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In Vitro Regeneration through Somatic Embryogenesis for Quality propagation of *Gerbera jamesonii*

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ABSTRACT

Our study presents an efficient system for *in vitro* plant regeneration via somatic embryogenesis from leaves of *Gerbera jamesonii* cultured on Murashige and Skoog (MS) medium supplemented with various combinations and concentrations of growth regulators (cytokinins or auxins). Nodular, greenish brown callus was obtained from leaf sections when cultured on MS basal medium supplemented with 2.0 mg l^{-1} 2, 4-D. Somatic embryo maturation and shoot regeneration was obtained in MS medium supplemented with 2.0 mg/l BAP and 3.0 mg/l IAA. Multiple shoot proliferation from the regenerated shoots was obtained under the influence of BAP (2.0 mg l^{-1}) and Kn (3.0 mg l^{-1}) followed by root induction using Indole-3-acetic acid (IAA 3.0 mg l^{-1}). Our results may facilitate mass production of high-quality Gerbera plantlets to meet up the demand of cut flowers in commercial market.

KEYWORDS- *Gerbera Jamesonii*; plant regeneration; Embryos; *in vitro* culture; Benzylaminopurine (BAP), Indole Acetic Acid

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INTRODUCTION

Gerbera belongs to family Compositae and is native to South Africa and Asiatic regions. It is commonly known as Transvaal daisy, Barberton daisy or African daisy, and produces very attractive flowers. Gerbera has gained popularity in the last year in many countries of the world and it is in great demand in the floral industry as cut flower as well as potted due to its beauty, colour, long vase life, and ability to rehydrate after long transportation. The genus consists of about 40 species. Out of the recorded species, only one species, *Gerbera jamesonii*, is under cultivation Ajithkumar[1] et al 2013. The mass cultivation of this specie faces natural barrier as seed propagation is typically adverse because it takes longer time to produce flowers Nhut[2] et al 2007. Vegetative propagation overcomes this problem and produce plants of better performance Topoonyanont and Debergh[3], 2001 but the process is slow and laborious. Other conventional methods like propagation through leaf cutting, stem cutting and divisions of clumps are also used Kumar and Kanwar[4], 2007 but these are inadequate for the production of large number of flowers to fulfil the demand in the market Kanwar and Kumar[5], 2008.

Tissue culture technique bears immense potential to overcome the natural hurdles and provide all the year round availability of such plants, rapid multiplication of valuable genotypes, release of improved varieties, fast production, germplasm conservation and facilitating their easy international exchange. Hence the purpose of this research is to develop a cost effective high reproducible protocol for *in vitro* regeneration of Gerbera to promote the commercial production and meet up the demand and supply ratio of this important cut flower crop.

EXPERIMENTAL SECTION

Establishment of primary culture:

The plants of *Gerbera jamesonii* were procured from the nursery, Pune (Maharashtra). Young leaves of approx. size 2×2 cm were excised and rinsed with water to remove soil and other foreign contaminants. They were surface sterilized in LAF with 0.1% $HgCl_2$ solution for 3-4 minutes to remove bacterial load. This was followed by 30 sec rinse with 70% alcohol. All the chemicals were removed by rinsing the leaves three times with sterilized-distilled water.

The surface sterilized leaf sections were inoculated in Murashige and Skoog (MS) medium having 30 g/l sucrose and supplemented with different concentrations of 2,4-D (table 1). The pH of the medium was maintained at 5.8. 0.5% agar (Himedia) was used as gelling agent and media was autoclaved at a pressure of 15 psi and 121°C temperature for 20 min.

Culture conditions

The cultures were placed in culture room under chambers illuminated with white light emitted by light emitting diodes (LEDs) with photon flux density of 17.7µmol/m^2/s at 16 h light and 8 h dark photoperiod using autotimer system. The callus was sub cultured regularly at 2-3 weeks interval. During subculture care was taken to remove pale and necrotic tissues, as their presence was inhibitory for cultures.

Somatic embryogenesis

The three week old callus was transferred to somatic embryogenesis medium containing lower concentrations of 2,4-D (0.25-2.0mg/l). After two weeks the SEs were transferred to MS medium fortified with BAP (0.25-2.0 mg/l) in combination with 2,4-D for maturation and germination.

Shoot induction and proliferation

After 2 weeks of shoot initiation the healthy shoots were carefully transferred to multiplication medium under sterile and controlled conditions. The shoots were uniformly separated from multiple shoot complex originated from the mature embryos and were transferred to medium containing BAP (1.0- 3mg/l^{-1}) and BAP in combination with Kn (1.0- 3mg/l^{-1}).

Rooting of the shoots and acclimatization of plantlets

The proliferated shoots were transferred to rooting medium consisting of MS supplemented with IAA (3.0-5.0 mg/l^{-1}).

