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Research Article

IN-VITRO MORPHOGENESIS STUDIES IN *Gerbera jamesonii BOLUS* EX HOOKER F.

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Abstract- During present investigation, mature embryos were used as explant sources and cultured on MS medium amended with diverse auxins and cytokinins in varying concentrations as sole as well as in different combinations Culture media MS3D.5B (MS + 3.0 mg. l^{-1} 2, 4 D + 0.5 mgl^{-1}BA + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar powder) proved well for callus initiation. Culture media MS2N.5iP/MS3N.5ip (MS + 2.0/3.0mg l^{-1} NAA + 0.5 mgl^{-1}2-ip + 30.0 gl^{-1} sucrose + 7.5 gl^{-1} agar) exhibited higher response *i.e.* number of shoot proliferating explants and number of shoot (s) per explant. However, shoot of higher length was recorded on nutrient medium MSB/MS2B (MS + 2.0/3.0 mgl^{-1} BA + 30.0 gl^{-1} sucrose + 7.5 g l^{-1} agar). Higher rooting response (root proliferating efficiency, number of roots and mean root length) was exhibited by rooting medium MS.1IB (MS + 0.1 mgl^{-1}IBA + 15.0gl^{-1} sucrose + 7.5 g l^{-1} agar). The plantlets were transferred to pots and hardened in Environmental Growth Cabinet and Net House during initial weaning period and transferred to field successfully. Phenotypic normal plants were recovered.

Keywords- *Gerbera jamesonii*, Mature embryo culture, Direct and indirect organogenesis, Direct and indirect somatic embryogenesis, Embryogenesis and plantlet regeneration

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Introduction

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 [1]. 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 honors 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 micropropagation offers a exclusive option for mass multiplication of true to type plantlets. Micropropagation 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 improved product. Clonal propagation of gerbera has effectively employed this technique to produce genetically pure plants ensuring a unique colour pattern for each variety. This method enables a million fold expansions per year of a desired plant [2-3].

Although, all plant cells are derived from the fertilized egg cell and contain identical information, callus derived from somatic cells varies in competence to express totipotency (*i.e.* their genetic ability to produce plants). In gerbera, various explants have been used efficiently to produce regenerable cultures *via* somatic embryogenesis and/or organogenesis are shoot tips [2 and 4-5], leaf discs [6-16], mature embryo [17], capitulum [5, 10, 11, 18-20, 21], petiole [12, 22], flower stack [8], floral buds [23-25], floral bracts [26], torus [20], inflorescence [27] and cell suspension cultures (13 , 17,28] with varying degree of success.

Although, a few studies have been conducted in some laboratories worldwide to obtain prolific *in-vitro* culture system of *Gerbera jamesonii* with varying regeneration potential, little work has been done so for in M.P. on these aspects. Furthermore, *in-vitro* response of plant tissues depends on genotype, the physiological status of the donor plant, the type of explant, components of culture medium and their interactions [29]. In view of this, an attempt was made to compute optimum quantity of plant growth regulators added in culture medium and other physical factors exhibiting higher *in vitro* morphogenesis by means of mature embryo culture.

MaterialsandMethods

Mature embryo explant of Pink cultivar of *Gerbera jamesonii* were cultured on different fortifications of two basal media *viz*: Murashige & Skoog (1962) [30] and Gerbera multiplication medium. Seeds of Pink cultivar were collected from Balaji Nursery, Indore (M.P.). During the course of preliminary investigations, MS basal medium was found more responsive than Gerbera medium (data not presented), hence, for later experimentations basal MS medium was used. All initial culture media were made using readymade basal medium (HiMidia™) and supplemented

with three different sets of plant growth regulators to fortify MS basal media. In first set: three different auxins, *namely*: 2, 4-D, NAA and 2, 4, 5-T (alone), in second set: three diverse cytokinins *viz*: BAP, Kn and 2-ip (sole) and in third set: different auxins NAA and 2,4-D in combinations with cytokinins BAP, Kn and 2-ip in varying concentrations. Apart from MS basal macro and micro salts, vitamins, all initial culture media was supplemented with 30.0 $g.l^{-1}$ sucrose and the final volume was made to 1000 ml and pH was adjusted to 5.8 ± 0.1 with 1N KOH solution. After adjusting the pH, agar powder @ 7.5 $g.l^{-1}$ was added to the media as a solidifying agent. Warm culture media, still in liquid state was poured into baby food bottles (50-60 ml / bottle) and culture tubes (15-20 ml/ tube) followed by autoclaving at 121 ^{o}C under 15 psi pressures for 25 minutes. However, in case of pouring in petridishes, autoclaved warm culture media was poured into pre-sterilized 100 x 17 mm glass petridishes (25-30 ml / dish) under aseptic conditions of Laminar Air Flow Clean Air Cabinet. Readymade basal media, plant growth regulators and other ingredients were procured from Hi-media Laboratories, Mumbai, India.

