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Studies on micropropagation of gerbera (*Gerbera jamesonii* Bolus)

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ABSTRACT *A study was carried out on micropropagation of Gerbera jamesonii Bolus cv. 'Harley'. Capitulum explants were tried to develop protocol for organogenesis and plant establishment during 2009-10. The earliest culture establishment of capitulum sections was obtained on modified MS medium supplemented with Benzyl-6-adenine (4 mg/l) and Indole-3-acetic acid (0.5 mg/l). The type, concentration and combination of cytokinin also showed profound effect on multiplication. Better proliferation of quality shoots was obtained on modified MS medium supplemented with combination of BA and kinetin. The optimum numbers of quality shoots were obtained on MS medium containing 4 mg/l BA and 2 mg/l kinetin. For in vitro rooting, half strength MS medium supplemented with 2 mg/l Indole-3-butyric acid was found best.*

Gerbera (*Gerbera jamesonii* Bolus) commonly known as Transvaal Daisy is a flower with increasing commercial significance. It is one of the leading cut flowers and ranks fourth among the top ten cut flowers of the world. During the Dutch flower auctions in 2008, the annual sale of quality cut flower gerbera was 1,142 million with an average price of 0.217 €/ stem (Evans, 2009). In India, commercial production of gerberas is centered around Pune and Bangalore, parts of Sikkim, Nagaland, Meghalaya and Uttarakhand, from where flowers are being sent to local and international market. The non-availability of good quality planting material of commercially important strains is a major constraint for its widespread cultivation in India. Though, it can be propagated both by sexual and asexual methods, seed propagation is not always satisfactory due to a great deal of variation (Schiva, 1975). The commercial cultivars are propagated through vegetative means so as to maintain uniformity, genetic purity and quality flower production (Peper et al. 1971). Among the vegetative means, multiplication through division of clumps is the most common method used for several decades. Its commercial propagation through division of clumps and other conventional methods of propagation is slow and inadequate for the production of large number of uniform propagules (Aswath & Choudhary, 2001). For commercialization of this crop, planting material is required on large scales, which further needs the development of an easier, quicker and economically viable method of propagation. Micropropagation is the only viable alternative for large-scale multiplication of gerbera. This method is free of seasonal bonds and enables manifold multiplication of the selected plants. The other advantages are product uniformity, disease-free plants, easy exchange of germplasm and planting material (Murashige et al. 1974).

The *in vitro* response in gerbera varies with cultivar, explants and composition of media. From many years gerbera is being propagated by direct or indirect organogenesis using various explants including stem tips, floral buds, leaf, capitulum etc. The plants are produced from explants of capitulum in red flower gerbera (Pierik et al. 1975, Pierik et al. 1982), leaves (Kumar et al. 2004, Jerzy & Lubomski, 1991), floral buds (Mandal et al. 2002), floral bracts (Maia et al. 1983) and inflorescence (Schum & Busold, 1985). Shoot tip culture is by far the most common *in vitro* method for commercial multiplication as shoot tip commences the growth more rapidly and contain more number of axillary buds (Murashige et al. 1974). The advantages of the capitulum method over shoot tip are the easier sterile isolation *in vitro*. It is also non-destructive method, as only inflorescences are used and no shoots are lost from the plant (Pierik et al. 1982). The present investigation was undertaken to develop an efficient and viable protocol for commercial micropropagation of gerbera.

MATERIAL AND METHODS

The gerbera cultivar 'Harley' was grown under polyhouse conditions at Experimental Farm of Department of Floriculture and Landscaping, Solan, Himachal Pradesh during 2009. All the recommended cultural practices were followed with regular spraying of plant protection chemicals to avoid diseases and pests. Capitulum explants of gerbera were collected at immature stage (0.5 to 1.0 cm diameter). The outer involucral bracts were removed and segmented into 4-8 sections. Excised explants were washed with teepol (0.1%) solution for 5 minutes followed by thorough washing under running tap water for 10-15 minutes to remove any residue of the detergent. These explants were pre-treated with 0.1% Bavistin ® (Carbendazim) for 15 minutes to minimize the contamination in the cultures followed by rinsing thrice with autoclaved distilled water. Thereafter, explants were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 5 minutes followed by washing thrice with sterile double-distilled water to remove the traces of sterilizing agent(s) immediately after treatment.

