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Callusing and regeneration response of *in vitro* derived leaf explants of gerbera (*Gerbera jamesonii*)

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ABSTRACT

Gerbera (*Gerbera jamesonii* Bolus ex. Hookes F.) is one of the most popular commercial flowers worldwide used both as cut flower and potted plant. Conventionally, it is propagated through vegetative means such as divisions of clump which is very slow process and not commercially viable. Rapid multiplication of elite hybrids/genotypes with improved quality traits, has been achieved by using both direct and indirect tissue culture methods. Gerbera hybrid seeds obtained by half-sib mating were raised in half strength MS medium. The earliest days for seed germination (2.00), first leaf development (3.00 days) and rooting (3.00 days) was recorded line IIHR1-1, IIHR 5-4 and IIHR1-1, IIHR1-2, IIHR1-5, IIHR3-2 and IIHR5-4. The response of *in vitro* leaf explants to callusing and regeneration was recorded when cultured on full strength Murashige and Skoog's media supplemented with different concentrations of 2,4-D or BAP or IAA and Kinetin. Leaf explants cultured on MS medium fortified with equal concentration of BA (1 mg l-1) and 2, 4-D (1 mg l-1) produced green and greenish white granular callus within 25 days, however, leaf explants cultured on MS medium supplemented with 2,4-D produced yellowish callus. Plant regeneration was also found earliest in the same media within 14 days.

Key words: Callus, Gerbera, *In vitro* leaf, Regeneration

Gerbera (*Gerbera jamesonii* Bolus ex. Hookes F.) is commercially grown as cut flower in different parts of the world under polyhouse/shade net to get quality flowers. *Gerbera jamesonii* belongs to the family Asteraceae and originated in South Africa. Although it is propagated through vegetative means such as divisions of clump which is very slow process and not commercially viable. Therefore, in recent years, most of the varieties of gerbera are multiplied through tissue culture. There has been increasing interest in tissue culture as an alternative to commonly used method of propagation of gerbera. This method enables a million fold expansions per year of a desired plant. Axillary shoot formation from excised capitulum explants (Pierik *et al*. 1973, 1974) or from shoot tips (Murashige *et al*. 1974) have been reported. It is possible to induce adventitious shoot formation on isolated young leaves derived from earlier developed axillary shoots under *in vitro* conditions. Plants regenerated from callus and adventitious shoots are required in mutation breeding as a tool for the production of solid mutants (Can *et al.* 2008) as reported in chrysanthemum (Datta *et al.* 2005) and rose (Madhu Bala and Singh 2015). Regeneration of adventitious shoots from leaf blades has also been reported (Cardoso and Silva 2013) in *Oncidium gower* (Chen *et al.* 1999) and *Rhynchostylis retusa* (Vij *et al.* 1984). The present investigation was carried out to study the response of *in vitro* derived leaf explants to callusing and regeneration in gerbera.

MATERIALS AND METHODS

The experiment was carried out in Division of Ornamental Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta Lake Post, Bengaluru during 2014-2015. Twenty different lines of gerbera, viz. IIHR-1 to IIHR-20 were used for hybridisation. Half sib method was used, where five genotypes IIHR-1, IIHR-2, IIHR-3, IIHR-4, IIHR-5 (Table 1) were used as female parents, while mixed pollen of remaining 15 genotypes were used as male parent.

The seeds obtained from the individual cross were surface sterilised, soaked in distilled water for 30 minutes with the addition of 1-2 drops of Tween-20 followed by 40% sodium chloride solution and gently agitated. The seeds were then rinsed three times with distilled water and then soaked in mercuric chloride (0.1%) for 3 to 4 minutes under laminar airflow. Finally, the seeds were again washed with autoclaved distilled water. Half MS medium (Murashige and Skoog 1962) was used for inoculation of seeds. The

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Table 1 Details of female parents used for crossing in gerbera

Characters	IIHR1	IIHR 2	IIHR 3	IIHR 4	IIHR 5
Diameter of flower head (cm)	12.00	11.00	14.00	11.00	12.00
Length of the flower stalk (cm)	50.00	60.00	55.00	60.00	45.00
Diameter of flower stalk (mm)	6.10	5.90	6.00	5.20	6.20
Type of flower head	Single	Double	Double	Double	Double
Colour of ray florets (RHS colour chart)	N67C (red purple group)	N42A (red group)	N28A (orange group)	NN155D (white group)	NN155C (white group)
Colour of disc florets	Yellow	Yellow	Black	Black	Yellow

observations were recorded on parameters like days taken for germination, days taken for first leaf emergence, days taken for rooting, number of leaves after 60 days, number of roots after 60 days and shoot length after 60 days.