Mature embryos were excised from seeds. For surface sterilization of seeds, seeds were washed with 2% Tween 20 (v/v) (a commercial detergent) for 15-20 minutes to remove dirt and residues. Then the seeds were treated with 70% (v/v) ethanol for 30 seconds. Then seeds were subjected to different concentrations of Bavistin® (BASF, Germany) followed by dipping in two surface sterilants i.e. $Ca(OCl)_2$ and $HgCl_2$ in different concentrations and combinations for diverse durations [Table-1]. Finally, seeds were rinsed 4-5 times with sterile double distilled water and soaked for 12 hours in sterilized double distilled water. Mature embryos from pre-soaked seeds were excised and cultured on the explant inoculation medium in 100 x 17 mm glass petri dishes and sealed with Lab film (Parafilm®) and incubated under complete darkness at 25±2°C for one week. Later *in vitro* cultured explants were subjected to photoperiod regime of 16 hours light / 8 hours dark cycle at an intensity of 2000-lux luminance provided by Photo-synthetically Active Radiation lamps at 25±2 ^{0}C and 60% RH. After 4-5 weeks of initial culturing, callus cultures were sub cultured on initial medium for regeneration of plantlets. Multiple shoots, obtained from direct organogenesis, (auxiliary bud proliferation) were transferred to elongation medium which was MS basal medium supplemented with 1.0 $mg.l^{-1}$ GA_3, 20.0 $g.l^{-1}$ sucrose and 7.5 $g.l^{-1}$ agar powder. Cultured baby food bottles /culture tubes were subjected to 25 ± 2^{o}C temperature and photoperiod regimes of 60μmol m^{-2} s^{-1} luminance provided by cool fluorescent tubes for 16 hr. When root formation was not attained on regeneration medium, plantlets were subsequently transferred to MS rooting medium supplemented with different concentrations of IBA, NAA, Kn and combination of IBA with BA and Kn, 15.0 g. l^{-1} sucrose and 7.5 $g.l^{-1}$ agar powder. For regeneration, elongation and rooting, reduced level of sucrose was applied on the basis of work conducted by various scientists as well as preliminary experiences of this laboratory.

The complete plants (shoot with roots) were carefully washed with running tap water to remove the adhering agar and were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were transferred under 30±2^{o}C and 60 ± 5% Relative Humidity for 20-25 days to an Environmental Growth Chamber for hardening. Latter these regenerants were transferred to the Green and Net House for 30-35 days for acclimatization. Acclimatized plants finally were transferred to field.

The observations were recorded for callus induction, number of shoot proliferating explants, average number of shoots per explant and mean shoot length for mature embryo culture and for *in-vitro* rooting, for root proliferation, number of root (s) and mean root length. The experiment was laid out in Completely Randomized Design to find out the significance of different culture medium combination. Each treatment was consisting of two replications. Per replication approximately 100-120 explants were excised and cultured on each media. The arc-sine transformations were made before the analysis of data, since all data were in percentage. The data were analyzed as per method suggested by Snedecor and Cochran (1967) [31].

Result and Discussion

Mature embryos of Pink cultivar of gerbera were cultured on different fortifications of MS medium. The importance of controlling contamination for achieving the goals of tissue culture, different methods with its own advantages and disadvantages have been suggested till now in different flowering cops. Applying Sodium hypochlorite, mercuric chloride, alcoholic and antibiotic solutions are the most common examples. The sterilization procedure for capitulum explants of gerbera earlier was applied by Shabanpour (2011) [6]. He pretreated explants with 70% ethanol for 90 seconds and 15 minutes with 1% aqueous solution of sodium hypochlorite and recovered only 34% disinfected explants. In our investigation, application of 0.5% bavistin in combination with 0.1% $HgCl_2$ exposed for 30 minutes supported higher percentage of aseptic culture (77.18%) as well as survival percentage (74.38%) [Table-1].