The explants were inoculated on basal Murashige and Skoog (1962) medium containing 1 mg/l Thiamine-HCl, 5 mg/l Pyridoxine-HCl and 5 mg/l Nicotinic acid. MS medium was supplemented with 30 g/l sucrose, pH was adjusted to 5.7 to

5.8 with drop-wise addition of 1 N NaOH or 1 N HCl using a digital *p*H meter and solidified with 8.0 g/l Agar with different concentrations of BA (1-6 mg/l), kinetin (1-6 mg/l), IAA (0.5 mg/l) and NAA (0.5 mg/l) for culture establishment. The contents were then sterilized in a vertical autoclave at 121°C for 20 minutes (15 lbs/inch^2). The cultures were maintained at 25±1°C under fluorescent white light at a photoperiod of 16:8 hours light and dark cycles. Twenty explants were inoculated per replication and each treatment was replicated thrice.

The divisions regenerated from capitulum explants were separated and multiplied on basal MS medium supplemented with different combinations of BA (2, 4 and 6 mg/l) and kinetin (2, 4 and 6 mg/l) either alone or in combination. Number of divisions/explants, number and length of shoots were recorded after four weeks of transfer to the shoot proliferation media. A total of 10 conical flasks (250 ml) were sub cultured in each treatment and each treatment was replicated thrice.

In vitro rooting of micro-shoots separated from the multiplication medium and individual shoots were transferred on half strength MS medium containing 30 g/l sucrose and 8g/l Agar. Different concentrations of auxins i.e. IAA (1-5 mg/l), IBA (1-5 mg/l) and NAA (1-5 mg/l) were tested for *in vitro* rooting. Rooted plantlets were carefully removed from flasks. Roots were rinsed with running water to eliminate residue from the culture media and then soaked in a fungicidal solution (Bavistin 0.1%) for 5 minutes. For hardening, rooted plants were transferred to different hardening media namely coco peat, coco peat: perlite (1:1, v/v) and coco peat: perlite: vermicompost (1:1:1, v/v) in plastic pots with polythene cover. The survival percentage of acclimatized plants was recorded four weeks after transplanting.

The data was analyzed employing completely randomized design (Gomez & Gomez, 1984). Percent data were subjected to arc sine transformation before calculating the ANOVA. The means were compared using Duncan's New Multiple Range test (DMRT).

RESULTS AND DISCUSSION

Culture establishment

Culture establishment is the most critical factor for *in vitro* morphogenesis and the success depends largely upon several factors like correct choice of explants, physiological state of the explants, growth, and biochemical composition, coupled with the presence of phytohormones, their ratio and level (Nugent et al. 1991). Initially no visible change in the morphology of the capitulum was observed except loosening of florets and drying of outer bracts. Subsequently, swelling and greening of the florets was seen (Fig 1). Later, shoot development occurred directly from these florets. This might be due to the formation of meristematic tissues in segment of immature flower heads (Mandal et al. 2002). The induction media had significant effect on initial culture establishment from capitulum explants (Table 1). Medium containing 4 mg/l BA + 0.5 mg/l IAA showed better potential for initial culture establishment. On this medium shoot emergence was observed after 91.74 ± 0.20 days of culturing.