Individual *in vitro* plants were raised under well defined culture room conditions. Individual seeds from the cross were considered as a single line and hence basic statistical measures such as mean was applied. *In vitro* leaf explants from fourteen hybrid lines were used. The Murashige and Skoog (1962) basal medium was used at full strength. Each basal medium was supplemented with different concentration of growth regulators in five treatments, viz. MS + BA (1 mg/l) (T_1), MS + BA (1 mg/l) + IAA (1 mg/l) (T_2), MS + BA (1 mg/l) + 2,4-D (1 mg/l) (T_3), MS +Kinetin (5 mg/l) +IAA (1 mg/l)+ 2,4-D (1 mg/l) (T_4) and MS + BA (5 mg/l) + Kinetin (5 mg/l) +2,4-D (1 mg/l) (T_5).

The pH of all media was adjusted to 5.7 to 5.8 before sterilization by the addition of 1N HCl or 1N NaOH as required. The containers having the medium were sterilized at 15 lbs/m^2 at 121°C for 20 min. The leaves with petiole from *in vitro* grown seedlings were used as explant. The leaves were cut into 4 to 5 mm in size, were placed in the culture bottles. Injury was made on the leaf before placing in the medium. The completely randomized design was followed with five replications. Observations were recorded

Table 2 Response of in vitro raised seeds of gerbera hybrids in half MS medium

Lines	Days taken for germination	Days taken for first leaf emergence	Days taken for first rooting	Number of leaves after 60 days	Number of roots after 60 days	Shoot length after 60 days
IIHR 1-1	2.00	4.00	3.00	5.00	3.00	6.00
IIHR 1-2	3.00	4.00	3.00	4.00	3.00	6.00
IIHR 1-3	4.00	6.00	4.00	4.00	3.00	6.00
IIHR 1-4	4.00	5.00	4.00	4.00	3.00	6.00
IIHR 1-5	3.00	5.00	3.00	3.00	4.00	5.00
IIHR 2-1	3.00	4.00	4.00	3.00	3.00	6.00
IIHR 2-2	3.00	6.00	4.00	4.00	5.00	6.00
IIHR 2-3	3.00	6.00	4.00	4.00	6.00	6.00
IIHR 2-4	4.00	6.00	5.00	5.00	5.00	6.00
IIHR 2-5	4.00	6.00	5.00	4.00	5.00	5.00
IIHR 3-1	4.00	5.00	5.00	5.00	5.00	6.00
IIHR 3-2	3.00	4.00	3.00	4.00	5.00	7.00
IIHR 3-3	7.00	8.00	8.00	3.00	4.00	6.00
IIHR 3-4	4.00	6.00	5.00	4.00	5.00	7.00
IIHR 3-5	5.00	6.00	5.00	6.00	5.00	4.00
IIHR 4-1	3.00	5.00	4.00	5.00	4.00	5.00
IIHR 4-2	6.00	6.00	5.00	4.00	5.00	5.00
IIHR 4-3	3.00	6.00	4.00	6.00	5.00	6.00
IIHR 4-4	3.00	5.00	4.00	6.00	5.00	6.00
IIHR 4-5	4.00	7.00	4.00	4.00	5.00	7.00
IIHR 5-1	3.00	5.00	4.00	5.00	5.00	6.00
IIHR 5-2	4.00	6.00	4.00	5.00	4.00	6.00
IIHR 5-4	3.00	3.00	3.00	4.00	4.00	8.00
IIHR 5-5	3.00	5.00	6.00	3.00	4.00	6.00
Mean	3.52	5.16	4.12	4.16	4.20	5.72

on days taken for callus initiation, callus production, days to develop plantlets from the callus, days to form shoots and roots per callus clump.

RESULTS AND DISCUSSION

Response of in vitro raised seeds in half MS medium

The data pertaining to response of *in vitro* raised seeds in half MS medium is presented in Table 2. The least days for seed germination was recorded in the line IIHR1-1 (2.00), whereas, IIHR3-3 took the highest days (7.00) to germinate, followed by IIHR4-2 (6.00), compared to other lines. The line IIHR 5-4 recorded earliest first leaf emergence (3.00 days) over other lines. The maximum number of days taken for first leaf emergence (8.00 days) was recorded from the line IIHR 3-3. Addition of auxins together with cytokinin becomes essential for shoot induction and multiplication depending on the plant type. The right combination of auxin and cytokinin in the culture medium determined the effectiveness of micropropagation of gerbera. Addition of strong auxin with BAP promoted better shoot formation compared to weak auxin (Pierik *et al.* 1973). The lines IIHR1-1, IIHR1-2, IIHR1-5, IIHR3-2 and IIHR5-4 took least number of days for rooting (3.00 days), however, IIHR3-3 recorded maximum number of days for first rooting (8.00). IAA proved to be more efficient in production of maximum number of good quality, healthy and thick roots. Pagnussat *et al*. (2004) reported that IAA increases the number of roots through the development of meristematic tissues and regulation of cell differentiation.