Table-1 *Effects of different surface sterilizing and antifungal agents on recovery of aseptic cultures*

Treatments	Concentration (%)	Exposure time (in minutes)	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^{i}(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^{j} (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.
Figures in parenthesis are transformed values (Arc-sine transformation).
Values with in column followed by different letters are significantly different at 5% probability level.

In present study, cultured mature embryos followed either direct or indirect pathway of plant regeneration depending upon the nature of different plant growth regulators supplemented in basal culture media. In direct approach, plantlets were regenerated on explant surface directly without callus formation (*via* direct organogenesis) and in indirect mode; plantlets were originated from callus mass (either *via* indirect somatic embryogenesis or organogenesis).

The first response of cultured mature embryo was similar after 4-7 days and mostly independent from culture media. All explants became swollen and no callus proliferation was observed during first few days. Callus proliferations usually started from embryonic axis after 4-7 days of culture [Fig-1A&B]. In indirect organogenesis, shootlets developed from the nodules arising on the surface of the callus [Fig-1C]. Shoot formation started approximately 10 days from initial culturing [Fig-1D], however, the duration varied from culture to culture and in a few cases shootlets formed after 35 days of initial culture. Plantlets also regenerated *via* indirect organogenesis [Fig-1E-F]. In case of direct organogenesis, adventitious structures were developed on explant surface and started approximately 10-14 days from initial culturing. However, the duration varied from culture to culture and in a few cases adventitious formed after 28-35 days. With time, these adventitious structures formed shootlets [Fig-1G-I]. In cases where, root formation was not attained, shoots were subsequently transferred to the rooting medium [Fig-1J]. Most of the calli, after prolonged culturing on the induction media gave rise to plants. However, transfer into elongation medium allowed higher plant formation and growth rate. During the present investigation, plantlets were regenerated in huge numbers, which may be used for mass *in vitro* propagation. Rooted plants were transferred under 28±2°C and 65±5% relative humidity for 20-25 days to an Environmental Growth Cabinet for hardening [Fig-1K]. Later these regenerants were transferred to the Net house/ Poly house [Fig-1L] for 25-30 days before transferring to the field.

Fig-1 Plant regeneration in *Gerbera jamesonii* from cultured mature embryo: A. Formation of callus after 15-20 days; B. Initiation of somatic embryo of globular stage; C. Shoot proliferation after 20-25 days; D. Multiple shoots formation *via* indirect organogenesis after 30-35 days; E. Multiple shoots formation *via* indirect somatic embryogenesis after 30-35 days; F. *Multiple shoots and root formation via indirect somatic embryogenesis;* G-I. Formation of multiple shoots *via* direct organogenesis; J. *In vitro* rooting; K. Regnerants transferred in Environmental Growth Chamber after 40-45 days and L. Plantlet transferred in Net House after 45-50 days for hardening.

The analysis of variance presented in [Table-2-5] revealed that there were highly significant ($p<0.01$) differences existing among the response of different culture media combinations in terms of overall callus induction, shoot proliferating efficiency, number of shoot (s) per explant, mean shoot length for mature embryo explant [Table-2-4] and root proliferating efficiency, number of root (s) and mean root length [Table-5] in terms of *in-vitro* rhizogenic response. It indicates the presence of considerable amount of variability amongst different culture media combinations.

Table-2 *Effect of different auxins alone in varying concentrations on in vitro response of mature embryo cultures*

Culture Media	Callus induction (%)	Shoot proliferating explant (%)	No. of shoot (s)/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^{j} (11.28)	1.79^{b} (7.62)	0.88^{b} (5.35)
MS5D	28.31^{d} (32.13)	2.20^{j} (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)
Mean	**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**

Figures in parenthesis are transformed values (Arc-sine transformation).
Values within column followed by different letters are significantly different at 5% probability level.

Table-3 *Effect of different cytokinins alone in varying concentrations on in vitro response of mature embryo cultures*

Culture media	Callus induction (%)	Shoot proliferating explant (%)	No. of shoot(s)/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**

Figures in parenthesis are transformed values (Arc-sine transformation).
Values within column followed by different letters are significantly different at 5% probability level.