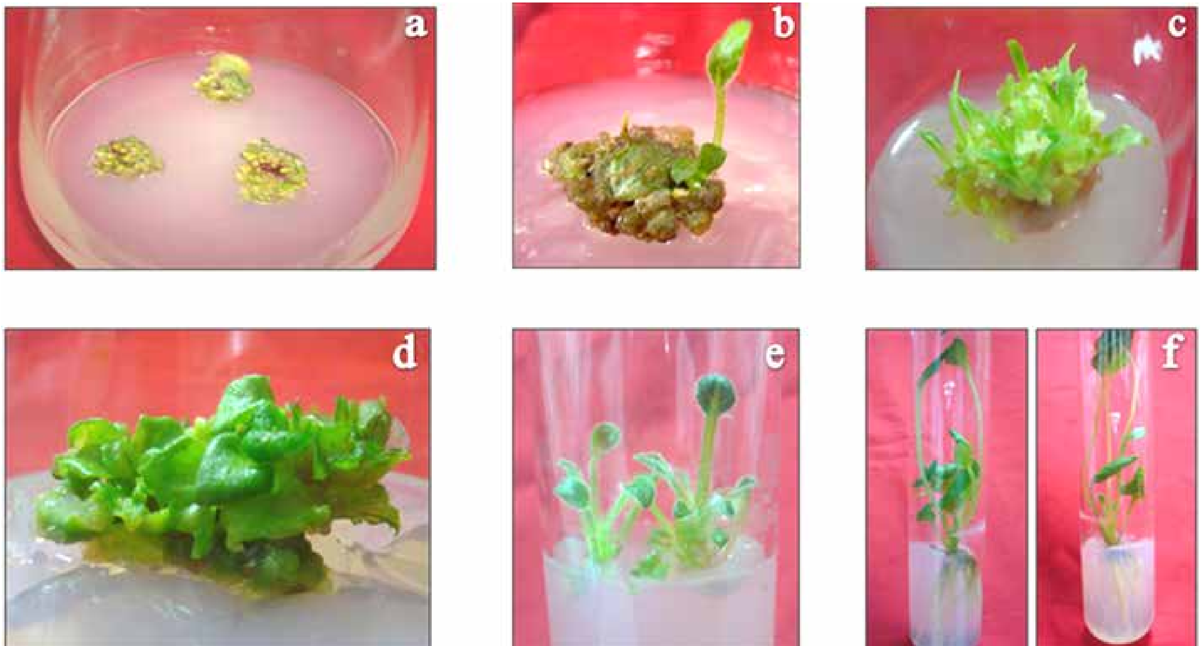

Fig 1. Stages of culture establishment: a. Immature capitulum explants; b. Swelling of floret and leaf initiation; c. Profuse shoot emergence from capitulum after 90 -100 days of culturing on MS + BA (4 mg/l) + IAA (0.5 mg/l); d. Shoot proliferation in gerbera after one month of sub culturing on MS + BA (4 mg/l) + Kinetin (2 mg/l); e. Multiplication of divisions derived from capitulum; f. *In vitro* rooting on Half strength MS + IBA (2 mg/l)

Shoot development from dormant buds situated in the axils of the bracts surrounding the receptacles of capitulum has also been reported by Pierik et al. (1975). With the increase in level of BA, significant reduction in days to sprout was observed. The role of auxins and cytokinin in micropropagation is well known and the best morphogenetic response can be obtained from synergistic effect of compatible auxins and cytokinin combination (Aswath & Choudhary, 2001). The favourable effect of cytokinins on shoot meristem initiation, axillary bud bursting and multiple shoot production have been demonstrated by Pierik et al. (1975). Warar et al. (2008) reported the best culture establishment of capitulum explants of gerbera cv. 'Sciella' in 60.73 days on MS medium supplemented with 3 mg/l BAP + 0.5 mg/l IAA. Laliberte et al. (1985) also obtained shoots on capitulum explants of gerbera cv. 'Pastourelle' on a medium containing 2 mg/l BA and 0.1 mg/l IAA after a culture period of 8 weeks.