After the seeds germinated, the plantlets were sub-cultured in a medium containing BAP (2 mg/l) along with IAA (1 mg/l). 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 and Choudhary 2001). The favorable effect of cytokinins on shoot meristem initiation, axillary bud bursting and multiple shoot production have been demonstrated by Pierik *et al.* (1975). The number of leaves after 60 days was found to be considerably increased in the line IIHR3-5, IIHR4-3 and IIHR4-4 (6 leaves per plant) when sub-cultured.

In the present study, it was noticed that several roots developed spontaneously from the *in vitro* grown shoots but the spontaneously developed roots were found to be inadequate for transplanting *in vitro* grown plants to the soil. Therefore, separate root induction was necessary. The plants were transferred in a rooting medium containing half MS along with 1.5 mg/l IBA. Highest number of roots (6) was recorded in line IIHR 2-3 after 60 days. Aswath and Choudhary (2001) also reported maximum root induction and average number of roots per shoot when cultured on MS medium containing 1.5 mg/l IBA. The line IIHR5-4 was found significantly superior for shoot length (8.00 cm), whereas, the line IIHR3-5 recorded the lowest shoot length (4.00 cm). Optimum concentration of growth regulators required varies with the cultivars as every genotype had a specific range of optimum growth regulator concentration (Deepaja 1999).

Callus induction from leaf explants

The effect of the MS treatments on callus induction is presented in Table 3. The callus was initiated in all the cultures within 26-32 days of culturing, irrespective

Table 3 Effect of different MS treatments on callus induction from leaf explants at 40 days in gerbera

Lines	MS+2,4-D (1.8 mg/l)	MS+2,4-D (2 mg/l) +BA (1 mg/l)	MS
IIHR 1-1	++	++	0
IIHR 1-2	++	++	0
IIHR 1-3	+	+++	0
IIHR 1-4	++	++	0
IIHR 1-5	++	++	0
IIHR 2-1	+	++	0
IIHR 2-2	++	+++	0
IIHR 2-3	++	++	0
IIHR 2-4	++	++	0
IIHR 2-5	++	+++	0
IIHR 3-1	+	++	0
IIHR 3-2	++	+++	0
IIHR 3-3	+	+++	0
IIHR 3-4	++	+++	0
IIHR 3-5	++	+++	0
IIHR 4-1	0	0	0
IIHR 4-2	++	++	0
IIHR 4-3	++	++	0
IIHR 4-4	+++	++	0
IIHR 4-5	++	++	0
IIHR 5-1	0	0	0
IIHR 5-2	0	0	0
IIHR 5-4	++	++	0
IIHR 5-5	0	0	0
Type of callus	Cream and creamy yellow	Green and greenish white	-

0: No callus, + : Poor growth of callus, ++: Moderate growth of callus, +++: Vigorous growth of callus

of strength of the nutrient medium. After 60 days of inoculation, callus produced from the explants were scored visually and analyzed. Explants cultured on full MS medium supplemented with lower levels of 2,4-D (2.0 mg/l) and BAP (1 mg/l) produced more amount of callus. There was no callus production on the basal medium and the leaf explants remained as it is. Growth regulator addition is a must to stimulate cell division. BAP is essentially required for the formation of callus. Kumar and Kanwar (2006) proved synergetic effect of BAP on gerbera. The 2,4-D and BAP in addition to full strength MS basal medium produced the best callus, both quantitatively and qualitatively.

However, there was difference in the type of callus produced. MS basal medium fortified with 2,4-D and BAP produced green and nodular callus and MS medium fortified with 2,4-D alone produced yellowish creamy callus in all the treatments irrespective of the lines. This result is in confirmation with Hasbullah *et al.* (2008).

The callus was observed under microscope for somatic embryogenesis validation. There was no vascular connection between the embryos and they were popping out from the clump, clearly indicated that they are somatic embryos. Naing *et al.* (2011) also reported that somatic embryos were indirectly induced from leaf derived callus. Similar results were also reported by Huan *et al*. (2004).

