1 to 4.2.3.

4.2.1 Number of days taken for shoot initiation

The results described here illustrate the potential of MS medium supplemented with plant regulators on shoot initiation in capitulum explant of gerbera. The various concentrations of BAP in combination with NAA exhibited significant difference among different treatments on number of days taken for initiation of shoots. The results (Table 4.2.1) revealed that early shoot intiation (10.66 days) was observed on MS medium supplemented with 3mg/L BAP + 0.5mg/L NAA (T_7) which was on par with MS medium + 2mg/L BAP + 0.5mg/L NAA (T_6) of 12.33 days followed by 3mg/L BAP + 0.1mg/L NAA (T_4) which recorded 14.66 days for shoot initiation . However, control (T_1) took maximum number of days (40 days) for shoot intiation. The results on number of days for shoot initiation in T_3- MS medium + 2mg/L BAP + 0.1mg/L NAA (17.66 days) was found to be on par with T_5- MS medium + 1mg/L BAP + 0.5mg/L NAA and T_2- MS medium + 1mg/L BAP + 0.1mg/L NAA with (17.66 days) and (20.33 days) respectively.

In the present study, the number of days taken for shoot initiation was decreased with increasing concentration of BAP and NAA. Auxin at 0.5 mg/L NAA is found to be beneficial in conjunction with high levels of cytokinin 3mg/L BAP on MS medium (T_7) which might be due to regulating growth and morphogenesis by removal of apical dominance at Stage II where shoot initiation took place at an early date.

The results are in conformity with that of Naz *et al.*, (2012) who obtained early shoot initiation (13 days) on MS medium with (8.68 µg/L BAP + 2.68 µg/L NAA) 2 mg/L BAP + 0.5 mg/L NAA using vegetative buds as explant in gerbera and contrary to that of Son *et al.*, (2011) who obtained early shoot intiation on MS medium with 3mg/L BAP + 0.1 mg/L NAA using flower bud as explant in gerbera.

4.2.2 Number of shoots per culture

The results described here illustrate the influence of MS medium supplemented with plant regulators on number of shoots per culture in capitulum explant of gerbera. The number of shoots produced after five weeks of inoculation differed significantly among the various treatments of BAP in combination with NAA. The results (Table 4.2.2) revealed that maximum shoots per culture (25.00) were produced on MS medium containing 2mg/L BAP + 0.5 mg/L NAA (T_6) followed by 1mg/L BAP + 0.5mg/L NAA (T_5) which was on par with 1mg/L BAP + 0.1mg/L NAA (T_2) with 21.00 and 18.33 shoots per culture respectively. However, control (T_1) has recorded minimum number (5) of shoots per culture. While, the results on number of shoots per culture on T_3- MS medium + 2mg/L BAP + 0.1mg/L NAA was found to be on par with T_4- MS medium + 3mg/L BAP + 0.1mg/L NAA with 11.66 and 10.33 shoots respectively.

In the present study, concentration of exogenous cytokinin appears to be the main factor affecting shoot multiplication. The number of shoots per culture was increased with optimum concentration of BAP (2 mg/L) in combination with higher concentration of 0.5 mg/L NAA (T_6) which might be due reduced apical meristem dominance which resulted in induction of both axillary and adventitious shoot formation from meristamatic explants of gerbera.

This results are in accordance with Naz *et al.* (2012) who obtained more number of shoots per culture at same concentration in gerbera using vegetative buds and also to that of Hasbullah *et al.* (2008) reported highest number of shoots from petiole explants cultured on MS medium supplemented with 2.0mg/L BAP + 0.5 mg/L NAA. While, Palai *et al.* (1998) obtained maximum number of shoots per callus on MS medium supplemented with 1 to 1.5mg/L BA + 0.2 to 0.5mg/L NAA using leaf as explants in gerbera. Son *et al.* (2011) obtained more number of shoots per culture at 3mg/L BAP + 0.1mg/L NAA using flower bud as explant in gerbera, Mishra *et al.* (2014) obtained more number of shoots per culture at 2.0mg/L BAP +

1.0 mg/L IAA using capitulum as explant in gerbera. Nazari *et al* (2014) reported more number of shoots per culture at 2 mg/L BA + 0.2mg/L NAA using shoot tips as explants obtained from gerbera seeds which were germinated under aseptic conditions on basal medium.

4.2.3 Shoot length (mm)

The results described here illustrate the potential of MS medium supplemented with plant regulators on shoot length in capitulum explant of gerbera. The results on shoot length (Table 4.2.3) after five weeks of inoculation differed significantly among different treatments and revealed that 1mg/L BAP + 0.5 mg/L NAA (T_5) has recorded significantly maximum shoot length (33.00 mm) followed by 2mg/L BAP + 0.5 mg/L NAA (T_6) with 26.66 mm shoot length. The results of shoot length on MS medium + 1 mg/L BAP + 0.1mg/L NAA (T_2) was found to be on par with T_7- MS medium + 3mg/L BAP + 0.5mg/L NAA with 23.00 mm and 21.33 mm shoot length respectively on capitulum explant respectively. Whereas, control (T_1) has recorded minimum shoot length (5.33 mm) in shoot multiplication media.

In the present study, MS supplemented with low concentration BAP and higher concentration of NAA on (T_5) 1 mg/L BAP + 0.5 mg/L NAA was found to be better for enhancement of shoot length due to fact that enhanced cell division, cell enlargement, increases plasticity of cell, promotes proteins synthesis coupled with higher apical dominance resulted in elongation of adventitious shoots.

These results are in conformity with that of Wassem *et al.* (2011) who obtained highest shoot length on MS medium supplemented with 1mg/L BAP + 0.5mg/L NAA and 1mg/L BAP using shoot tip as explant in chysanthemum. While, Warar *et al.* (2008) who reported maximum shoot length on MS medium supplemented with 3mg/L BAP + 0.5 mg/L IAA using capitulum as explant in gerbera and Rahman *et al.* (2013) obtained maximum shoot length on MS medium

supplemented with 2mg/L BAP using flower bud, flower stalk and leaf segments as explant in gerbera.

Experiment 3: 4.3 Effect of different concentrations of NAA and IBA on rooting of *in vitro* shoots

The data pertaining to rooting of seven treatments formulated with MS basal medium with different concentrations and combinations of IBA and NAA are depicted in table 4.3.1 to 4.3.4 while trends are depicted graphically in Fig 4.3.1 to 4.3.4.

4.3.1 Number of days taken for root initiation

The results described here illustrate the potential of MS medium supplemented with plant regulators on number of days for root initiation in capitulum explant of gerbera. The number of days taken for root intiation differed significantly among the various treatments after five weeks of inoculation of micro shoots into rooting media (Table 4.3.1) . Among seven treatments, early root initiation (19 days) was observed on MS medium supplemented with 2mg/L IBA (T_4) followed by 1 mg/L IBA (T_3) which was on par with 2 mg/L NAA (T_7) with 21.33 and 22.33 days respectively. However, the explants in control (T_1) took maximum number of days (68.33) for root intiation. It was observed that the results on T_2- MS medium + 0.5mg/L IBA for root initiation (23.33 days) was found to be on par with T_6- MS medium + 1.0mg/L NAA and T_5- MS medium + 0.5mg/L NAA with (24.00) and (25.66) days respectively.

Induction of roots at the base of *in vitro* grown shoots is essential and indispensable step to establish tissue culture derived plantlets in the soil. In the present study, MS medium supplemented with higher concentration of IBA at 2 mg/L (T_4) was found to be better for root initiation due to its stability and longer side chain which is more resistant to oxidation and initiate the cell division as a prelude to rooting at 3-4 orders of magnitude higher than other auxins. The results are in

agreement with Bhargava *et al.* (2013) who reported earlier root initiation on half strength MS medium supplemented with 2mg/L IBA using capitulum as explant in gerbera and Priyakumari and Sheela (2005) using cormels as explant in gladiolus on MS medium supplemented with 2mg/L IBA respectively.

4.3.2 Rooting percentage

The results described here illustrate the potential of MS medium supplemented with plant regulators on rooting percentage in capitulum explant of gerbera. The results on rooting percentage (Table 4.3.2) after five weeks of inoculation differed significantly among various treatments and revealed that 2mg/L of IBA (T_4) has recorded significantly maximum percentage (83.33) of rooting followed by 2mg/L NAA (T_7) and 1mg/L IBA (T_3) with 70% and 60.00% respectively. However, control (T_1) has recorded minimum percentage (6.66 %) in rooting media. While, the results on rooting percentage of the treatment T_2 - MS medium + 0.5mg/L IBA was found to be on par with T_6- MS medium + 1.0 mg/L NAA with (50%) of rooting respectively.

In the present study, MS medium supplemented with higher concentration 2mg/L IBA (T_4) found to be better for rooting percentage due to differentiation of phloem ray parenchyma cells into root primordial which exerts primary role in root formation by involving in successive and interdependent phases. It is clear from the results that rooting percentage showed the tendency to increase with increasing concentration of IBA.

