

TISSUE CULTURE OF GERBERA

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Abstract

Gerbera (*Gerbera jamesonii*) somatic tissues and seeds were tried for raising tissue cultures. The explants of shoot tips, immature inflorescences, leaf sections, capitulum explants, axillary buds and receptacles explants from field grown plants had contamination problems. Another trouble was slow growth of explant cultures as they were treated with sterilizing chemicals which damage their growing regions. However, callusing was in all explants and shoot regenerations were obtained from shoot tip and axillary buds. The calluses were obtained from clean seeds which were pre-soaked (20mg/liter for six hours) in colchicine for induction of polyploidy. The germinating seedlings were crushed and tissue mass was put on callusing medium containing MS with BA+2,4-D (each 3 mg/liter). After six weeks, the calli were transferred to MS+BA (4mg/liter) +IBA (1mg/liter). The developed plantlets were separated and cultured on MS containing BA and IBA (each @ 0.5mg/liter). The plantlets were transferred to pots under 100% humidity during initial weaning period, acclimatized and grown as normal plants.

Introduction

Gerbera (*Gerbera jamesonii*) belongs to sunflower family Asteraceae and is popular ornamental of commercial importance used as a decorative garden plant, container plant, or mostly as cut flowers. It ranks among the top ten cut flowers of the world (Parthasarathy & Nagaraju, 1999). There are single, semi double and double cultivars. A healthy, vigorous plant with flowers on appropriate height, attractive colors and flower size is important that make the surrounding environment beautiful and pleasant. Conventionally, they are propagated by dividing clumps or by seeds. If we are successful for developing effective and low cost *in vitro* system, tissue culture can establish plants with predictable selected traits for mass propagation. It can also provide desirable somaclones as bonus along with economy of time. Tissue culture of *Gerbera* has been studied by various scientists (Aswath & Wanzen, 2004; Kumar & Kanwar, 2005; Sharma & Srivastava, 2005; Thakur *et al.*, 2004 and Ray *et al.*, 2005). The objective of this study was to determine the effects of colchicine on callus production and regeneration of *Gerbera*. Colchicine was used to induce polyploids as it doubles the chromosomes by inhibiting the spindle formation during cell divisions.

Materials and Methods

Initially the shoot tips, immature inflorescences (0.6-0.8cm in diameter), leaf sections, capitulum explants, axillary buds, receptacle explants from the field were taken. The vegetative explant sources from the field had high contamination rates and slow culture growth due to harsh sterilization of explants with chemicals, which damage the growing points. It was identified as the root cause of this problem. Finally mature seeds were tried for establishment of *Gerbera* tissue culture because clean cultures can easily be obtained with seeds. The seeds were washed with detergent and sterilized with 0.1%

HgCl₂ solution for two minutes and were thoroughly washed with sterile water. Seeds were germinated on MS basal medium (Murashige & Skoog, 1962). The crushed germinated seed mass were used as explant for callus production in media containing Kn, BA and 2,4-D (each @ 1-5 mg/liter) supplemented with glutamine and proline (each 5mg/liter). The calli were regenerated in media containing BA, NAA and IBA (each 1-5 mg/liter) supplemented with 5mg/liter glutamine. The colchicine was used @ 0-50 mg/liter. The sterile seeds were dipped in filtered sterile colchicine solution for 6 hours. The seeds were kept on sieve to remove colchicine solution and washed thoroughly with sterile distilled water. The seeds were put on sterile filter paper to absorb unnecessary water and then sown on MS basal medium solidified with 1% agar. The pH of medium was 5.8 and the cultures were kept in normal day light at 20±5°C. After 10 days, the germinating seeds were crushed in Petri plates with sterile blades and the tissue mass was kept on media containing 2, 4-D. All operations were done in sterile environment in laminar air flow cabinet. After six weeks, calli were kept on BA, NAA, and IBA (each @1-5mg/l) containing media. After two months, the regenerated shoots were transferred to MS medium with BA, IBA (each 0.5mg/liter). The developing plantlets were finally transferred to pots in high humidity, acclimatized and grown like normal plants.

Results and Discussions

The recovery of sterile cultures was very low with field grown plant tissues like shoot tips, immature inflorescences, leaf sections, capitulum explants, axillary buds and receptacle explants because of contaminations. The other problem was very slow growth of cultures from sterilized explants because of effects of harsh treatments of chemicals like HgCl₂, NaOCl, ethanol etc. used for cleaning plant tissues. However, callus was obtained in all the explants and shoot regenerations were obtained from shoot tip and axillary buds. Mature seed is the hard tissue because of seed coat and embryo is protected within seed. A working method was developed for colchicine treated seeds (Table 1) for callusing and regeneration (Figs. 1&2).