For mature embryo culture, culture media MS3D.5B/ MS2D.5B/ MS3N.5ip (amended with relatively higher concentration of an auxin in combination with a lower concentration of a cytokinin) facilitate higher degree of callus induction. Culture media MS3D/MS4D/ MS3N/ MS2D (containing a moderate to higher concentration of 2,4-D and NAA) as well as MS3ip/ MS2ip/ MSip/ MS.5ip (containing a moderate to higher concentration of 2-ip) also initiated callus, however, frequencies were found moderate. Culture medium containing other auxin 2,4,5-T and cytokinins : BA and Kn in similar concentrations induced callus in lower frequencies which suggested that 2,4-D either sole or in combination with cytokinin is better for callus proliferation from cultured mature embryos. Aswath and Choudhary (2002) [8], Kumar *and* Kanwar (2006) [12] and Patidar *et al.* (2013) [17] also documented similar findings for diverse explants cultures in gerbera.

In terms of shoot proliferating ability, numbers of shoots and mean shoot length, culture media containing auxin alone in varying concentrations yielded lower shoot proliferation efficiency, *i.e.* lesser number of explants proliferated shoots, lesser numbers of shoot (s) per explant and shoots of minimum length. Shoot proliferation in very lower frequency has been evidenced on medium supplemented with 2,4-D as sole, however medium fortified with NAA responded much better. Earlier studies reported that cytokinin is required in shoot organogenesis In order to achieve the best *in-vitro* response basal medium was also fortified with different types of cytokinins in varying concentrations. During present study, culture media fortified with cytokin in as alone in moderate concentrations (BAP in rage of 1.0-2.0 mg.l^{-1} and 2-ip in range of 0.1-0.5 mg.l^{-1}) were responded well (more than 40% explants proliferated shoots) suggested that a moderate concentrations of cytokin in is required for this purpose. Earlier studies also demonstrated that lower level of BAP induced early bud sprouting in many species. Multiple shoot induction required the presence of cytokin in the culture medium, which is in closely relevant to finding in other studies in gerbera [10,14,16,17]. However, shoot proliferating ability and number of shoot (s) were

enhanced when auxins and cytokinins were added into culture medium in combinations. Culture media MS2N.5ip/MS3N.5ip containing relative higher concentrations of an auxin (NAA) in combination with a lower concentration of a cytokinin (BA), exhibited higher *in vitro* response (more than 53% explants proliferated shoots with 7 shoots/explant) than culture medium containing a higher concentrations of cytokinins as well as an auxin as sole. This finding is an accordance with Hasbullah *et al*. (2008) [22] and Patidar *et al*. (2013) [17] as they suggested that many commercial ornamental plants are being propagated by *in vitro* culture on the culture medium containing auxins and cytokinins. Furthermore, addition of strong auxin (NAA) with BAP promoted better shoot formation compared to weak auxin like IBA [18]. However, addition of auxin in higher concentration as compared to cytokinin in the culture medium resulted in the inhibition of shoot formation. In terms of mean shoot length culture medium fortified with BAP in range of 0.5-3.0 mgl^{-1} produced shootlet(s) of higher length.

Table-4 *Combined effects of added different auxins and cytokinins on in vitro response for cultured mature embryo.*

Culture Media	Callus induction (%)	Shoot proliferating explants (%)	No. of shoot(s)/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^{lm} (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**

Figures in parenthesis are transformed values (Arc-sine transformation).
Values within column followed by different letters are significantly different at 5% probability level.

Table-5 *Combined effect of different plant growth regulators added in MS basal media for induction of in vitro rooting.*

