International Journal of Bioresource Science
Citation: IJBS: 2(2): 195-204 December 2015
DOI: 10.5958/2454-9541.2015.00016.X

In vitro morphogenesis studies in gerbera jamesonii bolus ex hooker F.

Deepa Bhatt, M.K. Tripathi, Lal Singh*, P.K.S. Gurjar, A.K. Barholia, Rajesh Jatav and Narendra Vasure

RVSKVV, Department of Floriculture and Landscape Architecture, K.N.K. College of Horticulture, Mandsaur 458001 (M.P.), India

*Corresponding author: lalsingh_sagar@gmail.com

Abstract

An experiment was conducted during 2013 at the Horticultural Biotechnology Laboratory, KNK College of Horticulture, Mandsaur, RVSKVV, Gwalior (MP). The highest percentage of aseptic culture (83.54%) of explants were recorded with 0.5% bavistin in combination with 0.2% $HgCl_2$ when diverse explants were exposed for 30 minutes. followed by application of 0.5% bavistin in combination with 0.1% $HgCl_2$ (78.76%), while higher survival percentage (74.32%) of explants were evidenced with 0.5% bavistin in combination with 0.1% $HgCl_2$ with exposure time 30 minutes. Combination of 0.5% bavistin with 0.1% $HgCl_2$ exposed for the 30 minutes supported higher percentage of aseptic culture (78.76%) as well as survival of explants (74.13%) so that, this concentration and combination was used for surface sterilization of explants of gerbera during present investigation. The maximum callus was recorded on culture medium MS3D (35.09%), MS3N (33.83%) and MS2D (33.02%) with at par performance. Remaining culture media had low callus forming ability including MS5T (18.87%) that has significantly minimum counts. Maximum callus induction were recorded from cultured mature embryo on nutrients media MS3ip (35.06%) closely followed by a group of four nutrients media viz: MS2ip (29.95%), MSip (29.75%), MS.5ip (29.93%) and MS.2ip (29.12%). different culture media combination, inoculation media MS2N.5ip (55.22%), MS3N.5ip (53.17%) and MSN5ip (45.01%) were proved remarkably superior for shoots proliferating efficiency. Nutrients media MS5D.5Kn (3.05%) and MS4D.5KN (4.81%) was found low responding in this regard. Nutrient media MS2N (2.14cm), MSN (2.03cm) and MS2D (1.95) had produced shoot of higher length.

Keywords: Gerbera jamesonii, in vitro morphogenesis, MS media, multiplication, biotechnology, clonal propagation

The Barberton/Trranvaal daisy or gerbera which belongs to the family Asteraceae is one of the leading cut flowers and ranks among the top ten cut flowers in the world (Parthasarathy and Nagaraju, 1999). The species, a perennial herb and native to South Africa and Asia is grown all over world in a wide range of climatic conditions. The genus Gerbera was named in honour of a German naturalist Traugott Gerber, who travelled Russia in 1743. The genus consists of about forty species. Out of the recorded species, only one species *Gerbera jamesonii* is under cultivation. The flower makes an excellent choice for any gift basket intended to brighten someone's day, or celebrate joyous occasions. The gerbera daisy has long been a symbol of beauty, purity and innocence.Gerbera can be propagated by both sexual and asexual methods. Most of the commercially grown cultivars are propagated through vegetative means to maintain uniformity and genetic purity. Among the vegetative means, multiplication through division of clumps is

the most common method used for several decades. Gerbera can also be propagated through cuttings. The plants multiplication by these methods is too slow to be commercially practicable. For commercialization of this crop, planting material is required on large scale which requires the development of an easier, quicker and economically viable method of propagation. The use of biotechnological approaches especially micro propagation offers a unique alternative for mass multiplication of true to type plantlets. Micro propagation is a powerful tool for cloning and propagation of horticultural plants, especially ornamentals. Micro propagation techniques increase the scale and speed of production and yield a healthier product. Clonal propagation of gerbera has effectively employed this technique to produce genetically pure plants ensuring a unique colour pattern for each veriety. This method enables a million fold expansions per year of a desired plant (Murashige *et al.* 1974; Aswathi *et al.* 2003).