This result is similar to that of Bhargava *et al.,* (2013) who obtained by using capitulum as explant in Gerbera on half stength MS medium supplemented with 2mg/L IBA and Kabir *et al.* (2014) obtained maximum percent of rooting at higher concentration of IBA (2.5mg/L BAP) . However, Aswath and Chaudhary (2001) reported maximum rooting percentage when micro shoots cultured were on MS medium containing 1.75mg/L IBA using shoot tip as explant in gerbera. While, Ray *et al.,* (2005) obtained hundred percent rooting on MS medium containing 1.5mg/L

IAA + 0.5mg/L IBA using capitulum as explant in gerbera and Rahman *et al.,* (2013) obtained maximum percentage of rooting at 0.3mg/L IBA using flower bud, leaf segements and flower stalk in gerbera

4.3.3 Number of roots per shoot

The results described here illustrate the potential of MS medium supplemented with plant regulators on number of roots per shoot in capitulum explant of gerbera. There was significant difference with regard to number of roots produced among treatments of IBA and NAA after five weeks of inoculation to rooting media (Table 4.3.3). Maximum number of roots (7.66) were produced in shoots cultured on MS medium supplemented 0.5mg/L IBA (T_2) followed by 1mg/L IBA (T_3) which was on par with 2 mg/L IBA (T_4) with 6.00 and 5.33 number of roots respectively. However, control (T_1) has recorded minimum no of roots (1.33) per shoot.

In the present study, MS medium supplemented with low concentration 0.5mg/L IBA (T_4) found to be optimum concentration for obtaining more number of roots per shoot due to translocation of IBA down the stem to roots which stimulates the overall development of the roots by inducing growth of per-exisisting roots, adventitious root formation and branching of roots. The reduced effect of NAA on the root number might be due to its more persistent than IBA remains present in the tissue and may block further development of root meristemoids.

This results are in conformity with the report of Kharrazi *et al.* (2011) who obtained maximum number of roots per shoot on MS medium supplemented with low concentration of IBA in carnation using shoot explants and Nhut *et al.* (2007) obtained maximum number of roots on half strength of MS medium supplemented with low concentration of IBA (0.2mg/L IBA) using transverse thin cell layer culture of receptacles in gerbera. Ray *et al.,* (2005) obtained maximum number of roots per shoot on MS medium containing 1.5mg/L IAA + 0.5mg/L IBA using capitulum as explant in gerbera. While, Aswath and Chaudhary (2001) reported

average number of root per shoot when cultured on MS medium containing 1.75 mg/L IBA using shoot tips as explant in gerbera.

4.3.4 Root length (mm)

The results described here illustrate the potential of MS medium supplemented with plant regulators on root length in capitulum explant of gerbera. The root length was recorded after five weeks of inoculation and differed significantly among various treatments. The results (Table 4.3.4) revealed that 2mg/L of IBA (T_4) has recorded significantly maximum root length (54.66 mm) followed by (T_7) 2mg/L of NAA with (46.00 mm) root length. While, the results on 1mg/L IBA (T_3) was found to be on par with T_6- MS medium + 1.0 mg/L NAA with 36.33 mm and 32.33 mm of root length respectively.However, control (T_1) has recorded minimum root length (7.66 mm) in rooting media.

It was observed that concentration of auxin strongly influenced the quality of the root system at the end of the rooting period. In the present study, MS medium supplemented with higher concentration 2 mg/L IBA (T_4) found to be better for obtaining maximum root length due to superior effects of IBA on root elongation such as its preferential uptake, transport, metabolization and subsequent gene activation and promotes root length by influencing the synthesis of enzymes concerned in cell enlargement.

The results were similar to that of Bhargava *et al.,* (2013) who reported maximum root length on half MS medium supplemented with 2mg/L IBA and Priyakumari and Sheela (2005) obtained maximum root length on MS medium supplemented with 2mg/L IBA. While, Rahman *et al.,* (2013) who obtained maximum root length on MS medium supplemented with 0.3 mg/L IBA using flower bud, leaf segments and flower stalk in gerbera. While, Nhut *et al.* (2007) obtained maximum root length on MS medium supplemented with 1.0 mg/L IBA using receptacle transverse thin cell layer culture in gerbera.

Experiment 4: 4.4 Standardization of hardening material for acclimatization of plantlets

Rooted plantlets were planted in eight different media to study their survival and growth. The data pertaining on acclimatization of eight treatments formulated with sand as control, cocopeat, vermiculite and verimicompost individually along with different combinations are depicted in table 4.4.1 to 4.4.3 while trends are depicted graphically in Fig 4.4.1 to 4.4.3.

4.4.1 Survival percentage of plantlets

The results described here illustrate the potential of hardening media on survival percentage of plantlets in capitulum explant of gerbera. The results on survival percentage of plantlets (Table 4.4.1) observed after five weeks of transferring rooted *in vitro* plantlets to hardening media differed significantly among the various treatments. Among, eight treatments, maximum survival percentage of plantlets (80.00%) was observed in media containing of (T_7) vermiculite + vermicompost (1:1, v/v) followed by (T_8) cocopeat + vermiculite + vermicompost (1:1, v/v) which was on par with (T_6) cocopeat + vermicompost (1:1:1, v/v) with 70.00% and 63.33% of survival respectively. However, control (T_1) sand has recorded minimum survival percentage of plantlets (22.33%) in sand media. It was observed that the results on T_5- cocopeat + vermiculite was found to be on par with T_3- vermiculite with 60.00% and 56.66% of survival respectively.

In the present study, use of (T_7) vermiculite + vermicompost (1:1, v/v) found to be ideal for obtaining maximum survival percentage of plants might be due to the higher evapotranspiration in vermiculite because of its higher hydraulic conductivity which indicates good water holding capacity, good aeration combined with maximum supplementation of nutrients by vermicompost and also by maintaing plantlets under high humidity (80 – 90%) for the first 10-15 days inside glass house.

While, Rahman *et al.* (2013) who obtained maximum survival percentage of plants using vermicompost + coir dust + Garden soil (2:1:1) in gerbera while, Kashyap and Dhiman (2011) using cocopeat + perlite (3:1) in gloxinia and saintpaulia and Kadu (2013) using sand + soil + FYM + leaf mould in gerbera.

4.4.2 Plant height (mm)

The results described here illustrate the potential of hardening media on plant height in capitulum explant of gerbera. The results on Plant height (Table 4.4.2) after five weeks of transferring rooted *in vitro* plantlets to hardening media differed significantly among various treatments and revealed that (T_7) vermiculite + vermicompost (1:1, v/v) has recorded significantly maximum plant height (47 mm) which was on par with (T_6) cocopeat + vermicompost (1:1, v/v) and (T_8) cocopeat + vermiculite + vermicompost (1:1:1) with a plant height of 43.33 mm respectively. While, the results on minimum plant height (29.33 mm) was recorded with sand as control (T_1) in hardening media. While, the results on T_2- cocopeat which was found be on par with T_5- cocopeat + Vermiculite with a plant height of 40.66 mm and 37.00 mm.

In the present study, use of (T_7) vermiculite + vermicompost (1:1, v/v) was found to be better for obtaining maximum plant height might de due to better aeration, porosity, pH and drainage, which provided suitable conditions for further growth and development. However, sand proved to be the poor medium on account of poor water holding capacity, which resulted in the media dry and of high bulk density causing the compaction of roots.

However, Kashyap and Dhiman (2011) reported maximum plant height using cocopeat + Perlite (3:1) in gloxinia and saintpaulia, Kadu (2013) using sand + soil + FYM + leaf mould in gerbera and Muna (2016) using cocopeat + 3 pieces of coconut husk in dendrobium cv. Sonia – 17.

4.4.3 Number of leaves per plantlet

The results described here illustrate the potential of hardening media on numer of leaves per plantlet in capitulum explant of gerbera. The (Table 4.4.3) revealed that there was no significant difference on number of leaves per plantlet among various treatments after five weeks of transferring rooted *in vitro* plantlets to hardening media. Maximum no of leaves per plantlet (7.33) was recorded in (T_6) cocopeat + vermicompost (1:1, v/v). While, minimum number of leaves per plantlet (5.66) was recorded in sand (T_1) used as hardening media.

In the present study, use of (T_6) cocopeat + vermicompost (1:1, v/v) was found to be better for obtaining maximum number of leaves per plantlet. While, Kashyap and Dhiman (2011) reported more number of leaves using cocopeat + perlite (3:1) in gloxinia and saintpaulia, Bhargava *et al.* (2013) obtained maximum number of leaves per plant using cocopeat in gerbera and Kadu (2013) using sand + soil + FYM + leaf mould in gerbera.