Culture Media combinations ▼	Plant growth regulators mg l^{-1}				*In vitro* rooting response		
	IBA	NAA	BAP	Kn	Root proliferating shootlets (%)	No. of root (s) per shootlet	Root length (in cm)
MS.1IB	0.1	-	-	-	98.74^{a} (83.60)	10.71^{a} (19.08)	6.15^{a} (14.34)
MS.5IB	0.5	-	-	-	96.17^{a} (78.84)	10.33^{b} (18.73)	4.32^{b} (11.98)
MSIB	1.0	-	-	-	93.94^{b} (75.74)	8.54b^{c} (16.97)	5.07^{b} (13.00)
MS2IB	2.0	-	-	-	89.61^{c} (71.19)	6.81^{d} (15.08)	4.02^{b} (11.55)
MS3IB	3.0	-	-	-	86.50^{d} (68.43)	6.06^{e} (14.22)	2.56^{c} (9.19)
MS.1N	-	0.1	-	-	92.81^{b} (74.44)	7.42^{cd} (15.79)	2.37^{c} (8.82)
MS.5N	-	0.5	-	-	89.55^{c} (71.12)	8.32^{c} (16.74)	2.27^{c} (8.61)
MSN	-	1.0	-	-	86.21^{d} (68.24)	6.09^{e} (14.28)	1.82^{c} (7.68)
MS2N	-	2.0	-	-	84.88^{d} (67.10)	6.16^{de} (14.36)	2.21^{c} (8.50)
MS3N	-	3.0	-	-	79.49^{ef} (63.04)	5.51^{e} (13.56)	2.76^{c} (9.55)
MS.1Kn	-	-	-	0.1	84.81^{d} (67.04)	4.36^{f} (12.04)	2.04^{c} (8.12)
MS.5Kn	-	-	-	0.5	79.98^{e} (63.41)	4.69^{ef} (12.47)	2.33^{c} (8.74)
MSKn	-	-	-	1.0	78.76^{f} (62.55)	3.91^{f} (11.39)	3.54^{bc} (10.82)
MS2Kn	-	-	-	2.0	76.83^{f} (61.21)	3.72^{f} (11.10)	2.16^{c} (8.43)
MS3Kn	-	-	-	3.0	71.79^{g} (57.90)	3.96^{f} (11.46)	2.53^{c} (9.11)
MS.5IB.5B	0.5	-	0.5	-	79.63^{e} (63.17)	3.94^{f} (11.40)	2.56^{c} (9.20)
MSIB.5B	1.0	-	0.5	-	78.35^{f} (62.25)	3.66^{f} (11.02)	2.06^{c} (8.25)
MS2IB.5B	2.0	-	0.5	-	70.20^{g} (56.89)	3.16^{f} (10.23)	1.83^{c} (7.75)
MS.5IB.5Kn	0.5	-	-	0.5	84.82^{d} (67.07)	3.89^{f} (11.36)	3.44^{c} (10.66)
MSIB.5Kn	1.0	-	-	0.5	80.40^{e} (63.70)	4.21^{f} (11.82)	2.33^{c} (8.77)
MS2IB.5Kn	2.0	-	-	0.5	76.48^{f} (60.97)	3.05^{f} (10.04)	3.01^{c} (9.98)
Mean					**83.80 (67.04)**	**5.64 (13.48)**	**2.92 (9.67)**
CD(0.05)					**3.01**	**1.79**	**1.77**

Figures in parenthesis are transformed values (Arc-sine transformation).
Values within column followed by different letters are significantly different at 1% probability level

During present research, rhizogenesis frequency was found to be higher after transferring shootlets into rooting medium. In general, auxins like IBA [32, 14, 18, 5, 16, 17], IAA [10, 26] as well as NAA [14, 17] were effective in inducing *in vitro* rooting. In present study, full strength MS medium supplemented with 0.1-0.5 mg.l^{-1}IBA was found to be optimum for exhibiting higher *in vitro* rooting response. Maximum *in vitro* root proliferation, number of roots and root length were observed on culture media MS.1IB/ MS.5IB. The results clearly indicated that rooting of *in vitro* shoots of gerbera required lower concentrations of IBA at higher nutrient status. Auxins promoted adventitious root development on intact plants as well as excised stems. Of these, IBA was the most effective than any other plant growth regulators in the most of the cases apparently because it is not destroyed by IAA oxidase or other enzymes and therefore persists longer. The above results are in conformity with the earlier findings of Shabanpour *et al*. (2011) [5], Patidar *et al*. (2013) [17] and Nhut *et al.(*2007) [32].

Conclusion

The results of present study demonstrated that under conditions of these experimentations, *in-vitro* response of mature embryo cultures were under genetic control. As explants cultured on different culture media combinations, significant differences were observed for all the culture phases. Trend shown in the present investigation suggested that explant (s) with higher regeneration potential could be used for massive *in vitro* propagation and advance biotechnological work.

Abbreviations*:* MS: Murashige and Skoog medium; NAA:α-Naphthalene acetic acid; 2, 4-D: 2, 4-dichlorophenoxyacetic acid; 2, 4, 5-T: 2, 4, 5-trichlorophenoxyacetic acid; BA:6-benzylaminopurine; Kn: Kinetin; TDZ: Thidizuron; 2-ip : N-isopentenyl amino purine and IBA:Indole-3-butyric acid.

Conflict of Interest: None declared

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