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Research Article

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

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Abstract- An attempt has been made to determine the response of capitulum explants of gerbera for micropropagation with 3 mg/L BAP + 0.1 mg/L of IAA was found to be the best medium for culture establishment and primordial emergence (14.66 days). Early shoot initiation was observed on MS medium supplemented with 3mg/L BAP + 0.5mg/L NAA (10.66 days), MS medium supplemented 2 mg/L BAP + 0.5 mg/L NAA was found to produce maximum number of shoots in shortest time (26.00) and 1mg/L BAP + 0.5 mg/L NAA has recorded significantly maximum shoot length (33.00 mm). MS medium supplemented with 2mg/L IBA was best medium for *in vitro* rooting because it showed highest per cent rooting (83.33%), earlier rooting (19.00 days) with maximum root length (54.66 mm). In vermiculite + vermicompost (1:1, v/v) medium, survival percentage was maximum (80.00%) with maximum plant height (47.00 mm).

Keywords- Gerbera, Tissue culture, Capitulum, Culture establishment

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Introduction

Gerbera (*Gerbera jamesonii* L.) belongs to the family Asteraceae, is an important commercial flower grown throughout the world. Gerbera is generally propagated by division of suckers or clumps which gives true to type plants, Propagation through seeds is not preferred as the plants exhibit heterozygosity non-uniformity. 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. Micropropagation work in Gerbera was initiated by shoot tip in 1974 by Murashige and in 1985 by Huang and Chu [1,2]. However, capitulum explants are being used for micro propagation is used and no shoots are lost from the plant. 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.

Material and Methods

The experiment was carried out at the "AGRI BIOTECH FOUNDATION" Professor Jayashankar Telangana State Agrilcultural University Campus, Rajendranagar, Hyderabad, during the year 2015-2016 by adopting Complete Randomised Design. Capitulum explants of Gerbera were collected at immature stage (1.0 – 1.5 cm) diameter were washed under running tap water there by rinsing with 0.1% tween-20 for 15 minutes followed with double distilled water and further soaked in 0.1% bavistin solution for 20 minutes and later washed 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 to remove any traces of $HgCl_2$ [3]. In culture establishment, the sterilized explants were inoculated in jar bottles containing the MS medium supplemented with BAP (1, 3, and 5mg/L) individually and in combination (0.1mg/L) of IAA, NAA respectively. For multiple shoots, the regenerated capitulum transferred to BAP (1, 2 and 3mg/L) in combination with 0.1 and 0.5mg/L NAA respectively. For rooting the micro shoots of more than 1.0 cm transferred to IBA and NAA (0.5, 1 and 2mg/L). For hardening of *in vitro* shoots were transferred to cocopeat, vermiculite and vermicompost individually and in different combinations. Before the use of Laminar air flow cabinet, the working area was swabbed with 100% ethyl alcohol. They were kept in growth room at a temperature of 25 ± 2^{0}C and illuminated for 16 hrs of light at 3000 lux and 8 hour dark per day maintaining a relative humidity of 70%. The data was subjected to analysis of variance test as suggested by Panse and Sukhatme (1967) [4]. Critical difference values were tabulated at one per cent level of probability wherever F test was found to be significant.

Plate-1.1 Washing 4-5 times with sterile distilled water

Plate-1.2 Soaking of Capitulum explants in 0.1% bavistin solution (fungicide) for 20 minutes

Results and Discussion

Number of explants responded, Days taken for primordial emergence are presented under [Table-1]. Number of explants responded were maximum with optimum concentration of BAP 3 mg/L with 0.1 mg/L IAA due to Cytokinins 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. 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 [5]. 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.

Table-1 *Effect of different concentrations of BAP in combination with NAA and IAA on culture establishment*

Treatments	Number of Responded explants	Days taken for Primordial emergence
T1- Control (MS medium)	0.00 (1.00)	-
T2- MS medium + 1mg/L BAP	2.33 (1.82)	31.33 (5.68)
T3- MS medium + 3mg/L BAP	4.00 (2.23)	24.66 (5.06)
T4- MS medium + 5mg/L BAP	5.00 (2.44)	17.66 (4.31)
T5- MS medium + 1mg/L BAP + 0.1mg/L NAA	1.00 (1.41)	36.33 (6.11)
T6- MS medium + 3mg/L BAP + 0.1mg/L NAA	3.00 (2.00)	31.33 (5.68)
T7- MS medium + 5mg/L BAP + 0.1mg/L NAA	4.00 (2.23)	24.66 (5.06)
T8- MS medium + 1 mg/L BAP + 0.1mg/L IAA	3.66 (2.15)	26.00 (5.19)
T9- MS medium + 3mg/L BAP + 0.1mg/L IAA	6.00 (2.64)	14.66 (3.95)
T10- MS medium + 5mg/L BAP + 0.1mg/L IAA	4.66 (2.37)	21.00 (4.69)
SEm±1	0.18 (0.04)	0.78 (0.07)
CD @ 5%	0.54 (0.13)	2.32 (0.23)