Materials and methods

Experiment for the present investigations were conducted at the Horticultural Biotechnology Laboratory, KNK College of Horticulture, Mandsaur, RVSKVV, Gwalior (MP) during the session 2013-2014.Pink cultivar of *Gerbera jamesonii* was chosen for conducting present investigation. Explants viz: mature embryo was taken for study. Seeds of Pink cultivar was procured from Balaji Nursery, Indore (M.P.) Two different fortifications of basal media viz: MS (Murashige and Skoog, 1962) and Gerbera multiplication media were tested to find out better in vitro response. A general composition of basal MS and Gerbera medium is defined in Table 1. Apart from MS basal micro and macro salts, vitamins and agar powder, three different auxins (alone), namely : 2, 4- Dichlorophenoxy acetic acid (2,4-D), Nephthelene acetic acid (NAA) and 2,4,5- Trichlorophenoxy acetic acid (2,4,5-T) for both explants cultures (Table-3) as well as three diverse cytokinins (alone) viz. 6- Benzyl amino purine (BAP), Kinetin (KN) and N- isopentenyle amino purine (2-ip) for culturing both explants (Table 4) in varying concentrations were added to fortify MS media during present experiment. Therefore, for final experiment basal MS medium was fortified with both types of plant growth regulators (an auxin as well as a cytokinin) in verying concentrations and combinations.

Keeping all above facts in mind, for final experimentations, different culture media combinations were fortified by supplementing different concentrations and combinations of 2,4-D and NAA with BAP, KN and 2-iP for mature embryo explants culture (Table 5). Culture media combinations and other ingredients were short listed on the basis of work conducted by various scientists as well as preliminary experiments of this laboratory. Readymade MS/ Gerbera basal medium and all other supplements were procured from Hi media laboratories, Mumbai, India.

Experiment was conducted for mature embryo. Completely Randomized Design (CRD) was used to find out the significance of culture media combinations using different concentrations and combinations using different concentrations of plant growth regulators and other supplements. Each treatment was consisting of two replications. Per replication approximately 100-120 explants were excised and cultured on each media.For mature embryo explants culture, observation were recorded at 3 stages; stage-I: after 3-4 weeks of initial culturing, stage-II: after 4-5years weeks sub culturing of explants/calli on regeneration media and stage-III: when the complete plantlets were obtained. All the observation were based on initial culture media, irrespective of generation medium or rooting medium. During present experimentation following observation were recorded.

Number of callus forming explants/100 explants plated: Cultured mature embryos on different culture media were recorded for callus formation after 3-4 weeks stage-I observation.

Number of shoots (S) proliferating explants/100 cultured explants: Total number (S) of shoots profile rating mature embryos (direct and/or via callus) cultured on different culture media were recorded for shoots profile rating efficiency after 3-4 weeks.

Average number of shoots/explants: Average number of shoots (S) regenerated per responded mature embryo cultured on different culture media were recorded for number (S) of shoots per explants after 5-7 weeks.

Mean shoot length: Average length of shoots was recorded after 5-7 weeks.

Results and discussion

The highest percentage of aseptic culture (83.54%) of explants were recorded with 0.5% bavistin in combination with 0.2% $HgCl_2$ when diverse explants were exposed for 30 minutes followed by application of 0.5% bavistin in combination with 0.1% $HgCl_2$ (78.76%), while higher survival percentage (74.32%) of explants were evidenced with 0.5% bavistin in combination with 0.1% $HgCl_2$ with exposure time 30 minutes (Table 1). Combination of 0.5% bavistin with 0.1% $HgCl_2$ exposed for the 30 minutes supported higher percentage of aseptic culture (78.76%) as well as survival of explants (74.13%) so that, this concentration and combination was used for surface sterilization of explants of gerbera during present investigation.

Morphogenesis in diverse explants cultures

Mature embryo and leaf disc of pink cultivate of gerbera were cultured on different fortification of MS medium. In present study, cultured explants followed either direct or indirect pathway of plant regeneration depending upon the nature of different plant growth regulators supplied in culture media. In direct approach, plantlets were regenerated on explants surface directly without callus formation (via direct organogenesis) and in indirect mode, plantlets were originated from callus mass (either via indirect organogenesis or somatic embryogenesis).In mature embryo culture; plantlets were regenerated via direct (Plate 1) and indirect (Plate 2) organogenesis as well as via direct somatic embryogenesis (Table 2). In direct organogenesis, adventitious structures were developed on explants surface. Adventitious formation started approximately 5-7 days from initial culturing. However, the duration varied from culture to culture and in a few cases adventitious formed after 20-25 days. With time, these adventitious structure initiated single shoots (Plate 2A-1), elongated (plate 3 A-F; plate 3 A-F) and shootlets (plate 5 A-F). Formations of multiple shoots (plate 7 A-F) were also observed frequently. In indirect approach, plantlets were regenerated from callus mass. The first response to cultured mature embryo was similar after 4-7 days and mostly independent from explants and culture media. All explants became swallowen and no callus proliferation was observed during first few days. Callus proliferation usually started from embryonic axis after 4-7 days of culture (plate 8 A-F). In indirect organogenesis, shootlets developed from the nodules arising on the surfaces of the callus (plate 9 A-I). Shoots formation started approximately 7 days from initial culturing, however the duration varied from culture to culture and in a few cases shootlets formed after 30 days. In indirect embryogenesis, embryo formation started approximately 10 days from initial culturing to culture and in a few cases embryoids developed after 45 days of apparently undifferentiated growth. The embryoids like structure were rounded and cylindrical with irregular out lines usually appeared in clusters (plate 10A). After sometimes these somatic embryos germinated (plate 10 B-D) and formed shootlets (plate 10 E-F). In cultured leaf disc, mostly phenomenon of indirect organogenesis was observed (plate11 A-C). In cultured leaf disc, callus proliferation started from the cut edges after 7-10 days of culture (plate11 A-C). However, lesser number of shootlets initiated from cultured leaf discs in present investigation. Most of the calli, after prolonged culturing on the induction media gave rise to plants. However, sub culturing of these shoots/calli on regeneration medium allowed higher plantlet regeneration. In cases where there was no root formation, shoots were transferred to the rooting medium (plate 12 A-H). Rooted plantlets were elongated after transferring into elongation medium. Rooted plants were thoroughly washed with running tap water to remove the adhering agar and planted in 2.5 cm roots trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Roots trainers with transplanted plants were transferred under 28