Table 1- Combination of growth regulators utilized for various responses from leaf explants of *Gerbera jamesonii*

Response	2,4-D	BAP	Kinetin	IAA
Callogenesis	0.25	-	-	-
	0.5	-	-	-
	1.0	-	-	-
	1.5	-	-	-
	2.0	-	-	-
	2.5	-	-	-
Somatic embryogenesis	0.25	0.25	-	-
	0.5	0.5	-	-
	1.0	1.0	-	-
	1.5	1.5	-	-
	2.0	2.0	-	-
Shoot Induction and proliferation	-	1.0	1.0	-
	-	1.5	1.5	-
	-	2.0	2.0	-
	-	2.5	2.5	-
	-	3.0	3.0	-
Root Induction	-	-	-	3.0
	-	-	-	4.0
	-	-	-	5.0

RESULT AND DISCUSSION

The leaf explants failed to produce callus on MS medium without growth regulators. The callus was observed only when MS medium was supplemented with growth regulator auxin (2, 4-D, fig.1a). The first visible response was the initiation of callus along the wounded edges of leaf explants within 7 to 15 days of inoculation. It is because the cut ends of leaf explants provide a way for the nutrients and growth regulators to be absorbed efficiently from the medium and start functioning according to the nature of the growth regulator Reynoired[6] et al, 1993. The callus grew and turned greenish, gradually covering whole surface of the leaf (fig. 1b). Maximum callus induction and growth was recorded in cultures grown with 2 mg/1 2, 4-D, after one month of culture period (table 2). Though, 2, 4- D had positive effect on callus induction, but higher concentrations led to tough, compact and non morphogenic callus (fig.1c, table 2). After one month of culture period, all cultured callus commenced on to stage of embryogenesis initiated from the sides of callus mass (fig.1d). The cells turned nodular and acquired globular shape within seven days of culture period. This stage was confirmed by morphological observations done through stereo zoom microscope. The globular embryogenic mass was transferred to maturation medium containing 2,4-D in combination with BAP (0.25-1.0 mg/l). The embryos turned lustrous brown shiny and acquired maturity (fig.1e). Stimulation of somatic embryogenesis requires alteration and change in the vegetative cells which was induced by the addition of BAP. Among all the combinations tried (table 1), BAP (2.0 mg/l) in combination with 2,4-D (2.0 mg/l) was found best suited for somatic embryo maturation and germination.

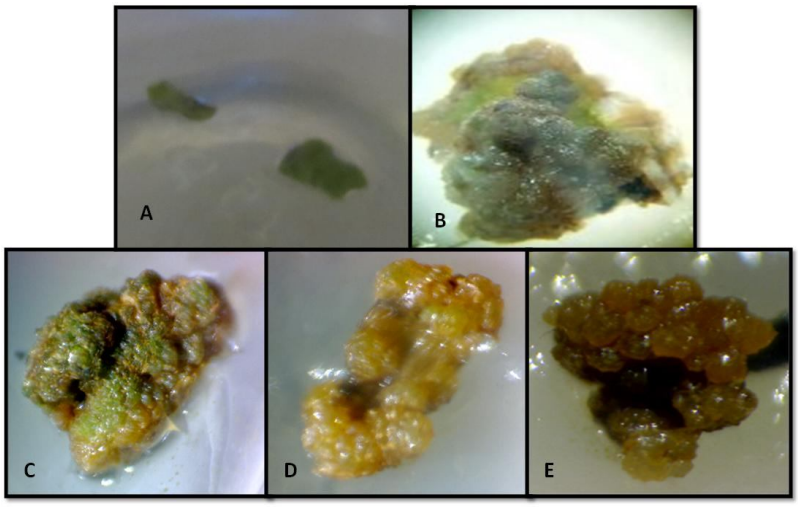

Fig 1. A- Leaf explant, B- Callus initiated from walls of leaf explant, C- Globular shape somatic embryos, D- Maturation stage of embryos, E- Mass of matured globular shiny somatic embryos

It was observed that the embryos failed to mature and germinate in the embryogenic medium containing 2,4-D alone. Addition of BAP was found essential for somatic embryo maturation and germination. Similar results are reported by Jain[7] et al 2010, where BAP was found essential for SE maturation and germination from leaf explants of *Bacopa monnieri*.