The growth regulator combination of 2,4-D with BAP proved to be the best compared to other treatments in the basal nutrient medium. The effect of the treatments on callus induction and regeneration is presented in Table 4. The earliest callus (25.87 days) was observed on the leaf explants cultured on MS + 1.0 mg/l 2,4-D + 1 mg/l BAP. The 2,4-D with BAP definitely stimulated callus production, whereas, IAA with BAP though produced callus took long time to initiate callus. Aswath *et al.* (2003) and Kumar *et al*. (2004) also reported similar findings.

The plantlets from callus were initiated from 13.75 days (MS + 1.0 mg/l 2,4-D + 1 mg/l BAP) to 22.00 days (MS + 5 mg/l BAP + 5.0 mg/l Kinetin + 1.0 mg/l 2,4-D) of culturing irrespective of strength of the nutritive medium. Medium MS and BA (1 mg/l) recorded maximum number of shoots per clump (3.50), however, maximum number of roots per clump (2.25) was recorded in MS + BA (1 mg^{-1}) + IAA (1 mg^{-1}). Martin (2004) reported that even low concentration of 2,4-D influenced somatic embryogenesis when added to the culture medium.

Among the lines, the earliest callus initiation was recorded in line IIHR1-1 (24.00 days), while it was recorded delayed in line IIHR2-2 (32.66 days) (Table 5). However, the line IIHR2-2 took least days to initiate plantlet from callus (13.33).

The number of shoots from the clump was ranged from 1 to 3. The line IIHR1-1 recorded 3.3 plantlets from each clump. BAP being the chemical analogue of cytokinin not only affect different phases of regeneration but also indicate cytokinin specificity for obtaining higher number of regenerants and shoots, which is in corroboration with the findings of Aswath and Wazneen (2004). The lines IIHR1-2, IIHR1-3, IIHR2-1, IIHR2-5 and IIHR3-1 recorded highest number of roots per callus clump (2.00). Kumar *et al.* (2004) also reported similar results. This variability between the lines could be attributed to genotypic difference and their response to phytohormones. Further, the study of histology of cultured leaves is necessary to understand the cells and factors contributing towards regeneration.

Table 4 Effect of the treatments on callus induction and regeneration in gerbera

Treatment	Days to initiate callus	Days to initiate plantlet from callus	Number of shoots/ clump	Number of roots/ clump
MS + BA (1 mg/l)	26.62	14.75	3.50	0.12
MS + BA (1 mg/l) + IAA (1 mg/l)	29.50	14.87	1.00	2.25
MS + BA (1 mg/l) + 2, 4-D (1 mg/l)	25.87	13.75	3.12	1.12
MS + Kinetin (5 mg/l) + IAA (1 mg/l) and 2, 4 D (1 mg/l)	29.62	22.00	0.75	0.87
MS + BA (5 mg/l) + Kinetin (5 mg/l) + 2, 4 D (1 mg/l)	32.12	22.40	0.75	0.62
SEm±	0.18	0.33	0.10	0.15
CD (P=0.01)	0.54	1.42	0.35	0.23

Table 5 Response of gerbera hybrid lines to callus formation and regeneration

Lines	Days to initiate callus	Days to initiate plantlet from callus	Number of shoots per clump	Number of roots per clump
IIHR 1-1	24.00	15.00	3.33	1.66
IIHR 1-2	28.00	17.00	2.33	2.00
IIHR 1-3	31.00	15.00	2.66	2.00
IIHR 2-1	32.00	14.00	2.66	2.00
IIHR 2-2	32.66	13.33	2.66	1.00
IIHR 2-5	30.00	17.00	3.00	2.00
IIHR 3-1	30.00	18.00	2.66	2.00
IIHR 3-2	24.66	20.00	2.00	1.00
IIHR 3-5	29.66	20.33	1.66	1.00
IIHR 4-1	29.33	20.00	2.00	1.66
IIHR 4-2	30.33	19.00	2.00	1.66
IIHR 4-4	29.33	20.66	2.00	1.66
IIHR 4-5	30.33	20.00	1.33	1.00
IIHR 5-4	30.33	19.66	1.33	1.66
SEm±	0.50	0.56	0.30	0.43
CD (P=0.01)	1.45	1.63	0.89	1.30

The production of adventitious shoots, which will be true to type to the mother plants, can be proposed as an alternative method of propagation. Competence of immature leaves for regeneration could also be exploited for creation of transgenics through *Agrobacterium* mediated genetic transformation, since it has been demonstrated that the juvenile tissue are genetically more susceptible for *Agrobacterium*.