± 2°c and 60 ± 5% relative humidity for 20-25 days in a environmental growth cabinet for hardening (plate 13 A). Later these regenerates were transferred in fields. During the presents investigation, plantlets were regenerated in huge numbers, which may be used for massive *in vitro* propagation of gerbera (plate 14 A-C; plate 15 A-C and plate 16 A-C). Mature embryos of pink cultivars of gerbera were cultured on different fortification of MS medium. The analysis of variance (appendices II-IV) revealed that the mean sum of squares due to the different culture media combination were highly significant at 5% probability level for different attribute investigated. It indicates the presence of considerable amount of variability amongst the different culture medium. Effects of different culture media combination are presented in (Table 3). Although callus induction, shoot proliferation, number of shoots (s) per explants and mean shoot length were recorded on all culture media combination.

Effects of different auxins (alone) in varying concentration on callus induction are presented in the Table 9. The maximum callus was recorded on culture medium MS3D (35.09%), MS3N (33.83%) and MS2D (33.02%) with at par performance. Remaining culture media had low callus forming ability including MS5T (18.87%) that has significantly minimum counts.

Effects of different cytokines (alone) in varying concentration on callus induction are presented in the Table 10. Maximum callus induction were recorded from cultured mature embryo on nutrients media MS3ip (35.06%) closely followed by a group of four nutrients media viz: MS2ip (29.95%), MSip (29.75%), MS.5ip (29.93%) and MS.2ip (29.12%). Remaining culture media had low callus forming ability including MS5K that has significantly minimum counts (8.79%).

Combined effects of different added auxins and cytokines in varying concentration on callus induction are presented in the Table 4. Maximum callus induction was recorded on a group of three induction media. Callus formation was exhibited by culture media combination MS5N.5B (23.79%) and MS.5N.5B (24.69%). Remaining culture media performed in between them.

Among different culture media combination amended with NAA, 2,4-D and 2,4,5-T culture media, MS.5N (40.31%) was found remarkably superior followed by nutrient media MSN (34.28%) and MS3N (33.96%) for shoots proliferating ability from cultured mature embryo. The lowest performance was exhibited by culture medium MS5D (2.20%). Remaining culture media performed intermediately. Among different culture media combination of a group of three nutrient media i.e. MSB (45.92%), MS5ip (44.90%) and MS2B (44.53%) were proved remarkably superior for shoot proliferating efficiency Nutrient medium MS3ip (33.70%) was responded poorest in this regard. Remaining culture media performed shoots in between them.

Combined effect of different added auxin and cytokines in varying concentration on shoot proliferating efficiency are presented in the Table 11. Among different culture media combination, inoculation media MS2N.5ip (55.22%), MS3N.5ip (53.17%) and MSN5ip (45.01%) were proved remarkably superior for shoots proliferating efficiency. Nutrients media MS5D.5Kn (3.05%) and MS4D.5KN (4.81%) was found low responding in this regard. Remaining culture media performed in between them. Inoculation media MS.5N (2.53), MSN (2.52), MS.5D (2.30), MS2N (2.15) and MS3N (2.08) had maximum count. Nutrient medium MS5T (0.950) produced shoot in minimum number. Among the different culture media combination supplemented with cytokines as sole, inoculation media MS.5ip (8.40) , MSB (6.25) and MS2B (5.65) were found significantly superior. Nutrient medium MS3ip (1.59) proved to be a lowest performer in this regard. Remaining nutrient media performed in between them.