Table 1. Effect of BA, kinetin and IAA on number of days taken for culture establishment of capitulum explants

*Media	Days to shoot emergence
MS + BA (1 mg/l) + IAA (0.5 mg/l)	102.42 ± 0.61[cd**]
MS + BA (2 mg/l) + IAA (0.5 mg/l)	102.11 ± 0.29[c]
MS + BA (3 mg/l) + IAA (0.5 mg/l)	104.82 ± 0.56[e]
MS + BA (4 mg/l) + IAA (0.5 mg/l)	91.74 ± 0.20[a]
MS + BA (5 mg/l) + IAA (0.5 mg/l)	94.70 ± 0.46[b]
MS + Kinetin (1 mg/l) + IAA (0.5 mg/l)	103.59 ± 0.23[de]
MS + Kinetin (2 mg/l) + IAA (0.5 mg/l)	106.81 ± 0.37[f]
MS + Kinetin (3 mg/l) + IAA (0.5 mg/l)	104.59 ± 0.26[e]
MS + Kinetin (4 mg/l) + IAA (0.5 mg/l)	95.84 ± 0.81[b]
MS + Kinetin (5 mg/l) + IAA (0.5 mg/l)	95.52 ± 0.69[b]

* Modified MS + 30.0 g/l sucrose + 8.0 g/l agar and *p*H 5.75

** Means followed by different letters within columns are significantly different at P=0.05, Duncan's Multiple Range Test

Shoot multiplication
Most of the divisions containing micro-shoots placed on MS medium devoid of hormones remained as such; however, few shoots proliferated into two or three shoots thus producing on an average of 2.59 ± 0.04 shoots per division which divided into 1.37 ± 0.02 divisions for further multiplication of micro-shoots. Significant improvement in shoot proliferation was observed with the use of growth hormones (Table 2). Linear increase in number of shoots was observed with increased concentration of cytokinins and their combinations. MS medium supplemented with 4 mg/l BA and 2 mg/l kinetin was found optimum for proliferation of quality shoots. On this media shoots were normal and healthy with a good proliferation and multiplication rate (6.41 ± 0.32 and 3.91 ± 0.01).

Shoot length was also recorded maximum in same media composition i.e. 3.67 ± 0.09 cm. Similar results were obtained by Warar et al. (2008) who obtained maximum number of shoots (7.0) on basal MS medium containing BA (3 mg/l) and kinetin (0.5 mg/l) in gerbera cv. 'Sciella'. Similar observations were made by Aswath et al. (2003) who obtained maximum number of shoots and shoot length (3.2 cm) using *in vitro* shoot explants on MS medium supplemented with 5 mg/l kinetin in all three varieties of gerbera viz., GJ-1, GJ-2 and GJ-3. Pierik et al. (1982) also obtained maximum number of shoots per capitulum explant on MS medium supplemented with 5 mg/l BA in gerbera cultivars 'Clementine' and 'Ronald'. The maximum proliferation and multiplication (11.11 and 4.89) was observed on MS medium supplemented with 6 mg/l BA + 2 mg/l kinetin. However, the shoots developed on this media were lanky exhibited vitrification like symptoms and also have less survival rate in further multiplication. Li et al. (2003) anticipated that an excess of cytokinins along with the high water potential of the medium were the major reasons for the vitrification of shoots. Though BA is best cytokinin for *in vitro* propagation of gerbera, however, its positive effect on shoot multiplication is related to detrimental effect on their growth. High concentration of BA coupled with high humidity often result in vitrification of *in vitro* raised shoots (Kataeva et al. 1991, Jerzy & Lubomski 1991).