REFERENCES

Aswath C and Choudhary M L. 2001. Effect of cytokinins on proliferation of multiple shoots in gerbera (*Gerbera jamesonii*). *Indian Journal of Horticulture* **58**(4): 383–6.

Aswath C and Wazneen S. 2004. An improved method for *in vitro* propagation of gerbera. *Journal of Ornamental Horticulture* 7: 141–6.

Aswath C, Deepa S M and Choudhary M L. 2003. Commercial multiplication of gerbera (*Gerbera jamesonii* Bolus) through *in vitro* shoot tip culture. *Journal of Ornamental Horticulture* **6**(4): 303–9.

Can E, Celiktas C and Hatipoglu R. 2008. Effect of auxin type and concentrations in different media on the callus induction and shoot formation of crested wheatgrass (*Agropyron cristatum*). *Biotechnology* **22**: 782–6.

Cardoso J C, Teixeira da Silva J A. 2013. Gerbera micropropagation. *Biotechnology Advances* **31**: 1344–57.

Chen J T, Chang C and Chang W C. 1999. Direct somatic embryogenesis on leaf explants of *Oncidium gower* Ramsey and subsequent plant regeneration. *Plant Cell Reports* **19**: 143–9.

Datta S K, Misra P, Mandal A K A. 2005. *In vitro* mutagenesis - a quick method for establishment of solid mutants in chrysanthemum. *Current Science* **88**: 155–8.

Deepaja S M.1999. Micropropagation of Gerbera (*Gerbera jamesonii* Bolus), M Sc Thesis, University of Agricultural Sciences, Bengaluru, India.

Hasbullah N A, Taha R M and Awal A. 2008. Growth optimization and organogenesis of *Gerbera jamesonii* Bolus ex. Hook F. *in vitro*. *Pakistan Journal of Biological Sciences* **11**(11): 1449–54.

Huan L V T, Takamura T and Tanaka M. 2004. Callus formation and regeneration from callus through somatic embryo structures in *Cymbidium* orchid. *Plant Science* **166**: 1443–9.

Kumar S and Kanwar J K. 2006. Regeneration ability of petiole, leaf and petal explants in gerbera cut flowers *in vitro*. *Folia Horticulturae* **18**: 57–64.

Kumar S, Kanwar J K and Sharma D R. 2004. *In vitro* regeneration of *Gerbera jamesonii* Bolus from leaf and petiole explants. *Journal of Plant Biochemistry and Biotechnology* **13**(1): 73–5.

Madhu Bala and Kanwar Pal Singh. 2015. *In vitro* mutagenesis in rose (*Rosa hybrid* L.) cv. Raktima for novel traits. *Indian Journal of Biotechnology* **14**: 525–31.

Martin K P. 2004. Benzyladenine induced somatic embryogenesis and plant regeneration of *Leptadenia reticulate*. *Plant Biology* **48**: 285–8.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* **15**: 473–97.

Murashige T, Serpa M and Jones J B. 1974. Clonal multiplication of Gerbera through tissue culture. *Hortscience* **9**(3): 175–80.

Naing A H, Chung J D and Lim K B. 2011. Plant regeneration through indirect somatic embryogenesis in *Coelogyne cristata* orchid. *American Journal of Plant Science* **2**: 262–7.

Pagnussat G C, Lanteri M L, Lombardo M C and Lamattina L. 2004. Nitric oxide mediates the indole acetic acid induction activation of mitogen activated protein cascade involved in adventitious root development. *Plant Physiology* **135**: 279–86.

Pierik R L M, Jansen J L M, Maasdam A and Binnendijk C M. 1975. Optimization of gerbera plantlet production from excised capitulum explants. *Scientia Horticulturae* **69**(4): 351–7.

Pierik R L M, Janson J L M and Masdam A. 1974. Vegetative propagation of gerberas in test tubes. *Vakblad-Voorde-Bloomisterij* **29**(39): 18–9.

Pierik R L M, Steegmans H H M and Marelis J J. 1973. Gerbera plantlets from *in vitro* cultivated capitalum explants. *Scientia Horticulturae* **1**(1): 117–9.

Vij S P, Sood A and Plaha K K. 1984. Propagation of *Rhynchostylis retusa* Bl. (Orchidaceae) by direct organogenesis from leaf segment cultures. *Botanical Gazzette* **145**: 210–4.