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Callus induction from leaf explants of *Gerbera jamesonii*

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Abstract

In present study, leaves of *Gerbera jamesonii* were used as explants for callus induction. Sterilization of explants was done by washing with bavistin (0.2 %) for 7-8 minutes followed by $HgCl_2$ (0.1 %) treatment for 3-4 minutes. This was the best treatment for sterilization as it produced maximum percentage of uncontaminated cultures. Callus induction was observed from leaf explants on Murashige and Skoog (MS) medium supplemented with 1.0 mg/l 2,4-D, 1.0 mg/l NAA, 2.0 mg/l IBA. Maximum callusing was found with this medium. Time required for callus initiation from leaf explants ranged from 17-18 days. Color of callus from leaf explants varied from light yellow to light green.

Keywords: *Gerbera jamesonii*, leaf explants, growth regulators, callus

Introduction

Gerbera jamesonii Bolus ex. Hooker f., is a flower with increasing commercial significance. *Gerbera* is one of the leading cut flower and ranks among the top of leading cut flowers of the world (*Parathasarathy* and Nagarajun, 1999). The genus consists of about 40 species (Das and Singh, 1989). Out of recorded species, only one species *Gerbera jamesonii* is under cultivation. *Gerbera* belongs to the family *Asteraceae* and 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 (Peper et al. 1971). Among the vegetative means multiplication through divisions of clumps is most common method used for several decades. *Gerbera* can also be propagated through cuttings (Schiva, 1975). The plant multiplication through these methods is too slow to be commercially practicable. For commercialization of this crop, however, planting material is required on large scale which requires the development of easier, quicker and economically viable methods of propagation. Now *in vitro* propagation is commonly used for rapid and large propagation (Pierk et al. 1975; Roger and Tija, 1990; Erwin et al. 1991).

A tissue culture procedure has been proven to be commercially practical method for *Gerbera* propagation. This method enables a million fold expansions per year of a desired plant (Murashige et al. 1974; Aswath and Choudhary, 2002). Like any other ornamental plant *Gerberas* are produced exclusively for their aesthetic values. Thus

improvement for quality attributes such as flower color, longevity and form, plant shape and the creation of novel variants are important economic goals. Regeneration of shoots from leaf blades was observed during *in vitro* propagation of *Gerbera* (Hedtrich, 1979). Genetic variability within the *Gerbera* genus is very limited and breeding potential for new flower colors patterns as well as resistance to biotic and abiotic stresses is also limited (Orlikowska et al. 1999).

The technique of *in vitro* cultivation of plant cells or organs is primarily devoted to solve two basic problems. Firstly to keep the plants cells and organs free from microbes like bacteria and fungi and secondly to ensure the desired development in the cells and organs by providing suitable nutrient media and other nutrient conditions. *Gerbera* was propagated by direct or indirect organogenesis using various explants including stem tips floral buds, leaf, capitulum. The plants were produced from explants of capitulum in red flower *Gerbera* (Pierik et al. 1973), leaves (Pierik et al. 1974; Barbosa et al. 1994), floral buds (Posada et al. 1999), floral bracts (Maia et al. 1983), torus (Zhang, 2002) and inflorescence (Schum and Busold, 1985). *Gerbera* is one of leading cut flower in the world. Due to high economic value the demand of *Gerbera* is increasing day by day. Therefore the present study has investigated callus induction from leaf explants of *Gerbera jamesonii.*

Materials and methods

Source of plant material

The plants of *Gerbera* (*Gerbera jamesonii*) were procured from local nursery, Solan, HP.

Nutrient medium and culture conditions

For all *in vitro* studies, the basic nutrient medium Murashige and Skoog, MS (Murashige and Skoog, 1962) supplemented with various concentrations of growth regulators were used. After inoculation the cultures were incubated in culture room at 25+-2^{o}C under 16 hrs photoperiod.

Surface sterilization of leaf explants of *Gerbera*

Leaves of *Gerbera* were collected and thoroughly washed under running tap water. Leaves were then dipped in teepol (1 %) for 10 minutes and then treated with bavistin (0.2 %) for 5-8 minutes. To remove detergent, leaves were rinsed in sterilized distilled water. Under aseptic conditions, leaves were cut into small pieces (5-6 mm). These pieces were used as explants. Subsequent to this the explants were treated with $HgCl_2$ (0.1 %) for 1-4 minutes. Then surface sterilized explants were rinsed 4-5 times with sterilized distilled water and inoculated onto culture media.