Among the different culture media combination tested, nutrient media MS2N.5ip (9.30), MSN.5ip (7.98) and MS5N.5B (1.54) and MS5D.5K (1.57) proved to be a lowest performer in this regard. Remaining nutrient media performed immediately. Nutrient media MS2N (2.14cm), MSN (2.03cm) and

MS2D (1.95) had produced shoot of higher length. Culture medium MS5D (0.61cm) produced shoot of minimum length. Remaining culture media performed in between them. Effect of different cytokines (alone) in varying concentration on mean shoot length are presented in the table 4. The shoot of higher length was recorded in media MSB (2.88cm) and MS3B (2.65cm). The culture medium MS3ip (0.95) produced shoots of minimum length.

The shoot of higher length was recorded on a group of 12 culture media including MS2N.5ip (2.50cm) and MSB.5ip (2.25). Culture medium. MS5N.5ip (0.87cm) produced shoots of minimum length. Remaining culture media responded intermediately.

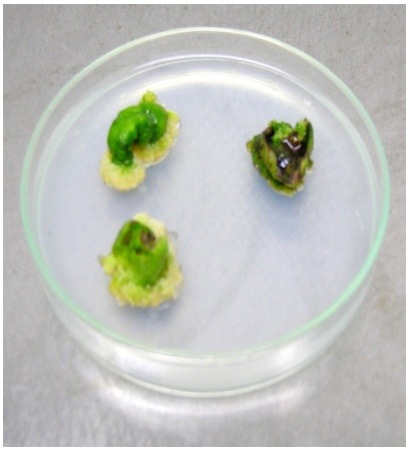

Plate-1

Plate-2

Plate-3

Table 1: Effects of different surface sterilizing and antifungal agents on recovery of aseptic cultures in gerbera.

Treatments	Concentration (%)	Exposure Time (In minute)	Aseptic culture (%)	Survival of explants (%)
$Ca(OCl)_2$	5	10	12.37^{p} (20.56)	23.64^{k} (29.08)
$Ca(OCl)_2$	5	15	15.42^{o} (20.56)	28.01^{j} (31.94)
$Ca(OCl)_2$	5	20	36.49^{l} (23.10)	43.97^{f} (41.52)
$Ca(OCl)_2$	10	10	24.91^{n} (37.15)	45.98^{ef} (42.68)
$Ca(OCl)_2$	10	15	28.92^{m} (29.92)	54.54^{d} (47.59)
$Ca(OCl)_2$	10	20	38.05^{kl} (35.52)	62.57^{c} (52.26)
$Ca(OCl)_2$	15	10	37.25^{l} (38.07)	30.42^{i} (33.46)
$Ca(OCl)_2$	15	15	44.99^{j} (37.60)	44.15^{f} (41.62)
$Ca(OCl)_2$	15	20	46.01^{ij} (42.11)	24.37^{k} (29.56)
$Ca(OCl)_2$	20	10	64.59^{e} (53.46)	65.46^{b} (53.99)
$Ca(OCl)_2$	20	15	47.03^{i} (43.28)	64.17^{c} (53.21)
$Ca(OCl)_2$	20	20	48.75^{hi} (44.27)	62.71^{a} (52.34)
$HgCl_2$	0.1	2	39.92^{k} (39.17)	59.15^{d} (50.25)
$HgCl_2$	0.1	5	43.02^{j} (40.97)	25.62^{ik} (30.39)
$HgCl_2$	0.2	2	50.23^{gh} (45.11)	74.13^{a} (59.41)
$HgCl_2$	0.2	5	53.02^{g} (46.71)	34.51^{h} (35.96)

$HgCl_2$	0.2	10	76.86^{b} (61.23)	62.63^{c} (52.30)
Bavistin + Ca $(OCl)_2$	0.5 + 10	30	71.29^{cd} (57.58)	29.91^{i} (33.14)
Bavistin + Ca $(OCl)_2$	0.5 + 15	30	73.68^{c} (59.11)	37.64^{g} (37.83)
Bavistin + Ca $(OCl)_2$	0.5 + 20	30	64.72 ^{e} (53.54)	47.20^{e} (43.38)
Bavistin + $HgCl_2$	0.5 + 0.1	20	56.32^{f} (48.61)	67.35^{b} (55.13)
Bavistin + $HgCl_2$	0.5 + 0.2	20	69.09^{d} (56.20)	63.61^{c} (52.88)
Bavistin + $HgCl_2$	0.5 + 0.1	30	78.76^{b} (62.54)	74.32^{a} (59.53)
Bavistin + $HgCl_2$	0.5 + 0.2	30	83.54^{a} (66.05)	64.61^{bc} (53.48)
Mean			50.22 (45.06)	49.61 (44.70)
CD (0.05)			2.99	2.85

- **Ca $(OCl)_2$**: Calcium hypochlorite, **$HgCl_2$**: Mercuric chloride
- Values with in column followed by different letters are significantly different at 5% probability level.