Plate 1: Explant and preparation

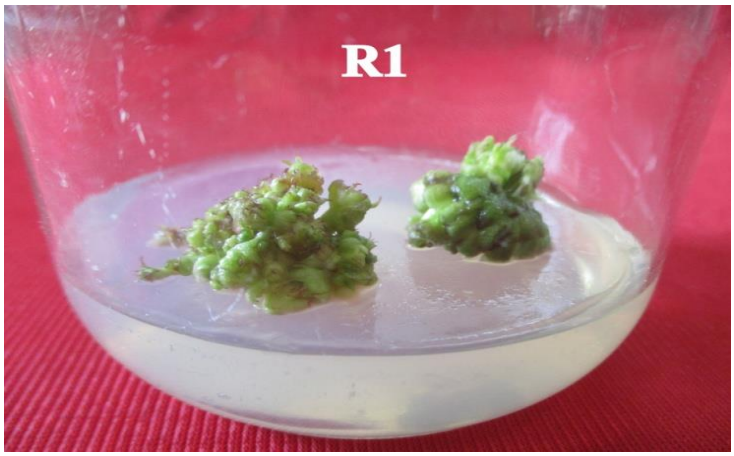
Plate 1.1: Collection of capitulum explants from field

Plate 1.2: Washing 4-5 times with sterile distilled water

Plate 1.3: Soaking of Capitulum explants in 0.1% bavistin solution (fungicide) for 20 minutes

Plate 1.4: Complete set up of explants before inoculation into media

Plate 2: Primordial emergence on different concentrations of Growth regulators



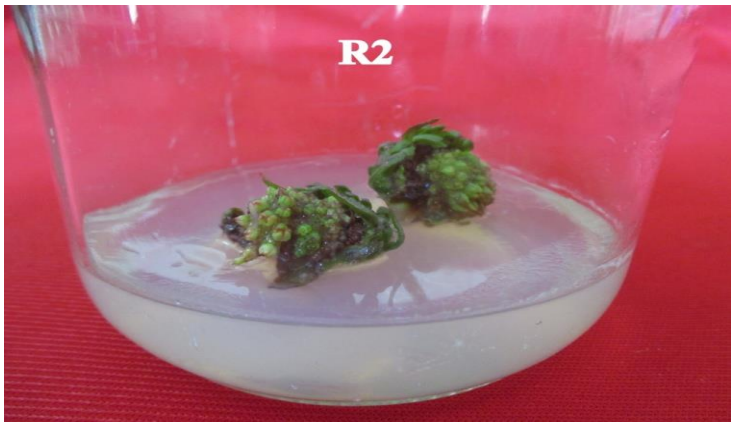


Plate 2.1: T_9 - MS medium + 3mg/L BAP + 0.1mg/L IAA

Plate 2.2: T_4- MS medium + 5mg/L BAP

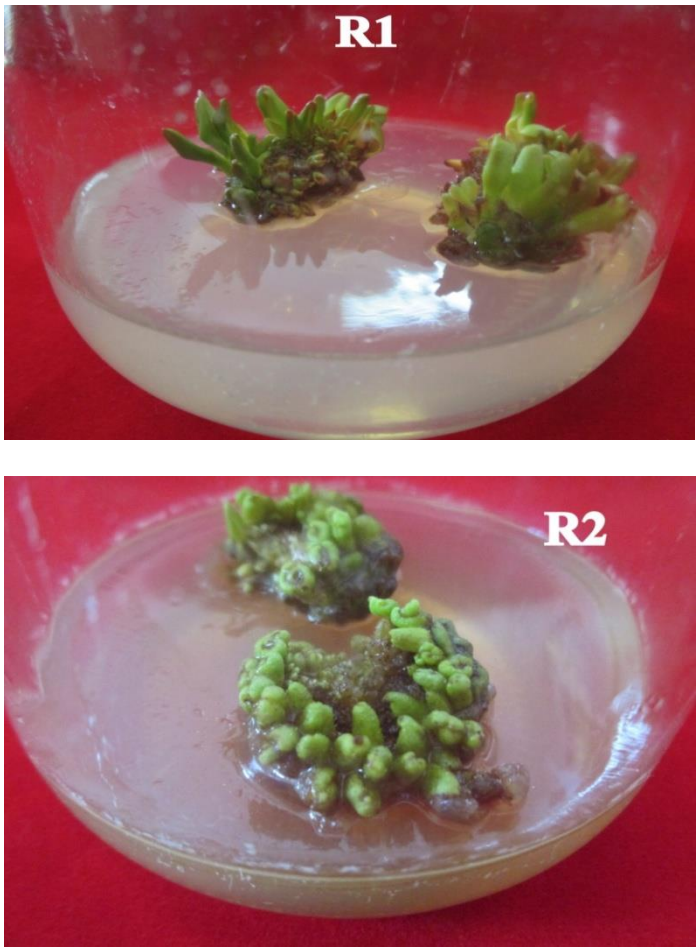
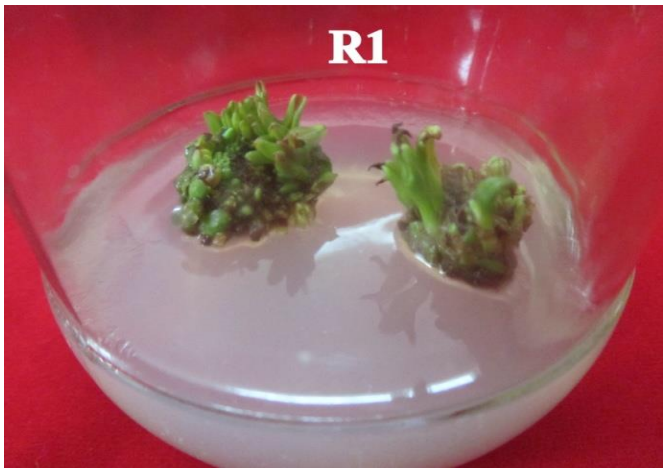


Plate 2.3: T_{10}- MS medium + 5mg/L BAP + 0.1mg/L IAA



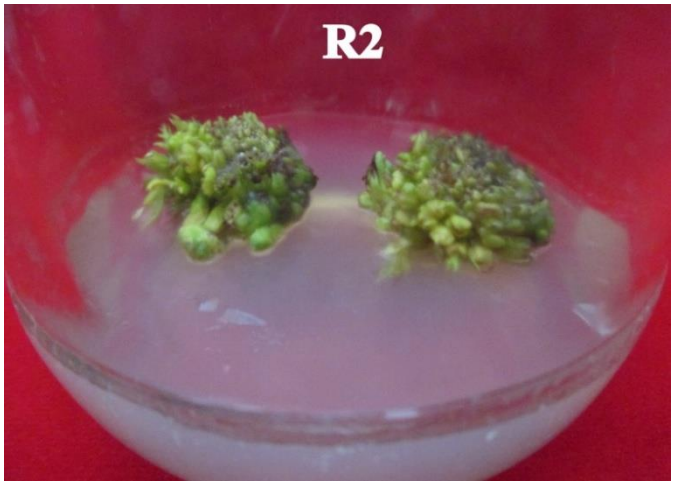
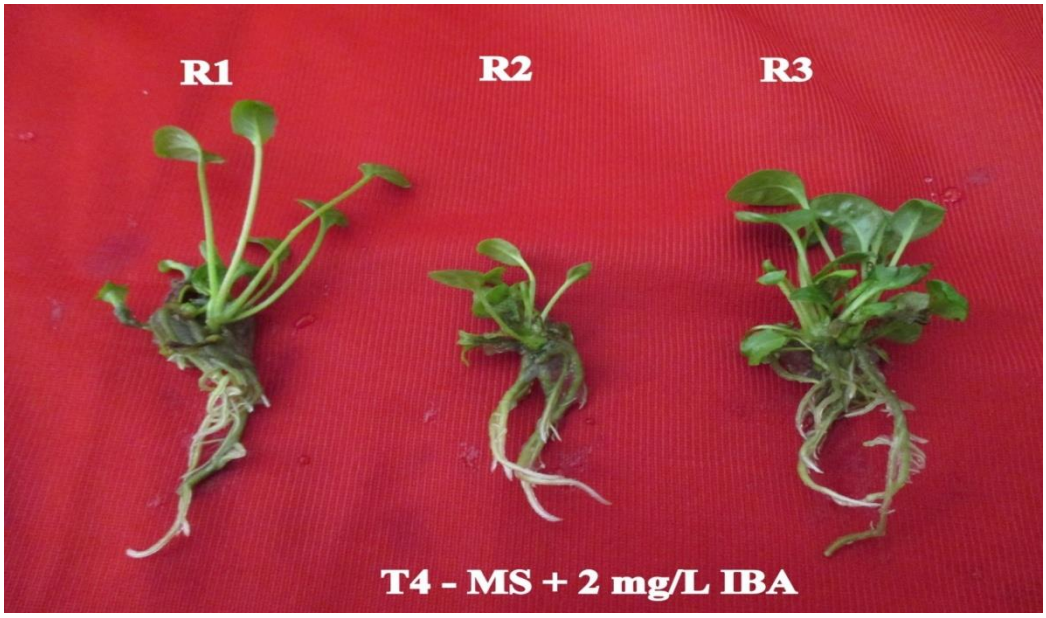