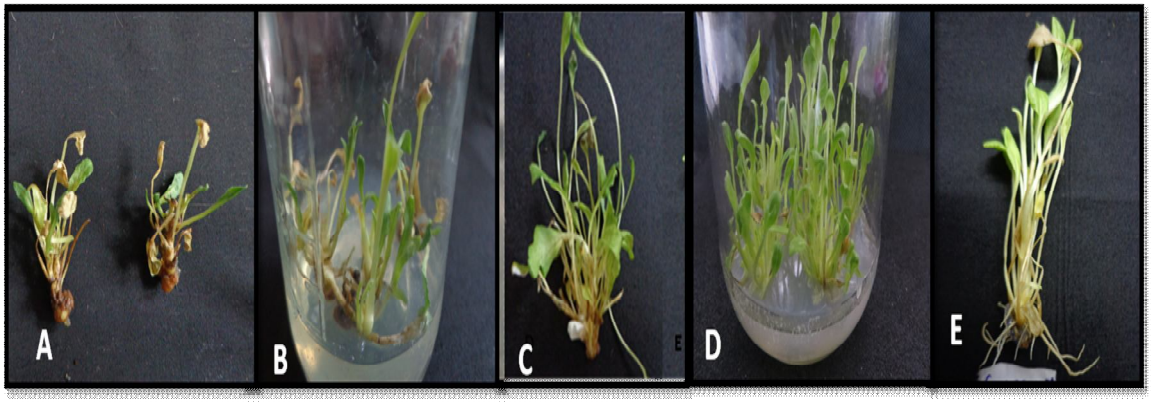

Fig 2. A-Shoot emergence from embryos, B-C shoot induction, D- Shoot proliferation, E- Rooted shoots

Table 2- Response of various concentrations of 2,4-D on callogenesis from leaf explants of *Gerbera jamesonii*

2,4-D (mg/l^{-1})	Callus growth
0.25	-
0.5	-
1.0	+
1.5	++
2.0	+++
2.5	++

Callus growth: +: good, ++: very good, +++: excellent

The shoots emerged from the embryos were uniformly isolated from multiple shoot complex (fig. 2a) and proliferated in the proliferation medium containing Kinetin (1.0- 3.0 mg/l) in addition to BAP (fig. 2b-c). Enhanced proliferation was obtained in the medium containing 2.0mg/l BAP and 3.0mg/l Kinetin (fig. 2d). BAP in isolation was not found effective for enhanced shoot proliferation and we observed that kinetin acted in synergy with BAP. It is reported that cytokinins transmit their signals by a receptor component system which consists of histidine kinase proteins and response regulators Jenifer and Kieber[8] 2008. Various scientists have reported more than one type of cytokinin receptor in various plant species Du[9] et al., 2007; Inoue[10] et al., 2001; Suzuki[11] et al., 2001; Yonekura-Sakakibara[12] et al., 2004. The ligand binding activities to specific types of cytokinins

hence is restricted due to the diversity of receptor structures Spíchal[13] et al 2009 which may sequentially activate different developmental responses. There are reports that cytokinins affect various aspects of plant development, especially vascular development and organ formation (Mähönen[14] et al., 2006; Barciszewski[15] et al., 2007). In our study, BAP in isolation generated shoots but the shoots were small and failed to develop further. However, with the addition of kinetin, multiple shoots initiated leading to high frequency regeneration of plantlets. Similar results have been reported by Kanokporn S[16] et al 2016 on regeneration of vetiver grass where BAP and kinetin worked synergistically to produce high plant regeneration efficiency.

Rooting of the shoots was obtained by transferring shoots in rooting media containing IAA in various concentrations (3.0-5.0 mg/l^{-1}). Excellent rooting was obtained in shoots cultured in MS medium containing 3.0 mg l^{-1} IAA within 4 weeks of culture period (fig. 2e).

Various reports on tissue culture of Gerbera from various explants are available. Regeneration from callus culture of *Gerbera Jamesonii* is reported by Aswath and Choudhary[17] 2002, organogenesis through callus in suspension by Kumar and Kanwar[18], 2005,2007 embryogenesis in suspension culture from leaf explants by Hasbulla[19] et al 2011, organogenesis from leaf derived callus and shoot initiation from immature and mature capitulum by Reeta[20] et al 2008 and 2011 respectively. We present effective somatic embryogenesis and enhanced regeneration of *Gerbera jamesonii* plantlets from leaf explants cultured in semisolid MS medium fortified with auxin and cytokinin growth regulators. This efficient somatic embryos induction protocol could be useful for development, improvement and conservation of *Gerbera jamesonii*.

CONCLUSION

Somatic embryogenesis is an influential tool for plant propagation and improvement of ornamental plants of commercial value. We report somatic embryogenesis and successful regeneration from leaf explants of *Gerbera jamesonii*. Our results can be utilised for plant propagation and to generate foundation stocks of elite planting material to meet up the demand of cut flowers in commercial market.

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ABBREVIATIONS:

BAP- Benzyl Amino Purine,
2, 4-D – 2, 4-Dichlorophenoxyacetic acid
IAA – Indole-3-acetic acid
Kn – Kinetin
MS- Murashige and Skoog

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