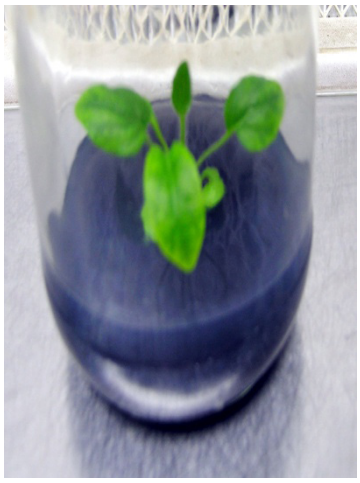

Plate-4

Plate-5

Plate-6

Table 2: Effect of different auxins (alone) in varying concentrations on in vitro response of mature embryo cultures in gerbera.

Culture Media	Callus Induction (%)	Shoot Proliferating explant (%)	No. of Shoots/ explant	Mean shoot length (In cm)
MS.5D	24.67^{e} (29.76)	11.01^{g} (19.35)	2.30^{a} (8.68)	1.41^{ab} (6.78)
MSD	29.52^{c} (32.89)	9.80^{h} (18.20)	2.01^{b} (8.12)	1.62^{a} (7.27)
MS2D	33.02^{ab} (35.06)	7.12^{hi} (15.43)	1.87^{b} (7.84)	1.95^{a} (7.96)
MS3D	35.09^{a} (36.31)	5.21^{ij} (13.13)	1.95^{b} (7.97)	1.18^{b} (6.21)
MS4D	34.32^{a} (35.84)	3.89^{i} (11.28)	1.79^{b} (7.62)	0.88^{b} (5.35)
MS5D	28.31^{d} (32.13)	2.20^{i} (8.28)	0.55^{b} (3.77)	0.61^{b} (4.42)
MS.5N	24.00^{e} (29.32)	40.31^{a} (39.40)	2.53^{a} (9.09)	1.73^{a} (7.50)
MSN	28.44^{cd} (32.21)	34.28^{b} (35.82)	2.52^{a} (9.10)	2.03^{a} (8.07)
MS2N	30.30^{bc} (33.38)	33.96^{bc} (35.63)	2.15^{a} (8.38)	2.14^{a} (8.29)
MS3N	33.82^{a} (35.54)	31.00^{c} (33.82)	2.08^{a} (8.21)	0.87^{b} (5.33)

MS4N	28.38^{d} (32.17)	25.98^{d} (30.62)	1.80^{b} (7.69)	0.75^{b} (4.90)
MS5N	24.96^{de} (29.95)	9.67^{h} (18.09)	1.81^{b} (7.71)	0.48^{b} (3.90)
MS.5T	16.44^{g} (23.90)	24.70^{d} (29.78)	1.85^{b} (7.79)	1.37^{b} (6.66)
MST	19.68^{fg} (26.32)	17.56^{e} (24.75)	2.07^{ab} (8.25)	1.78^{a} (7.63)
MS2T	22.01^{f} (27.96)	16.11^{e} (23.64)	1.86^{b} (7.83)	1.72^{a} (7.49)
MS3T	22.88^{ef} (28.56)	14.59^{ef} (22.43)	1.62^{b} (7.30)	1.13^{b} (6.08)
MS4T	23.39^{e} (28.91)	11.99^{fg} (20.23)	1.05^{bc} (5.86)	1.07^{b} (5.91)
MS5T	18.87^{g} (25.72)	9.97^{gh} (18.37)	0.95^{c} (5.57)	1.02^{b} (5.79)
Maen	**26.56 (23.24)**	**17.19 (23.24)**	**1.82 (7.60)**	1.32 (6.42)
CD(0.05)	3.49	3.21	1.02	1.00

Values within column followed by different letters are significantly different at 5% probability level.

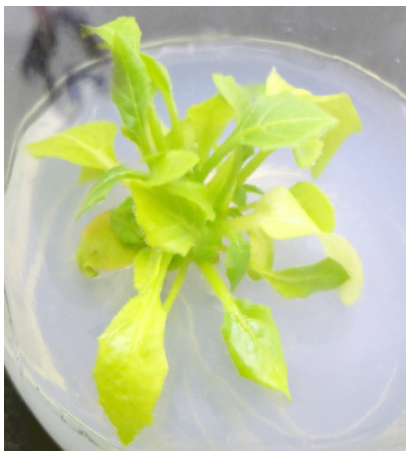

Plate-7

Plate-8

Plate-9

Table 3: Effect of different Cytokinins (alone) in varying concentrations on in vitro response of mature embryo cultures in gerbera.