Plate 3: Shoot multiplication on different concentration of growth regulators

Plate 3.1: Shoot multiplication on T_6- MS medium + 2mg/L BAP+0.5mg/L NAA

Plate 3.2: Shoot multiplication on T_5- MS medium + 1mg/L BAP + 0.5mg/L NAA

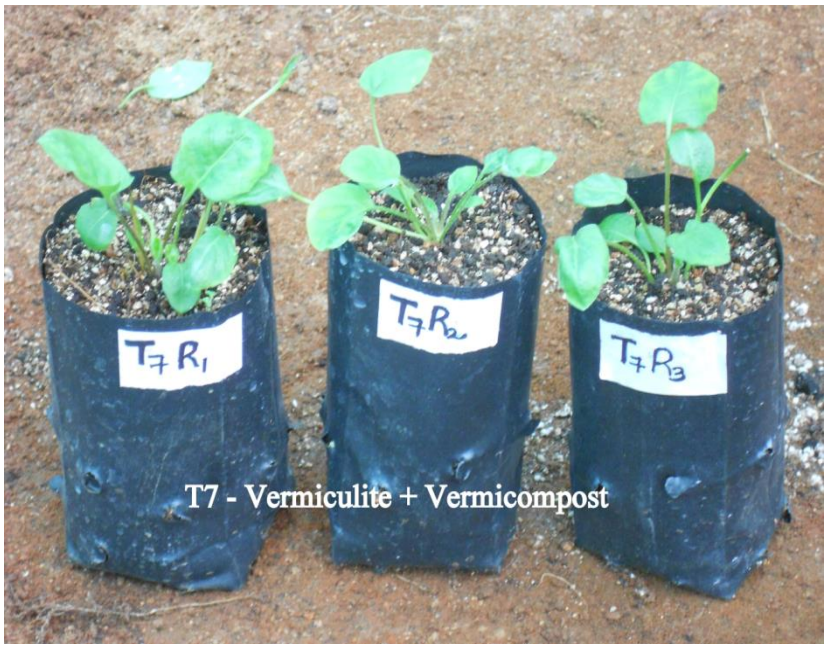
Plate 4: Rooting of *in vitro* shoots in different concentrations of Auxins

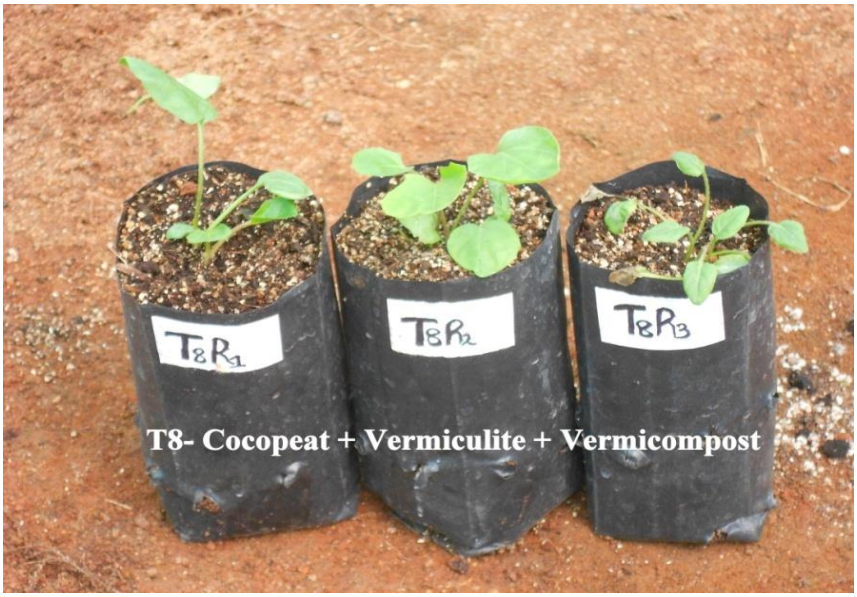

R1
R2
R3
T4 - MS + 2 mg/L IBA

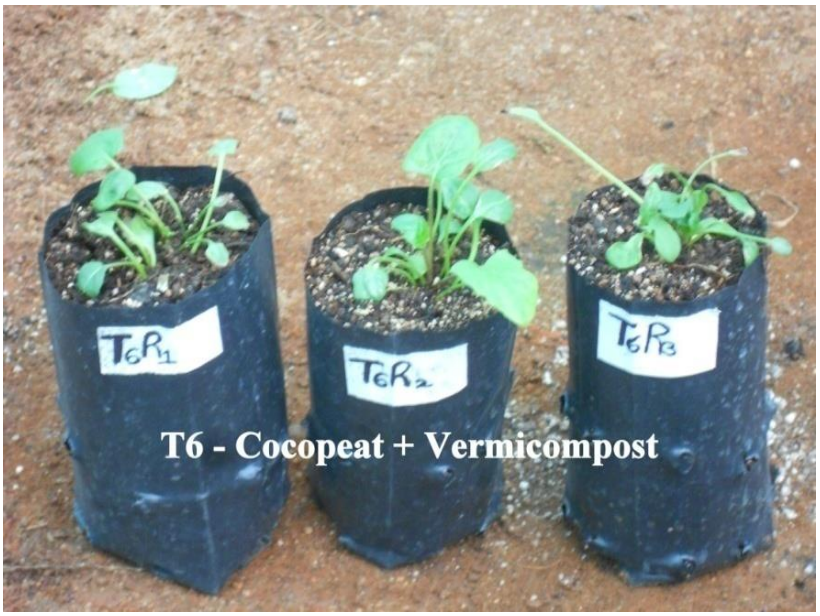

R1
R2
R3
T7 - MS + 2 mg/L NAA


T1- MS + 0.5mg/L IBA

Plate 5: Establishment of plantlets on different hardening medium







T6 - Cocopeat + Vermicompost

Experiment 1: 4.1 Effect of different concentrations of BAP in combination with NAA and IAA on culture establishment

Table 4.1.1: Number of explants responded

Treatments	Mean
T_1- Control (MS medium)	0.00 (1.00)
T_2- MS medium + 1mg/L BAP	2.33 (1.82)
T_3- MS medium + 3mg/L BAP	4.00 (2.23)
T_4- MS medium + 5mg/L BAP	5.00 (2.44)
T_5- MS medium + 1mg/L BAP + 0.1mg/L NAA	1.00 (1.41)
T_6- MS medium + 3mg/L BAP + 0.1mg/L NAA	3.00 (2.00)
T_7- MS medium + 5mg/L BAP + 0.1mg/L NAA	4.00 (2.23)
T_8- MS medium + 1 mg/L BAP + 0.1mg/L IAA	3.66 (2.15)
T_9- MS medium + 3mg/L BAP + 0.1mg/L IAA	6.00 (2.64)
T_{10}- MS medium + 5mg/L BAP + 0.1mg/L IAA	4.66 (2.37)

SEm±1	0.18 (0.04)
CD @ 5%	0.54 (0.13)

The values in parentheses are Square root transformation

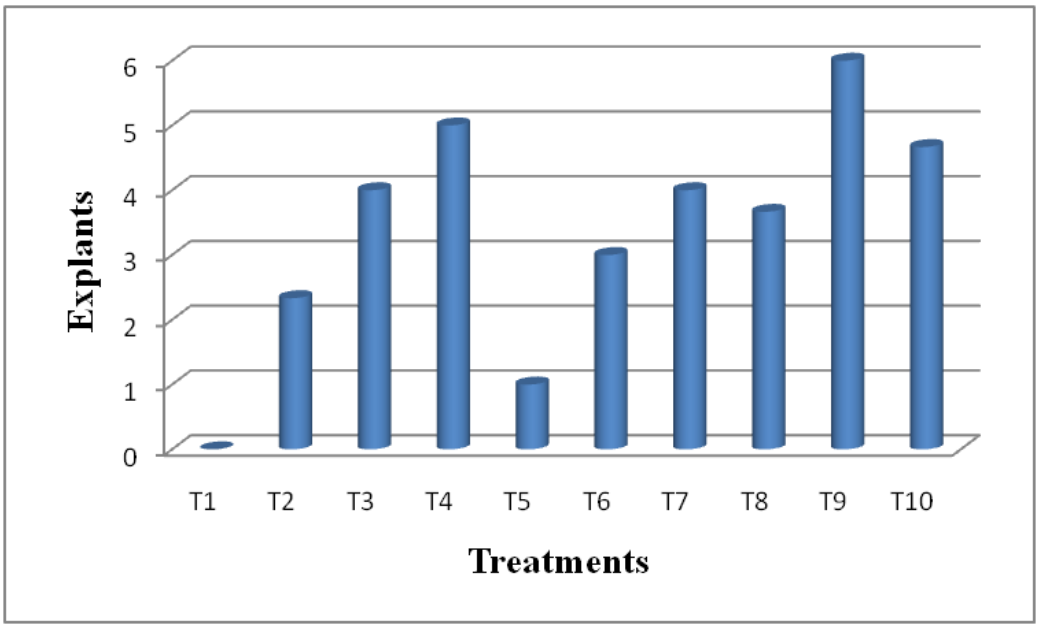


Fig 4.1.1 : Number of explants responded

Table 4.1.2: Number of days taken for primordial emergence

Treatments	Mean
T_1- Control (MS medium)	–
T_2- MS medium + 1mg/L BAP	31.33 (5.68)
T_3- MS medium + 3mg/L BAP	24.66 (5.06)
T_4- MS medium + 5mg/L BAP	17.66 (4.31)
T_5- MS medium + 1mg/L BAP + 0.1mg/L NAA	36.33 (6.11)
T_6- MS medium + 3mg/L BAP + 0.1mg/L NAA	31.33 (5.68)
T_7- MS medium + 5mg/L BAP + 0.1mg/L NAA	24.66 (5.06)
T_8- MS medium + 1 mg/L BAP + 0.1mg/L IAA	26.00 (5.19)