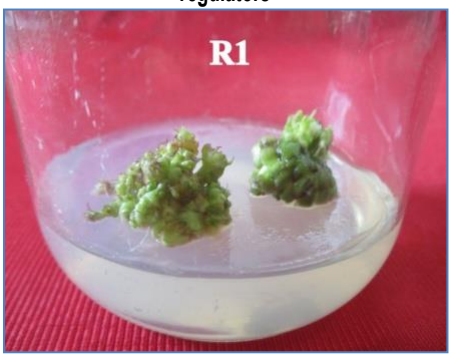
The values in parentheses are Square root transformation

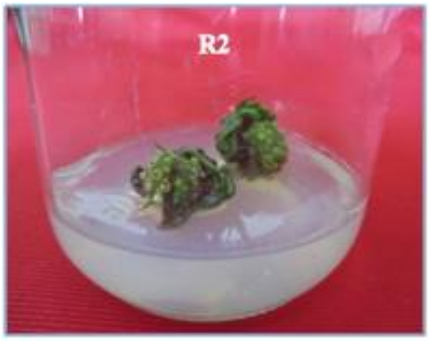
Days taken for shoot initiation, Number of shoots per culture and Shoot length (mm) are furnished under [Table-2]. Auxin at 0.5 mg/L NAA is found to be beneficial in conjunction with high levels of cytokinin 3mg/L BAP on MS medium (T_7) which might be due to regulating growth and morphogenesis by removal 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). 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) [6]. 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 chrysanthemum [7]. Days taken for root initiation, Rooting percentage, Number of roots per shoot, Root length (mm). 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) using capitulum as explant in gerbera [8] and Priyakumari and Sheela (2005) using cormels as explant in gladiolus [9]. 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. This result is similar to that of Bhargava *et al.,* (2013) who obtained by using capitulum as explant in Gerbera and Kabir *et al.* (2014) [10]. 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. This results are in conformity with the report of Kharrazi, *et al.,* (2011) in carnation using shoot explants and Nhut *et al.* (2007) using transverse thin cell layer culture of receptacles in gerbera [11, 12]. 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 and Priyakumari and Sheela, (2005).

Survival percentage of plantlets, Plant height (mm), Number of leaves per plantlet were depicted under [Table-3]. 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. While, Rahman, *et al.,* (2013) who obtained maximum survival percentage of plants using vermicompost + coir dust + Garden soil (2:1:1) in gerbera. Vermiculite + vermicompost (1:1, v/v) in (T_7) 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 [13]. 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 [14, 15]. 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-2 Primordial emergence on different concentrations of Growth regulators





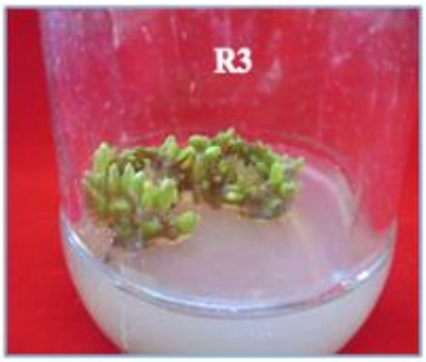


Plate-2.1 T9 - MS medium + 3mg/L BAP + 0.1mg/L IAA

Plate-3 Shoot multiplication on different concentration of growth regulators

Plate-3.1 Shoot multiplication on T6- MS medium + 2mg/L BAP+0.5mg/L NAA

Table-2 *Effect of different concentrations of BAP and NAA on shoot multiplication*