Culture Media	Callus Induction (%)	Shoot Proliferating expladnt (%)	No. of Shoots/ explant	Mean shoot length (In cm)
MS.5B	13.05^{f} (21.16)	35.80^{d} (36.73)	4.32^{b} (11.55)	2.63^{a} (9.27)
MSB	15.34^{f} (23.04)	45.92^{a} (42.64)	6.25^{a} (14.33)	2.88^{a} (9.70)
MS2B	19.86^{d} (26.44)	44.53^{a} (41.84)	5.65^{a} (13.59)	2.83^{a} (9.61)
MS3B	18.20^{e} (25.24)	38.28^{c} (38.20)	4.63^{ab} (12.37)	2.65^{a} (9.31)
MS4B	15.90^{ef} (23.48)	36.71^{cd} (37.28)	2.41^{b} (8.77)	2.58^{a} (9.18)
MS5B	14.52^{f} (22.38)	35.44^{d} (36.52)	2.32^{b} (8.67)	2.52^{a} (9.11)
MS.5Kn	19.18^{de} (25.93)	34.72^{d} (36.09)	2.41^{b} (8.84)	2.53^{a} (9.13)
MSKn	14.62^{f} (22.43)	34.35^{d} (35.86)	3.46^{b} (10.72)	2.55^{a} (9.15)
MS2Kn	20.13^{d} (26.65)	39.90^{bc} (39.16)	3.95^{b} (11.13)	2.65^{a} (9.33)
MS3Kn	24.22^{c} (29.46)	37.54^{c} (37.77)	3.49^{b} (10.76)	2.59^{a} (9.23)

MS4Kn	13.80^{f} (21.79)	35.62^{d} (36.63)	2.35^{b} (8.74)	2.55^{a} (9.16)
MS5Kn	8.79^{g} (17.21)	34.92^{d} (36.21)	1.97^{b} (7.91)	2.52^{a} (9.09)
MS.1ip	25.15^{c} (30.08)	34.92^{d} (36.20)	3.59^{b} (10.66)	1.89^{ab} (7.90)
MS2ip	29.12^{b} (32.64)	40.42^{b} (39.46)	4.20^{b} (11.73)	2.25^{a} (8.62)
MS.5ip	29.13^{b} (32.65)	44.90^{a} (42.06)	8.40^{a} (16.77)	2.20^{a} (8.52)
MSip	29.75^{b} (33.03)	39.12^{c} (38.70)	1.79^{b} (7.68)	0.95^{b} (5.59)
MS2ip	29.95^{b} (33.15)	35.65^{d} (36.64)	1.73^{b} (7.55)	1.30^{b} (6.50)
MS3ip	35.06^{a} (36.29)	33.70^{d} (35.47)	1.59^{b} (7.24)	1.00^{b} (5.73)
Maen	**20.88**	**37.91**	**3.58**	**2.28**
CD(0.05)	**3.86**	**3.41**	**3.17**	**1.18**

Values within column followed by different letters are significantly different at 5% probability level.