T_9- MS medium + 3mg/L BAP + 0.1mg/L IAA	14.66 (3.95)
T_{10}- MS medium + 5mg/L BAP + 0.1mg/L IAA	21.00 (4.69)
SEm±1	0.78 (0.07)
CD @ 5%	2.32 (0.23)

The values in parentheses are Square root transformation

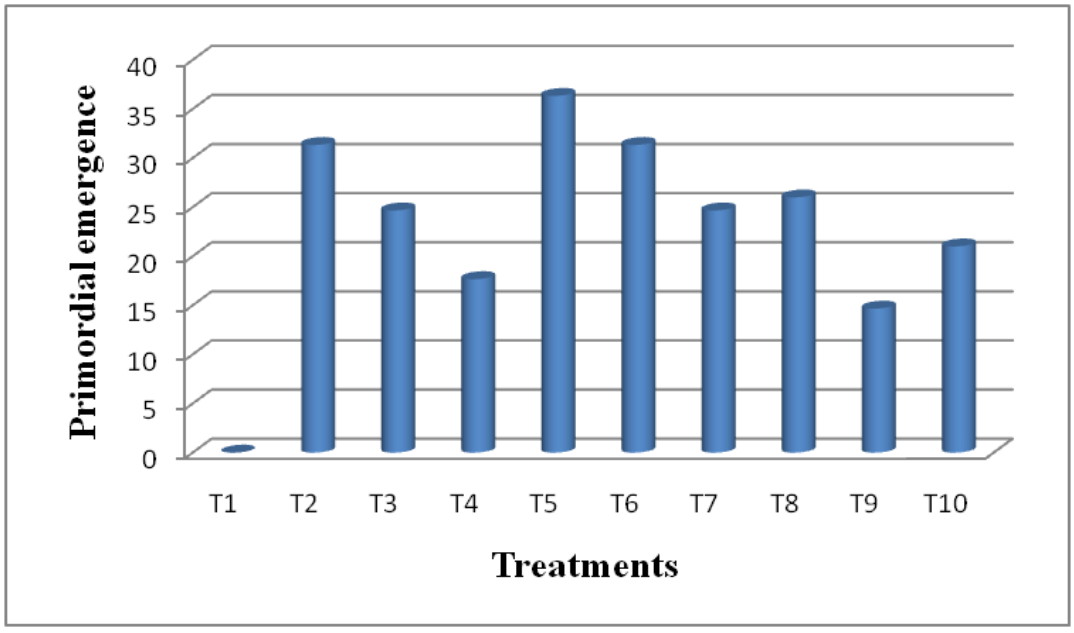


Fig 4.1.2: Number of days taken for primordial emergence

Experiment 2: 4.2 Effect of different concentrations of BAP and NAA on shoot multiplication

Table 4.2.1: Number of days taken for shoot initiation

Treatments	Mean
T_1- Control (MS medium)	40.00 (6.40)
T_2- MS medium + 1mg/L BAP + 0.1mg/L NAA	20.33 (4.61)
T_3- MS medium + 2mg/L BAP + 0.1mg/L NAA	17.66 (4.31)

T_4- MS medium + 3mg/L BAP + 0.1mg/L NAA	14.66 (3.95)
T_5- MS medium + 1mg/L BAP + 0.5mg/L NAA	17.66 (4.32)
T_6- MS medium + 2mg/L BAP + 0.5mg/L NAA	12.33 (3.64)
T_7- MS medium + 3mg/L BAP + 0.5mg/L NAA	10.66 (3.41)
SEm±1	0.816 (0.099)
CD @ 5%	2.501 (0.303)

The values in parentheses are Square root transformation

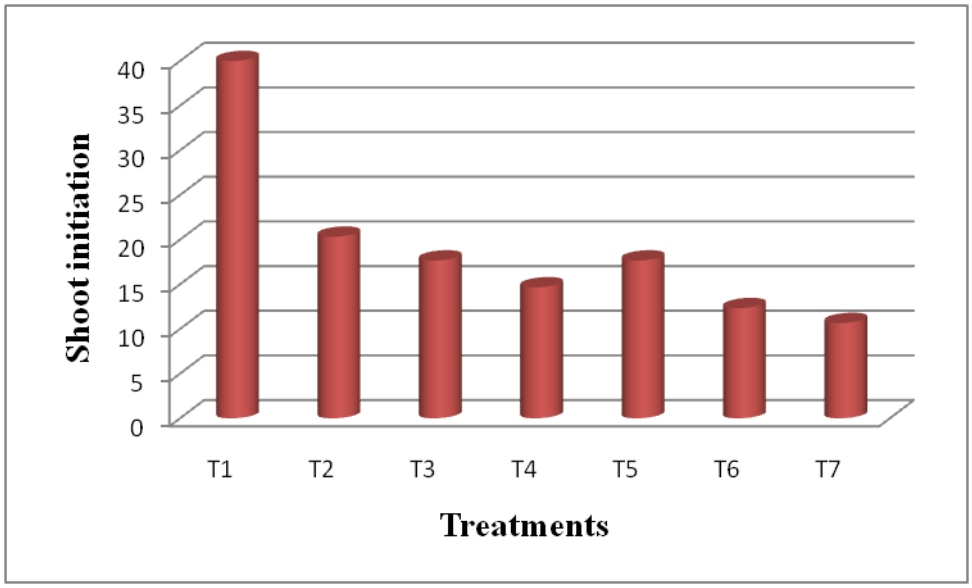


Fig 4.2.1: Number of days taken for shoot initiation

Table 4.2.2: Number of shoots per culture

Treatments	Mean
T_1- Control (MS medium)	4.33 (2.30)
T_2- MS medium + 1mg/L BAP + 0.1mg/L NAA	18.33 (4.39)
T_3- MS medium + 2mg/L BAP + 0.1mg/L NAA	11.66 (3.55)
T_4- MS medium + 3mg/L BAP + 0.1mg/L NAA	10.33 (3.36)
T_5- MS medium + 1mg/L BAP + 0.5mg/L NAA	21.00 (4.68)
T_6- MS medium + 2mg/L BAP + 0.5mg/L NAA	25.00 (5.09)
T_7- MS medium + 3mg/L BAP + 0.5mg/L NAA	15.33 (4.04)
SEm±1	0.86 (0.09)
CD @ 5%	2.64 (0.29)

The values in parentheses are Square root transformation

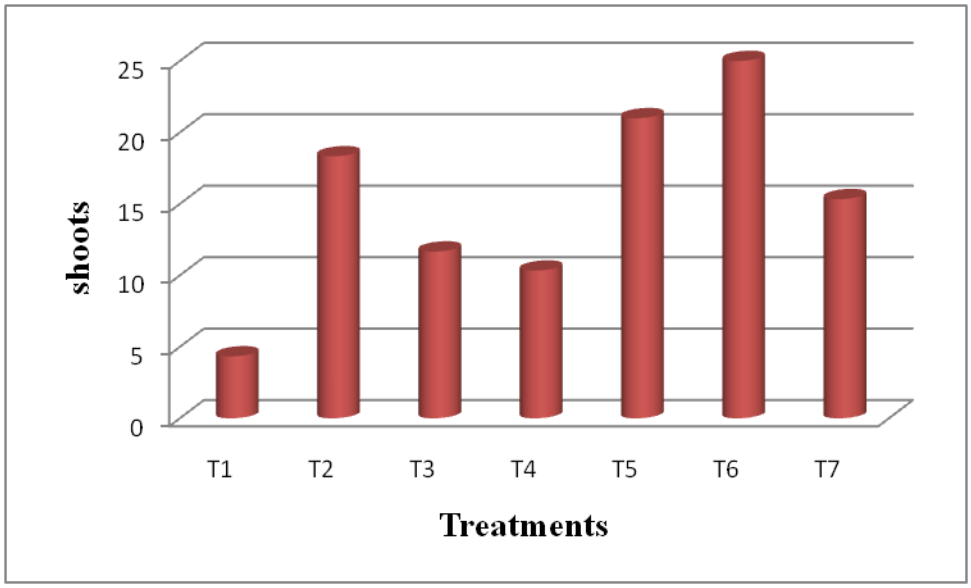


Fig 4.2.2: Number of shoots per culture

Table 4.2.3: shoot length (mm)