Table 2. Effect of different combinations of BA and kinetin on number of divisions per explant, number of shoots and length of shoots per division in gerbera cv. 'Harley'

*Media	Number of divisions/ explant	Number of shoots/ division	Average shoot length (cm)
MS + no hormone (Control)	1.37 ± 0.02[a**]	2.59 ± 0.04[a]	1.39 ± 0.06[a]
MS + BA (2 mg/l)	2.63 ± 0.02[b]	3.07 ± 0.04[a]	2.26 ± 0.13[b]
MS + BA (4 mg/l)	3.46 ± 0.04[d]	6.15 ± 0.27[c]	3.27 ± 0.13[d]
MS + BA (6 mg/l)	3.65 ± 0.02[d]	10.56 ± 0.07[f]	2.95 ± 0.20[c]
MS + Kinetin (2 mg/l)	2.54 ± 0.02[b]	2.81 ± 0.04[a]	2.16 ± 0.13[b]
MS + BA (2 mg/l) + Kinetin (2 mg/l)	3.39 ± 0.03[c]	6.22 ± 0.13[c]	3.04 ± 0.13[d]
MS + BA (4 mg/l) + Kinetin (2 mg/l)	3.91 ± 0.01[e]	6.41 ± 0.32[c]	3.67 ± 0.09[e]
MS + BA (6 mg/l) + Kinetin (2 mg/l)	4.89 ± 0.03[f]	11.11 ± 0.23[g]	3.37 ± 0.13[d]
MS + Kinetin (4 mg/l)	3.39 ± 0.09[c]	5.78 ± 0.13[b]	3.11 ± 0.17[d]
MS + BA (2 mg/l) + Kinetin (4 mg/l)	3.54 ± 0.05[d]	5.96 ± 0.07[b]	3.60 ± 0.13[de]
MS + BA (4 mg/l) + Kinetin (4 mg/l)	3.57 ± 0.07[d]	8.15 ± 0.07[d]	3.13 ± 0.17[d]
MS + BA (6 mg/l) + Kinetin (4 mg/l)	3.61 ± 0.03[d]	9.33 ± 0.11[e]	2.89 ± 0.09[c]
MS + Kinetin (6 mg/l)	3.61 ± 0.08[d]	9.96 ± 0.10[e]	2.83 ± 0.14[c]
MS + BA (2 mg/l) + Kinetin (6 mg/l)	4.33 ± 0.03[f]	10.30 ± 0.10[f]	3.04 ± 0.12[d]
MS + BA (4 mg/l) + Kinetin (6 mg/l)	3.57 ± 0.02[d]	9.26 ± 0.10[d]	3.26 ± 0.08[d]
MS + BA (6 mg/l) + Kinetin (6 mg/l)	3.54 ± 0.02[d]	8.19 ± 0.20[d]	3.04 ± 0.13[d]

* Modified MS + 30.0 g/l sucrose + 8.0 g/l agar and *p*H 5.75

** Means followed by different letters within columns are significantly different at *P*=0.05, Duncan's Multiple Range Test

In vitro root induction
Fortification of half strength MS medium with either of IAA, IBA or NAA had a profound effect on inducing early rooting (Table 3). The shoots cultured on medium supplemented with 1 mg/l IAA of rooting hormone showed poor rooting (37.04%) and took maximum time (26.90 ± 0.38 days) to root initiation. Rooting was significantly improved with increasing the concentration of auxins into the media. Half strength MS medium containing 2 mg/l IBA was optimum for root induction (Fig 2). On this medium root initiation occurred at the earliest (16.77 ± 0.29 days) with maximum rooting (70.37%). The longest roots (3.31 ± 0.03 cm) and number of roots (6.22 ± 0.23) with good root growth were also recorded on this medium. The root formation is markedly influenced by the presence of auxins and sugars, whereas presence and absence of macro-elements did not influence rooting (Pierik *et al*. 1975). Studies carried out by Palai *et al*. (1998) also indicated that IBA is the best auxin for rooting in gerbera. Among the three auxins tried, IBA was found better when compared with IAA and NAA for inducing good quality roots. However, Aswath and Chaudhary (2001) reported maximum root induction and average number of roots per shoot when cultured on MS medium containing 1.75 mg/l IBA.

Fig 2. Hardening of *in vitro* raised plants: a. Rooted plantlets just before transplanting; b. Plants in polythene covered pots containing coco peat; c. Plants after 4 weeks of hardening

Hardening of in vitro raised plants
Out of the three potting mixture, maximum survival of plantlets (90.00 %) after 4 weeks was observed in coco peat containing pots which were covered with polythene (Fig 2). Whereas, only 80.00% of plants survived when hardening was done in coco peat, perlite and vermi-compost mixture.