Plate-10

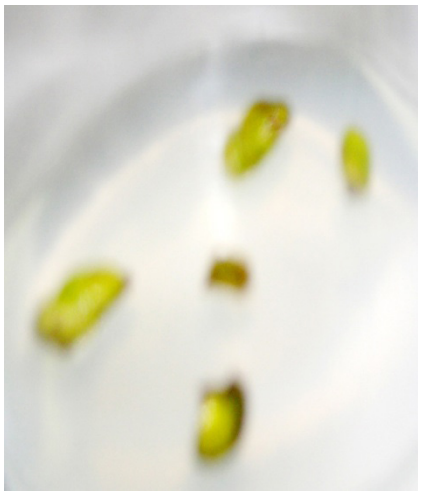

Plate-11

Plate-12

Plate-13

Plate-14

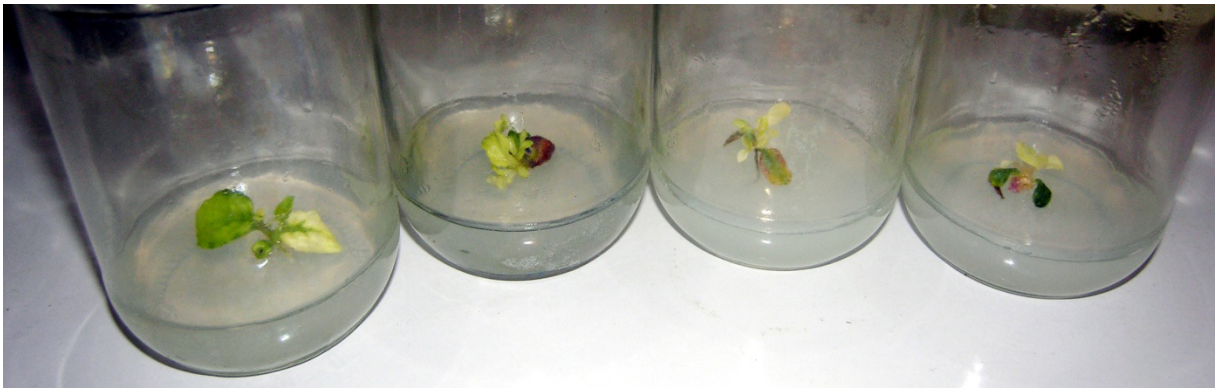

Plate-15

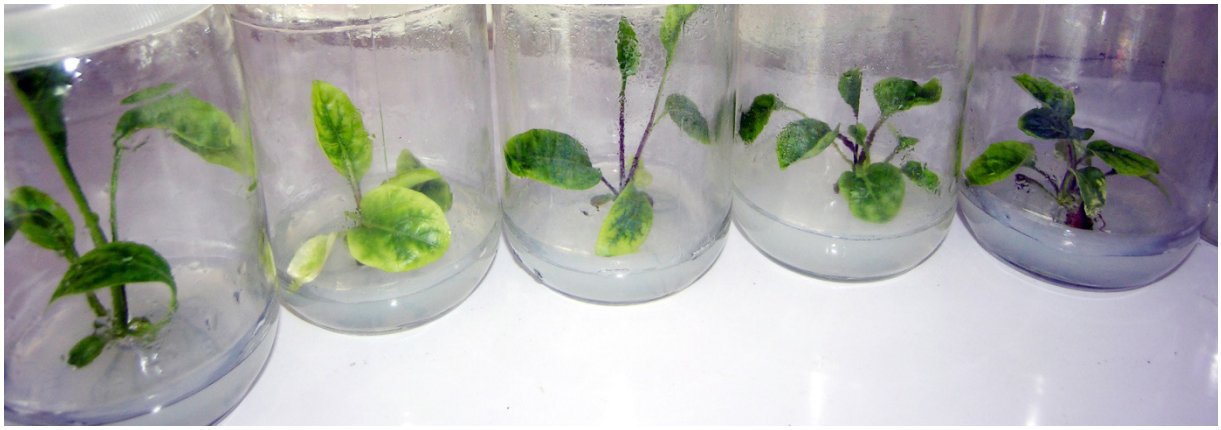

Plate-16

Table 4: Combined effects of added different auxins and Cytokinins on in vitro response for cultured mature embryo in gerbera.

Culture Media	Callus Induction (%)	Shoot Proliferating explant (%)	No. of Shoots/ explant	Mean shoot length (In cm)
MS.5D.5B	27.01^{a} (31.30)	19.25^{ij} (26.01)	2.54^{d} (8.98)	1.79^{a} (7.39)
MSD.5B	30.36^{d} (33.42)	22.64^{h} (28.40)	2.61^{d} (9.11)	1.87^{a} (7.56)
MS2D.5B	41.12^{a} (39.87)	24.73^{g} ()29.80	2.32^{d} (8.62)	1.95^{a} (7.76)
MS3D.5B	43.59^{a} (41.30)	19.12^{j} (25.91)	2.36^{d} (8.70)	1.12^{b} (5.96)
MS4D.5B	35.22^{c} (36.38)	17.11^{jk} (24.42)	2.31^{d} (8.58)	0.89^{b} (5.41)
MS5D.5B	31.02^{d} (33.83)	13.44^{im} (21.48)	1.23^{d} (6.36)	0.86^{b} (5.32)
MS.5D.5Kn	25.03^{e} (30.00)	14.70^{kl} (22.52)	2.14^{d} (8.36)	2.02^{a} (8.17)
MSD.5Kn	30.00^{d} (33.19)	11.97^{m} (20.22)	2.44^{d} (8.85)	2.08^{a} (8.29)
MS2D.5Kn	33.62^{c} (35.41)	9.20^{n} (17.63)	2.02^{d} (8.100	1.69^{ab} (7.47)
MS3D.5Kn	34.92^{c} (36.19)	6.82^{no} (15.09)	1.89^{d} (7.90)	1.72^{a} (7.53)
MS4D.5Kn	33.62^{c} (35.41)	4.81^{op} (12.61)	1.83^{d} (7.77)	0.86^{b} (5.32)
MS5D.5Kn	34.92^{c} (36.19)	3.05^{p} (10.05)	1.57^{d} (7.19)	0.67^{b} (4.69)
MS.5N.5B	32.19^{c} (34.98)	36.51^{d} (37.16)	4.82^{bc} (12.47)	1.72^{a} (7.53)