Treatments	Mean
T_1- Control (MS medium)	5.33 (2.51)
T_2- MS medium + 1mg/L BAP + 0.1mg/L NAA	23.00 (4.89)
T_3- MS medium + 2mg/L BAP + 0.1mg/L NAA	18.33 (4.39)
T_4- MS medium + 3mg/L BAP + 0.1mg/L NAA	16.00 (4.12)
T_5- MS medium + 1mg/L BAP + 0.5mg/L NAA	33.00 (5.82)
T_6- MS medium + 2mg/L BAP + 0.5mg/L NAA	26.66 (5.25)
T_7- MS medium + 3mg/L BAP + 0.5mg/L NAA	21.33 (4.71)
SEm±1	0.98 (0.10)
CD @ 5%	3.01 (0.31)

The values in parentheses are Square root transformation

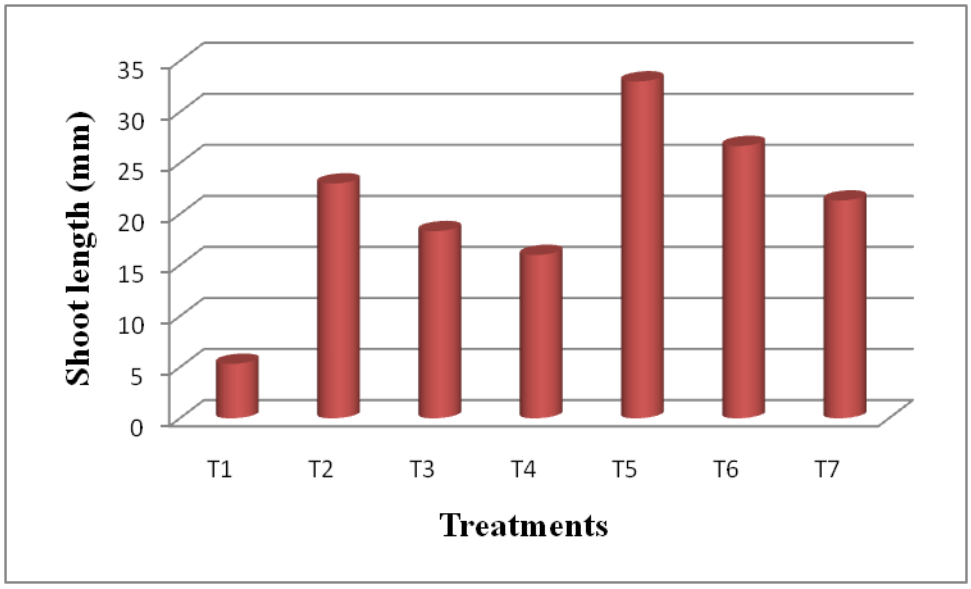


Fig 4.2.3: Shoot length (mm)

Experiment 3: 4.3 Effect of different concentrations of NAA and IBA on rooting of *in vitro* shoots

Table 4.3.1: Number of days for root initiation

Treatments	Mean
T_1- Control (MS medium)	68.33 (8.32)
T_2- MS medium + 0.5mg/L IBA	23.33 (4.93)
T_3- MS medium + 1.0mg/L IBA	21.33 (4.72)
T_4- MS medium + 2.0mg/L IBA	19.00 (4.47)
T_5- MS medium + 0.5mg/L NAA	25.66 (5.16)
T_6- MS medium + 1.0mg/L NAA	24.00 (4.99)
T_7- MS medium + 2.0mg/L NAA	22.33 (4.82)
SEm±1	0.99 (0.08)
CD @ 5%	3.03 (0.26)

The values in parentheses are Square root transformation

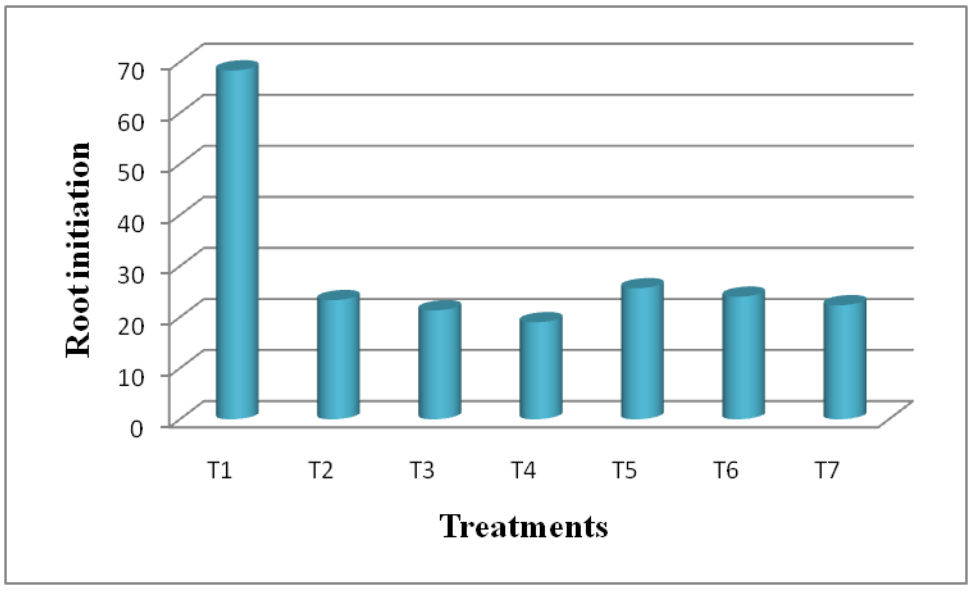


Fig 4.3.1: Number of days for root initiation

Table 4.3.2: Rooting Percentage (%)

Treatments	Mean
T_1- Control (MS medium)	10.00 (18.42)
T_2- MS medium + 0.5mg/L IBA	50.00 (44.98)
T_3- MS medium + 1.0mg/L IBA	60.00 (50.74)
T_4- MS medium + 2.0mg/L IBA	83.33 (66.11)
T_5- MS medium + 0.5mg/L NAA	40.00 (39.21)
T_6- MS medium + 1.0mg/L NAA	50.00 (44.98)
T_7- MS medium + 2.0mg/L NAA	70.00 (56.76)
SEm±1	1.26 (1.02)

CD @ 5%	3.85 (3.13)

The values in parentheses are Angular transformation

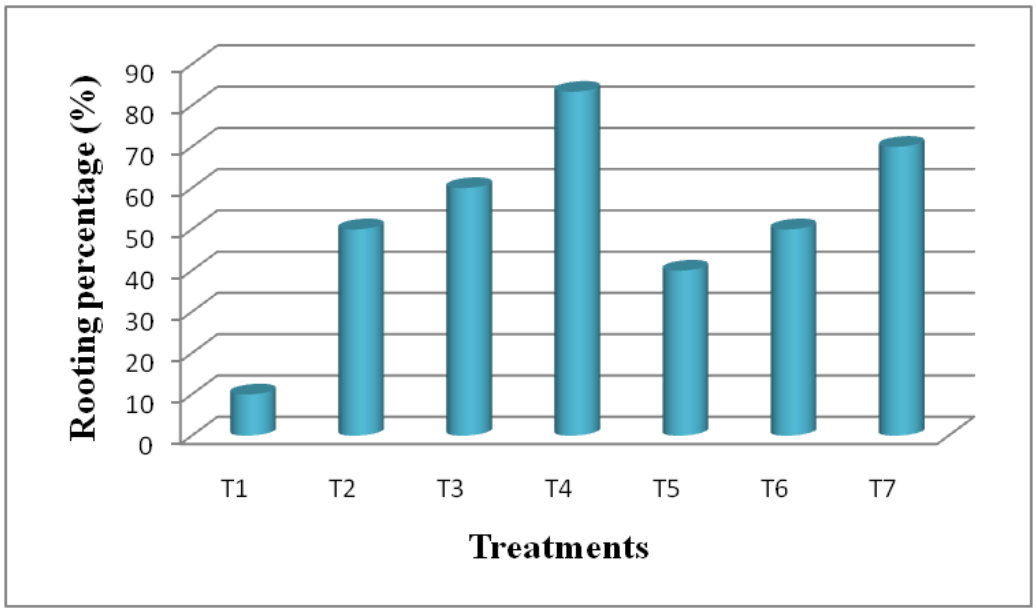


Fig 4.3.2: Rooting Percentage (%)

Table 4.3.3: Number of roots per shoot

Treatments	Mean

T_1- Control (MS medium)	1.00 (1.41)
T_2- MS medium + 0.5mg/L IBA	7.66 (2.94)
T_3- MS medium + 1.0mg/L IBA	6.00 (2.64)
T_4- MS medium + 2.0mg/L IBA	5.33 (2.51)
T_5- MS medium + 0.5mg/L NAA	4.33 (2.30)
T_6- MS medium + 1.0mg/L NAA	3.00 (2.00)
T_7- MS medium + 2.0mg/L NAA	2.00 (1.73)
SEm±1	0.30 (0.05)
CD @ 5%	0.94 (0.18)