Disinfected mature seeds soaked in colchicine dose (20mg/liter) for 6 hours → Seeds dried on filter paper and germinated on MS basal medium → Germinated seedlings crushed and kept on MS medium containing BA+2,4-D (each 3mg/liter) → After six weeks, calli transferred to MS+ BA (4mg/liter) +IBA (1mg/liter)→ After 2 months, shoot, root regeneration subcultured in MS containing BA+ IBA(each 0.5mg/liter)→ Plants were transferred to pots in 100% humidity during weaning period → Acclimatized and grown like normal plants. The regeneration in callus of colchicine treated seeds is mentioned in Table 1. The maximum seed germination, callusing and regeneration in callus was obtained in control seeds (No colchicine soaking) while these parameters declined with increasing doses of colchicine and finally there was no germination in 40 mg/liter presoaked seeds. The initial calli were pale yellow, which were turning brown on long keeping and upon sub culturing, the calli became cream white and friable. The regenerations started in callus as green points. The leafy shoots developed with delicate thin roots at the base. The shoots were separated aseptically and further grown in culture before transfer to pots with 98% survival rate. Tissue culture studies have been reviewed by Kanwar & Kumar, (2008) who concluded that Gerberas are highly amenable to *In vitro* studies and favorably respond to tissue culture media. Two clones of *G. jamesonii* were propagated from fragments of young capitulum (Laliberte *et al.*, 1985).

Table 1. Regeneration in colchicine treated seed through callus.

Treatment	Colchicine (mg/liter)	% Seed germinated	% Explant forming callus	% Regenerated callus
C ₁	0	48	41	29
C ₂	10	31	28	17
C ₃	20	23	19	11
C ₄	30	9	4	2
C ₅	40	0	0	0



Fig. 1. Regeneration in callus from colchicine soaked seeds.



Fig. 2. Potted plants.

The multiple shoot cultures were obtained from elongating axillary buds of *Gerbera aurantiaca* (Meyer & Staden, 1988). Commercial multiplication of *Gerbera hybrida* was obtained through shoot tip culture (Huang & Chu, 1985). Plant regeneration was obtained from callus (Aswath & Choudhary, 2002) and plants were regenerated from cell suspension cultures of calli derived from leaf tissue culture (Kumar & Kanwar, 2007). The plants were regenerated from receptacle transverse thin cell layer cultures of *G. jamesonii* (Nhut *et al.*, 2007). All these reported studies clearly indicated that *In vitro* propagation of Gerbera can be obtained on commercial scale with improvement in field explant sterilization/ cleaning methodology.

References

- Aswath, C. and S. Wazneen. 2004. An improved method for *In vitro* propagation of *Gerbera*. *J. of Ornamental Hort.*, 7: 141-146.
- Aswath, C.R. and M.L. Choudhary. 2002. Rapid plant regeneration from *Gerbera jamesonii* Bolus callus cultures. *Acta Bot. Croat.*, 61(2): 125-134.
- Huang, M.C. and C.Y. Chu. 1985. A scheme for commercial multiplication of *Gerbera hybrida* Hort., through shoot tip culture. *J. Japan. Soc. Hort. Sci.*, 54(1): 94-100.
- Kanwar, J.K. and S. Kumar. 2008. *In vitro* propagation of *Gerbera* – A Review. *Hort. Sci. (Prague)*, 35(1): 35-44.
- Kumar, S. and J.K. Kanwar. 2005. Plant regeneration from callus and cell suspension cultures of *Gerbera jamesonii* Diablo. *European J. of Hort. Sci.*, 70: 265-270.
- Kumar, S. and J.K. Kanwar. 2007. Plant regeneration from cell suspensions in *Gerbera jamesonii* Bolus. *J. Fruit & Ornamental Plant Research*. 15: 157-166.

- Laliberte, S., L. Chretien and J. Vieth. 1985. *In vitro* plantlet production from young capitulum explants of *Gerbera jamesonii*. *Hort. Sci.*, 20(1): 137-139.
- Meyer, H.J. and J.V. Staden. 1988. The *In vitro* culture of *Gerbera aurantiaca*. *Plant cell, tissue and organ culture*, 14(1): 25-30.
- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiologia Plantarum*, 15(5): 473-497.
- Nhut, D.T., T.T.T. An., N.T.D. Huong., N.T. Don., N.T. Hai., N.Q. Thien and N.H. Vu. 2007. Effect of genotype, explant size, position and culture medium on shoot generation of *Gerbera jamesonii* by receptacle transverse thin cell layer culture. *Scientia Horticulturae*, 111(2, 4): 146-151.
- Parthasarathy V.A. and V. Nagaraju. 1999. *In vitro* propagation in *Gerbera jamesonii* Bolus. *Indian Journal of Horticulture*, 56: 82-85.
- Ray, T., P. Saha and S.C. Roy. 2005. *In vitro* plant regeneration from young capitulum explants of *Gerbera jamesonii*. *Plant Cell Biotechnology and Molecular Biology*, 6: 35-40.
- Sharma, G. and R. Srivastava. 2005. Combinations and concentrations of growth regulators for somatic embryogenesis in gerbera. *Annals of Agricultural Research*, 26: 214-217.
- Thakur, P.S., R.B. Ghorade and T.H. Rathod. 2004. Micropropagation studies in Gerbera. *Annals of Plant Physiology*. 18: 133-135.

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