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Maximum number of newly emerged leaves after 4 weeks (3.10) was recorded in coco peat. This indicated that coco peat might have proved to be a better hardening mixture for the *in vitro* raised plantlets of gerbera in comparison to the other mixture tested. Superiority of coco peat has been proved earlier in gerbera (Shailja *et al*. 2004). Thus the present study describes an efficient protocol for rapid *in vitro* multiplication of gerbera. This protocol can be successfully employed for commercial multiplication of gerbera.

Table 3. Effect of different concentrations of IAA, IBA and NAA on root induction in gerbera cv. 'Harley'

Treatment	Number of days taken for root initiation	Per cent rooting (%)	Number of roots per clump or division	Root length (cm)
½ MS + IAA (1 mg/l)	26.90 ± 0.38^{g}**	37.04 (37.44)*^{b}	1.48 ± 0.10^{a}	2.06 ± 0.32^{b}
½ MS + IAA (2 mg/l)	21.37 ± 0.46^{d}	40.74 (39.63)^{b}	3.07 ± 0.26^{b}	2.36 ± 0.04^{c}
½ MS + IAA (3 mg/l)	16.93 ± 0.35^{a}	55.56 (48.25)^{d}	4.67 ± 0.36^{d}	2.93 ± 0.02^{f}
½ MS + IAA (4 mg/l)	19.93 ± 1.14^{c}	48.15 (43.94)^{c}	5.00 ± 0.32^{e}	2.54 ± 0.05^{d}
½ MS + IAA (5 mg/l)	17.43 ± 0.67^{a}	66.67 (54.94) ^{f}	3.85 ± 0.13^{c}	3.27 ± 0.03^{c}
½ MS + IBA (1 mg/l)	18.97 ± 0.71^{b}	62.96 (52.56)^{de}	4.81 ± 0.13^{d}	2.68 ± 0.09^{e}
½ MS + IBA (2 mg/l)	16.77 ± 0.29^{a}	70.37 (57.12)^{g}	6.22 ± 0.23^{f}	3.31 ± 0.03g
½ MS + IBA (3 mg/l)	21.23 ± 0.76^{d}	59.26 (50.37)^{de}	4.26 ± 0.13^{d}	2.99 ± 0.05^{f}
½ MS + IBA (4 mg/l)	23.97 ± 0.15^{e}	44.44 (41.81)^{b}	2.93 ± 0.22^{b}	2.17 ± 0.05^{b}
½ MS + IBA (5 mg/l)	24.53 ± 0.46^{f}	37.04 (37.44)^{b}	1.85 ± 0.10^{a}	1.65 ± 0.02^{a}
½ MS + NAA (1 mg/l)	20.77 ± 0.23^{d}	51.85 (46.06)^{cd}	1.19 ± 0.07^{a}	2.76 ± 0.05^{e}
½ MS + NAA (2 mg/l)	18.40 ± 0.50^{b}	59.26 (50.37)^{de}	1.37 ± 0.14^{a}	2.56 ± 0.04^{d}
½ MS + NAA (3 mg/l)	18.70 ± 0.40^{b}	40.74 (39.63)^{b}	2.78 ± 0.23^{b}	2.28 ± 0.08^{c}
½ MS + NAA (4 mg/l)	19.73 ± 0.34^{c}	37.04 (37.44)^{b}	3.00 ± 0.19^{b}	2.08 ± 0.05^{b}
½ MS + NAA (5 mg/l)	19.80 ± 0.21^{c}	29.63 (32.88)^{a}	5.41 ± 0.07^{e}	1.57 ± 0.02^{a}

* Figures in parentheses are arc sine transformed values

** Means followed by different letters within columns are significantly different at *P*=0.05, Duncan test

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