Treatments	Days taken for shoot initiation	Shoots per culture	Shoot length (mm)
T1- Control (MS medium)	40.00 (6.40)	4.33 (2.30)	5.33 (2.51)
T2- MS medium + 1mg/L BAP + 0.1mg/L NAA	20.33 (4.61)	18.33 (4.39)	23.00 (4.89)
T3- MS medium + 2mg/L BAP + 0.1mg/L NAA	17.66 (4.31)	11.66 (3.55)	18.33 (4.39)
T4- MS medium + 3mg/L BAP + 0.1mg/L NAA	14.66 (3.95)	10.33 (3.36)	16.00 (4.12)
T5- MS medium + 1mg/L BAP + 0.5mg/L NAA	17.66 (4.32)	21.00 (4.68)	33.00 (5.82)
T6- MS medium + 2mg/L BAP + 0.5mg/L NAA	12.33 (3.64)	25.00 (5.09)	26.66 (5.25)
T7- MS medium + 3mg/L BAP + 0.5mg/L NAA	10.66 (3.41)	15.33 (4.04)	21.33 (4.71)
SEm±1	0.816 (0.099)	0.86 (0.09)	0.98 (0.10)
CD @ 5%	2.501 (0.303)	2.64 (0.29)	3.01 (0.31)

The values in parentheses are Square root transformation

Table-3 *Effect of different concentrations of NAA and IBA on rooting of in vitro shoots*

Treatments	Days for root initiation	Rooting percentage (%)	Number of roots per shoot	Root length (mm)
T1- Control (MS medium)	68.33 (8.32)	10.00 (18.42)	1.00 (1.41)	8.33 (3.04)
T2- MS medium + 0.5mg/L IBA	23.33 (4.93)	50.00 (44.98)	7.66 (2.94)	30.66 (5.62)
T3- MS medium + 1.0mg/L IBA	21.33 (4.72)	60.00 (50.74)	6.00 (2.64)	36.33 (6.10)
T4- MS medium + 2.0mg/L IBA	19.00 (4.47)	83.33 (66.11)	5.33 (2.51)	54.66 (7.46)
T5- MS medium + 0.5mg/L NAA	25.66 (5.16)	40.00 (39.21)	4.33 (2.30)	21.00 (4.68)
T6- MS medium + 1.0mg/L NAA	24.00 (4.99)	50.00 (44.98)	3.00 (2.00)	32.33 (5.77)
T7- MS medium + 2.0mg/L NAA	22.33 (4.82)	70.00 (56.76)	2.00 (1.73)	46.00 (6.85)
SEm±1	0.99 (0.08)	1.26 (1.02)	0.30 (0.05)	1.32 (0.11)
CD @ 5%	3.03 (0.26)	3.85 (3.13)	0.94 (0.18)	4.04 (0.35)

The values in parentheses are Angular transformation

Plate-4 Rooting of in vitro shoots in different concentrations of Auxins

Plate-5 Establishment of plantlets on different hardening medium

Table-4 *Standardization of hardening material for acclimatization of plantlets*

Treatments	Survival percentage of plants (%)	Plant height (mm)	Number of leaves per plantlet
T1- Sand (Control)	13.33 (21.13)	28.33 (5.41)	5.66 (2.58)
T2- Cocopeat	50.00 (44.98)	40.66 (6.45)	6.33 (2.70)
T3- Vermiculite	56.66 (48.82)	34.66 (5.97)	6.33 (2.70)
T4- Vermicompost	50.00 (44.98)	33.00 (5.83)	6.33 (2.70)
T5- Cocopeat + Vermiculite	60.00 (50.74)	37.00 (6.16)	6.33 (2.70)
T6- Cocopeat + Vermicompost	63.33 (52.75)	43.33 (6.65)	7.33 (2.88)
T7- Vermiculite + Vermicompost	80.00 (63.40)	47.00 (6.91)	6.33 (2.70)
T8- Cocopeat + Vermiculite + Vermicompost	70.00 (56.76)	43.33 (6.65)	6.33 (2.70)
SEm±1	2.04 (1.37)	1.74 (0.12)	0.33 (0.06)
CD @ 5%	6.17 (4.14)	5.26 (0.39)	N.S

The values in parentheses are Square root transformation

Conclusion

Conclusively, it can be suggested that for regeneration of gerbera from capitulum explants, a combination of MS medium supplemented with 3mg/L BAP + 0.1mg/L IAA was best for initial culture establishment while for shoot multiplication in shortest time, MS medium supplemented with 2mg/L BAP + 0.5mg/L NAA was best. Further rooting of *in vitro* explants, the MS medium supplemented with 2mg/L IBA was found to be the best media. While, vermiculite + vermicompost (1:1) was found to be the the best media for acclimatization of *in vitro* raised gerbera plantlets.

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Author Contributions

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Abbreviations

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
$Hgcl_2$	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
L	Liter
et al.	and others
Mg	Milligram
Mm	Millimeter
v/v	volume/volume

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors – yes

Conflict of Interest: None declared

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