The values in parentheses are Square root transformation

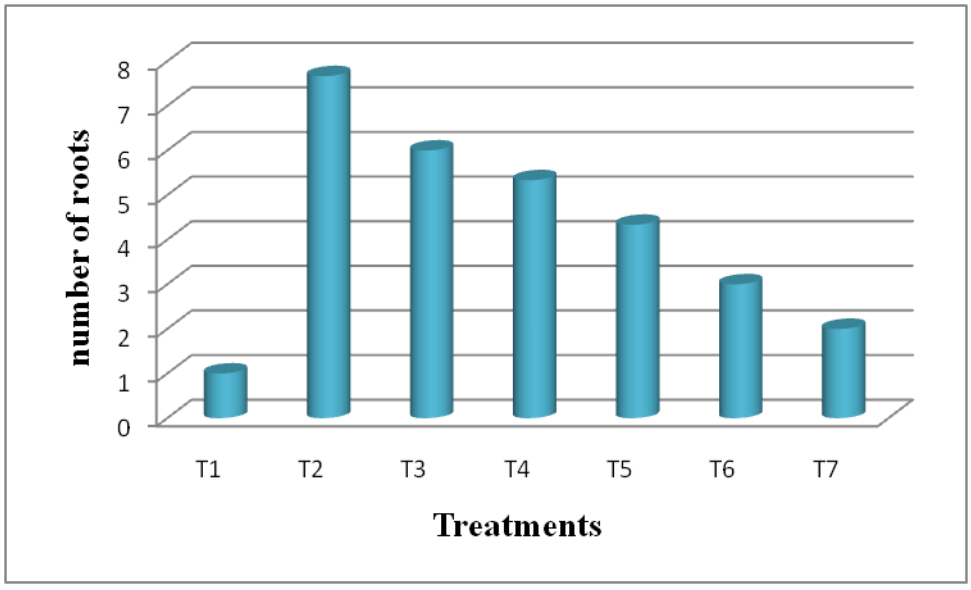


Fig 4.3.3: Number of roots per shoot

Table 4.3.4: Root length (mm)

Treatments	Mean
T_1- Control (MS medium)	8.33 (3.04)
T_2- MS medium + 0.5mg/L IBA	30.66 (5.62)
T_3- MS medium + 1.0mg/L IBA	36.33 (6.10)
T_4- MS medium + 2.0mg/L IBA	54.66 (7.46)
T_5- MS medium + 0.5mg/L NAA	21.00 (4.68)
T_6- MS medium + 1.0mg/L NAA	32.33 (5.77)
T_7- MS medium + 2.0mg/L NAA	46.00 (6.85)
SEm±1	1.32 (0.11)
CD @ 5%	4.04 (0.35)

The values in parentheses are Square root transformation

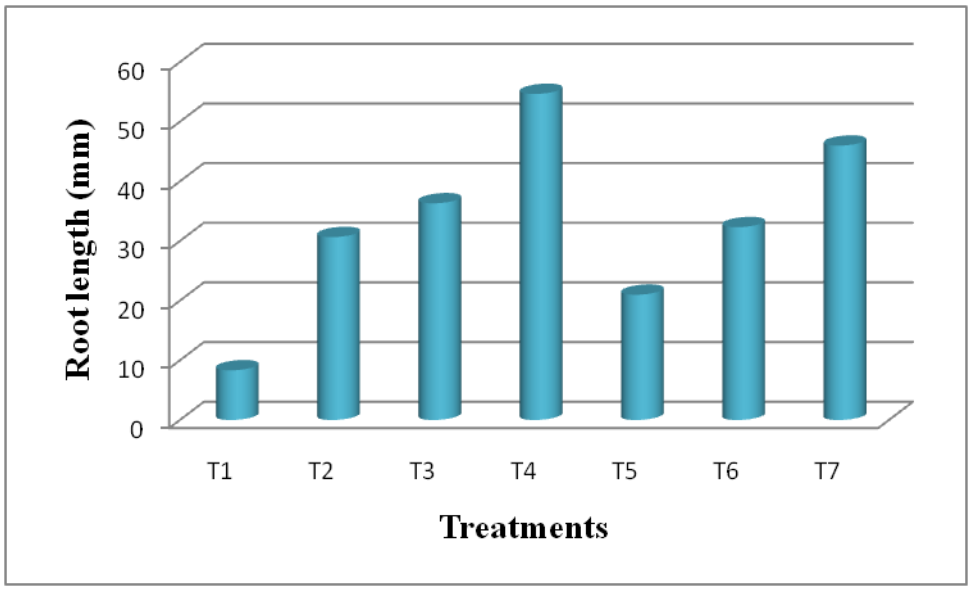


Fig 4.3.4: Root length (mm)

Experiment 4: 4.4 Standardization of hardening material for acclimatization of plantlets

Table 4.4.1: Survival percentage of Plants (%)

Treatments	Mean
T_1- Sand (Control)	13.33 (21.13)
T_2- Cocopeat	50.00 (44.98)
T_3- Vermiculite	56.66 (48.82)
T_4- Vermicompost	50.00 (44.98)
T_5- Cocopeat + Vermiculite	60.00 (50.74)
T_6- Cocopeat + Vermicompost	63.33 (52.75)
T_7- Vermiculite + Vermicompost	80.00 (63.40)
T_8- Cocopeat + Vermiculite + Vermicompost	70.00 (56.76)
SEm±1	2.04 (1.37)
CD @ 5%	6.17 (4.14)

The values in parentheses are Angular transformation

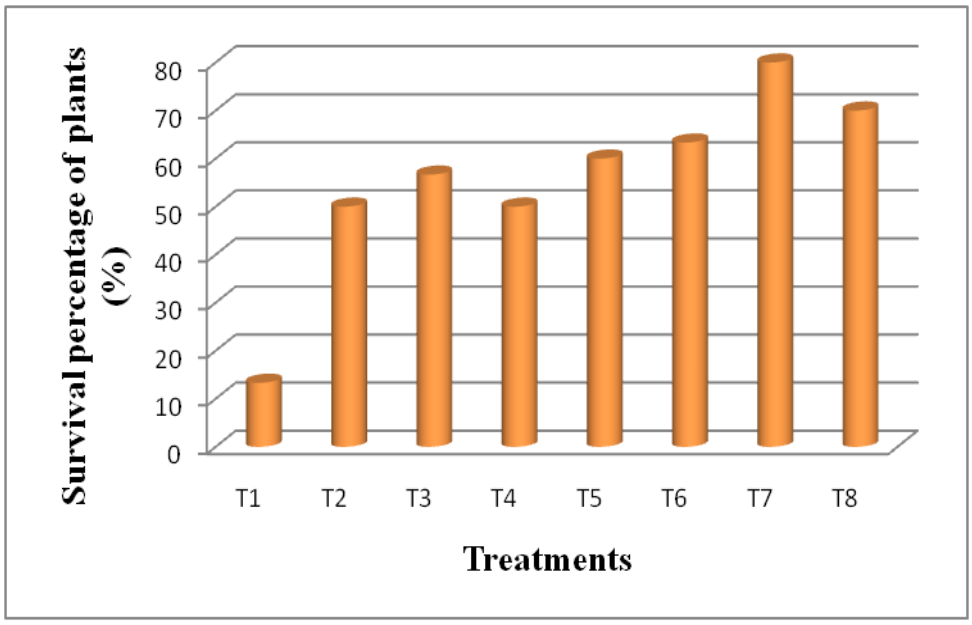


Fig 4.4.1: Survival percentage of Plants (%)

Table 4.4.2: Plant height (mm)

Treatments	Mean
T_1- Sand (Control)	28.33 (5.41)
T_2- Cocopeat	40.66 (6.45)
T_3- Vermiculite	34.66 (5.97)
T_4- Vermicompost	33.00 (5.83)
T_5- Cocopeat + Vermiculite	37.00 (6.16)
T_6- Cocopeat + Vermicompost	43.33 (6.65)
T_7- Vermiculite + Vermicompost	47.00 (6.91)
T_8- Cocopeat + Vermiculite + Vermicompost	43.33 (6.65)
SEm±1	1.74 (0.12)
CD @ 5%	5.26 (0.39)

The values in parentheses are Square root transformation

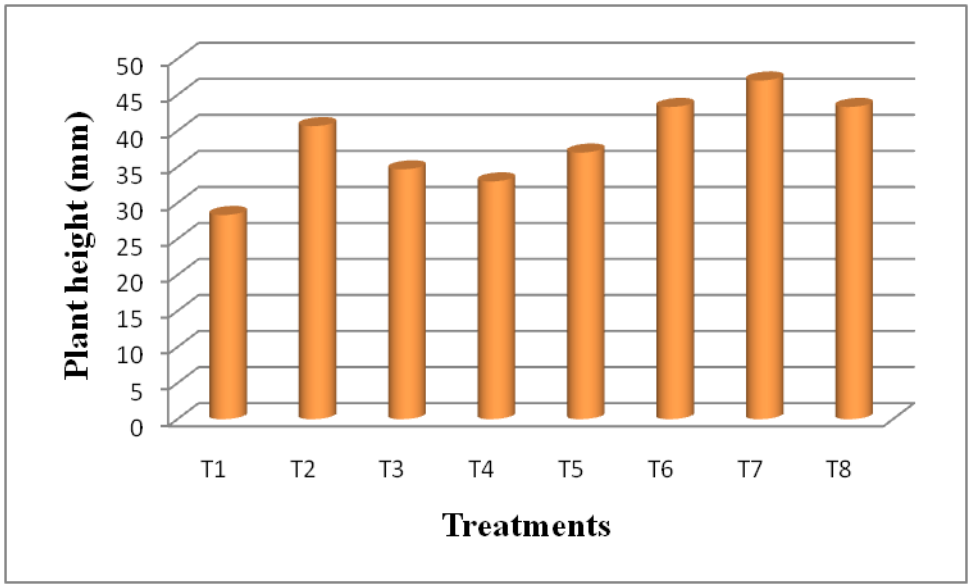


Fig 4.4.2: Plant height (mm)