MSN.5B	27.97^{de} (31.90)	41.67^{c} (40.19)	6.80^{ab} (14.97)	1.85^{a} (7.81)
MS2N.5B	24.69^{e} (29.77)	44.08^{bc} (41.58)	6.72^{b} (14.87)	1.59^{b} (7.24)
MS3N.5B	31.12^{d} (33.89)	33.35^{e} (35.26)	2.65^{d} (9.36)	1.54^{b} (7.13)
MS4N.5B	31.56^{cd} (34.16)	30.52^{f} (33.52)	2.57^{d} (9.22)	1.19^{b} (6.26)
MS5N.5B	26.80^{e} (31.14)	21.54^{hi} (27.64)	1.54^{d} (7.13)	1.02^{b} (5.79)
MS.5N.5ip	25.02^{e} (29.98)	38.20^{d} (38.16)	2.72^{cd} (9.49)	1.82^{s} (7.75)
MSN.5ip	23.79^{e} (29.17)	45.01^{a} (42.12)	7.98^{a} (16.28)	2.25^{a} (8.62)
MS2N.5ip	26.43 (30.91)	55.22^{a} (47.98)	9.30^{a} (17.69)	2.50^{a} (9.09)
MS3N.5ip	33.21^{c} (35.17)	53.17^{a} (46.80)	7.82^{a} (16.23)	2.12^{a} (8.37)
MS4N.5ip	36.12^{bc} (36.93)	36.12^{d} (36.92)	2.03^{d} (8.19)	0.97^{b} (5.65)
MS5N.5ip	39.00^{ab} (38.63)	21.20^{i} (27.40)	1.70^{d} (7.49)	0.87^{b} (5.35)
Mean	**31.18 (33.85)**	**25.98^{i} (29.54)**	**3.41 (10.08)**	**1.54 (6.98)**
CD (0.5%)	**4.73**	**2.71**	**2.77**	**1.03**

Values within column followed by different letters are significantly different at 5% probability level.

References

Parthasarthy, V.A., Parthasarthi, U. and Nagaraju, V. 1996. Morphogenetic response of gerbera shoots to media and benzyl amino purine. *Annals of plant physiology* **10**: 34-39.

Murashige, T., Sepra, M. and Jones, J.B 1974. Clonal multiplication of Gerbera through tissue culture. *Horti Science*, 9: 175-180

Aswath, C., Deepa, S.M. and Chaudhary, M.L. 2003. Commercial multiplication of gerbera (*Gerbera jasmesoni* Bolus) through in vitro shoot tip culture. *Journal of Ornamental Horticulture* **6**: 303-309

Bhaskaran, S. and Smith, R.H. 1990. Regeneration in tissue culture a review. *Crop Science* **30**: 1328-1337.

Bhatia, R., Singh, K.P., Jhang, T. and Sharma, T.R. 2009. Assessment of clonal fidelity of micro propagated gerbera plants by ISSR markers. *Scientia Horticulturae,* 208-211.

Chu, C.Y. and Huang, M.C. 1983. *In vitro* formation of Gerbera (*Gerbera hybrid* Hort.) plantlets through excised scape culture. *J. Jpn. Soc. Hort. Sci.* **52**: 45-50.

Debergh, P.C.A. (1991). Acclimatization techniques of plants from *in vitro.* Acta Hort. **289:**291-299.

Eduardo, F.A., Moacir, P., Josh, E.B.P.P. and Marcio, H.P.B. 1991. *In vitro* establishment and plantlet regeneration of *Gerbera jamesonii* Bolus ex. Hook by tissue culture technique. *Peaq. Agropec. Brac. Brasillia* **26**(2): 269-273.

Fakharfeshani, M., Bhagheri, A. and Sharifi, A. 2012. Disinfection effects of nano silver fluilds in Gerbera *(Gerbera jamesonii*) capitulum tissue culture, *J. Biol. Environ. Sci.* **6**(17): 121-127.

Ghani, M., Kumar, S. and Thakur, M. 2012. *In vitro* propagation and assessment of plant genetic fidelity by RAPD and SSR markers in gerbera, *Research journal of biotechnology* **7**(4).

Hedtrich, C.M. 1979. Production of shoots from leaves and propagation of Gerbera jamesonii. *Gartenbau wissenschaft* **44**: 1-3.

Kageyama, K., Kazunori, Y. and Shiggestoshi, M. 1991. Sometic embryogenesis in suspension culture of mature seed of Cucumis melo L. *Japan J. Breed* **41**: 273-278

Laliberte, S., Chretinn, I. and Veith, J. 1985. In Vitro plantlet production from young captulum explants of Gerbera jamesonii. *Horti Sci*. **20**: 137-139

Nagarajun, P., Mckenzie, J.S. and Walton, P.D. 1986. Embryogenesis and plant regeneration of *Medicago spp.* in tissue culture. *Plant cell Rep*. **5**: 77-80.

Patidar, H., Vidhya Sankar, M., Tripathi, M.K. and Patel, R.P. 2013. Effect of auxin and Cytokinin on shoot formation of Gerbera jamesonii. *Plant Cell Biotech. Mol. Biol*. **14**(3&4): 128-138.