Table 4.4.3: Number of leaves per plantlet

Treatments	Mean
T_1- Sand (Control)	5.66 (2.58)
T_2- Cocopeat	6.33 (2.70)
T_3- Vermiculite	6.33 (2.70)
T_4- Vermicompost	6.33 (2.70)
T_5- Cocopeat + Vermiculite	6.33 (2.70)
T_6- Cocopeat + Vermicompost	7.33 (2.88)
T_7- Vermiculite + Vermicompost	6.33 (2.70)

T_8- Cocopeat + Vermiculite + Vermicompost	6.33 (2.70)
SEm±1	0.33 (0.06)
CD @ 5%	N.S

The values in parentheses are Square root transformation

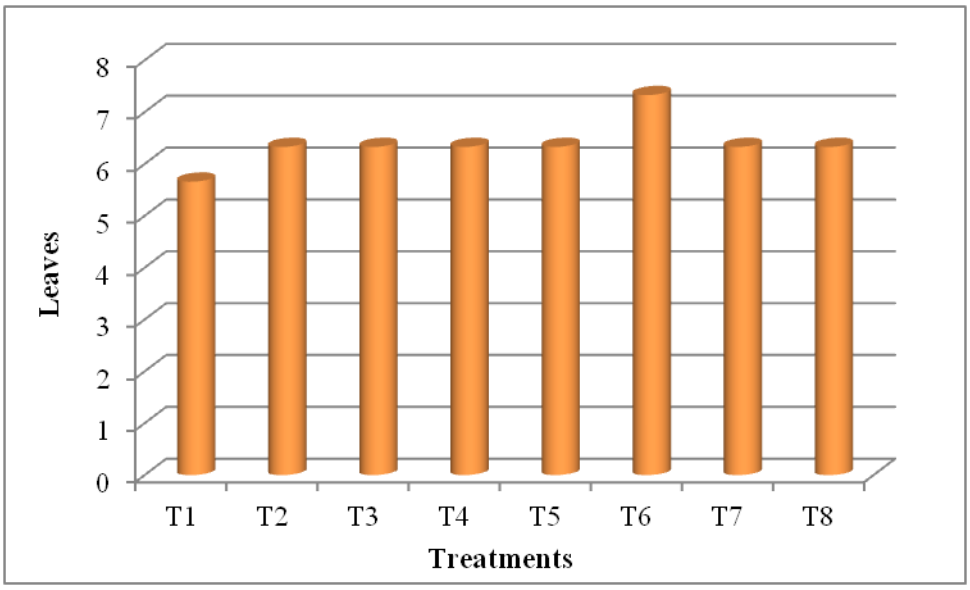


Fig 4.4.3: Number of leaves per plantlet

Chapter-V

Summary and conclusion

Chapter V

SUMMARY AND CONCLUSION

The present investigation titled **"STUDIES ON TISSUE CULTURE IN GERBERA (*Gerbera jamesonii* L.)''** was carried out "AGRI-BIOTECH FOUNDATION" in PJTSAU campus and College of Horticulture, Rajendranagar, Hyderabad during the year 2015-2016.

Gerbera is one of the important cut flowers having commercial significance. Conventionally propagated Gerbera are heterozygous in nature and their seedlings do not set seeds. Vegetative propagation is possible through division of clumps, but rate of multiplication is too slow to be commercially practicable, as divisions can produce only 5 - 10 fold increase per year of selected plant. Each year several new cultivars are introduced into the market. Micropropagation is an important tool for acceleration of plant multiplication and the plants produced through tissue culture are true to type and are free from diseases. Keeping this in view, the present investigation was carried out in four experiments and was aimed at determining the optimum hormonal combinations in nutrient medium for establishment, multiplication, rooting and acclimatization of the *in vitro* produced plantlets.

5.1 Effect of different concentrations of BAP in combination with NAA and IAA on culture establishment

In the first experiment, the sterilized explants were inoculated in MS medium for culture establishment. The cut ends of explants were kept in such a way that they are in maximum contact with the medium. The explants on the MS medium supplemented with 3mg/L BAP + 0.1mg/L IAA (T_9) had maximum number of responded explants (6.00) and with early primordial emergence (14.66 days). While,

there were no responded explants and primordial emergence in MS basal medium (control).

5.2 Effect of different concentrations of BAP and NAA on shoot multiplication

The regenerated captilum explants was cut into 2-4 equal pieces under sterile environment of laminar air flow cabinet with sterile dissecting needle and forceps and were inoculated into freshly prepared shoot multiplication medium. Among different treatments, explants cultured on MS medium supplemented with 3mg/L BAP + 0.5mg/L NAA (T_7) took shortest time for shoot initiation (10.66 days). While, the explants cultured on MS medium supplemented with 2mg/L BAP + 0.5mg/L NAA (T_6) produced more number of multiple shoots (25.00) in shortest time. While, maximum shoot length (33.00 mm) was observed on MS medium supplemented with 1mg/L BAP + 0.5mg/L NAA (T_5). Maximum number of days for shoot initiation (40.00 days) with less number shoots per culture (4.33) and minimum shoot length (5.33 mm) was observed with control (T_1) MS basal medium with devoid of hormones.

5.3 Effect of different concentrations of NAA and IBA on rooting of *in vitro* shoots

Under this study, the number of roots generated per shoot was recorded in shoots derived from capitulum explant of gerbera. Among the different treatments formulated MS medium supplemented with 2mg/L IBA (T_4) recorded minimum number of days (19.00 days) for root initiation with maximum rooting percentage (83.33%) and root length (54.66 mm). While, MS medium supplemented with 0.5mg/L IBA (T_2) produced more number of roots (7.66) per shoot. Maximum number of days for root initiation (68.33 days), minimum rooting percentage (10.00%) provided with less number of roots per shoot (1.00) and minimum root length (8.33 mm) was observed with control (T_1) MS basal medium that is devoid of hormones.

5.4 Standardization of hardening material for acclimatization of plantlets

Well rooted Plantlets were studied for acclimatization in different hardening media namely sand (control), cocopeat, vermiculite and vermicompost individually and in combinations. The present study revealed that (T_7) vermiculite + vermicompost (1:1) recorded in obtaining highest survival percentage of plants (80.00%) with maximum plant height (47.00 mm). While, maximum number of leaves per plantlet (7.33) was reported in (T_6) cocopeat + vermicompost (1:1). Lowest survival percentage of plants (13.33%) with minimum plant height (28.33mm) and less number of leaves per plantlet (3.33) was observed in (T_1) sand as control media for hardening.

Conclusively, it can be suggested that for micropropagation of gerbera through capitulum explants in which MS medium supplemented with different concentrations of growth regulators used, MS medium supplemented with 3 mg/L BAP + 0.1 mg/L IAA is best medium for culture establishment. The best treatment to produce higher shoot number in shortest time was MS medium supplemented with 2mg/L BAP + 0.5 mg/L NAA. MS medium supplemented with 2 mg/L IBA was the best medium for rooting of *in vitro* shoots. The vermiculite + vermicompost was found to be the best media for hardening of plantlets with maximum survival percentage and better plant height.

FUTURE LINE OF WORK

- Use of capitulum of different ages as explants may be studied for better regeneration in different varieties.
- Establishment and multiplication of Gerbera can be studied with different auxins at varied concentration along with cytokinin.

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Appendices

APPENDIX - I

Meteorological data for the period of experimentation (September 2015 to March 2016) recorded at the meteorological observatory of the Agricultural Research Station, Rajendranagar.

Month	Temperature (^{0}C)		Relative Humidity (%)		Rainfall (mm)	Rainy days	Sun Shine (hrs)	Wind Speed (km/hr)	Evaporation (mm)
	Minimum	Maximum	Morning	Evening					
September 2015	31.01	22.4	89.93	65.36	168.2	9	5.23	1.49	4.07
October 2015	32.33	19.7	90.61	45.67	35.6	2	7.92	0.83	4.21
November 2015	30.45	17.6	88	55.3	17.3	1	7.35	1.65	4.03
December 2015	30.9	14.8	86.5	34.8	1.4	0	7.9	0.7	3.8
January 2016	29.69	13.68	78.94	31.10	0.00	0	8.34	1.33	3.84
February 2016	33.6	17.4	78.9	29.6	0.00	0	8.8	1.9	5.8
March 2016	36.2	22.6	72.5	29.1	0.09	0.03	7.9	2.07	28.3