Callus induction from leaf explants of *Gerbera* using different concentrations and combinations of growth regulators

MS basal media supplemented with different concentrations and combinations of growth regulators (2,4-D, NAA and IBA) were prepared and optimized for best callus induction from leaf explants of *Gerbera* (Table 1). The cultured test tubes were kept in culture room under suitable conditions for callus induction.

Results

Surface sterilization of leaf explants of *Gerbera*

The leaf explants of *Gerbera* used for callus induction were surface sterilized by treating with bavistin (0.2 %) and $HgCl_2$ (0.1 %) at varying time intervals. The results showed that maximum (87.88 %) of uncontaminated cultures were reported with bavistin (0.2 %) for 7-8 minutes followed by $HgCl_2$ (0.1 %) for 3-4 minutes. The least (62.22 %) effective treatment was with bavistin (0.2

%) for 5-6 minutes followed by $HgCl_2$ (0.1 %) for 3-4 minutes (Table 2).

Table 1: Different concentrations of growth regulators (2,4-D, NAA and IBA) used for callus induction from leaf explants of *Gerbera*.

Medium code	Growth Regulators (mg/l)		
	2,4-D	NAA	IBA
MS 1	1.0	1.5	2.0
MS 2	2.0	1.5	2.0
MS 3	1.0	1.0	2.0
MS 4	1.5	2.0	1.0
MS 5	2.0	1.5	2.0
MS 6	2.0	2.0	2.0
MS 7	2.0	2.5	1.0

Callus induction

It was observed that maximum callus induced from leaf explants of *Gerbera* when MS basal medium containing 2,4-D (1.0 mg/l), NAA (1.0 mg/l), IBA (2 mg/l) was used (Table 3). Time required for callus initiation from leaf explants ranged from 17-18 days. Maximum callus observed after 30 days of incubation. Color of callus varied from light yellow to light green (Figure 1-4).

Discussion

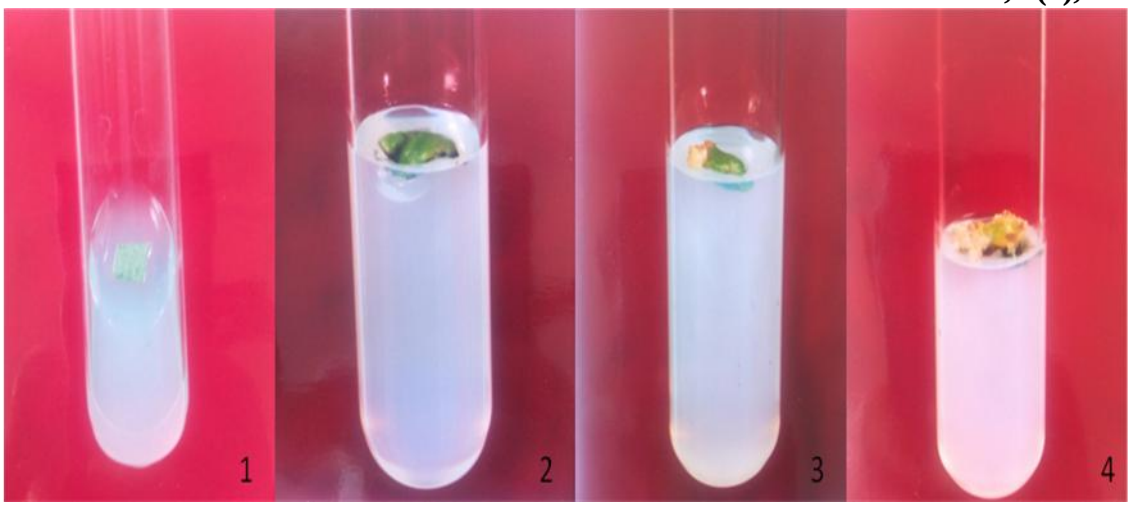
The present investigation revealed that *Gerbera* took 17-18 days for initiation of callus from leaf explants when MS basal medium was supplemented with growth regulators 2,4-D (1.0 mg/l), NAA (1.0 mg/l), IBA (2mg/l). With these concentrations of 2,4-D, NAA and IBA, *Gerbera* showed best callus formation as compared to other concentrations of growth regulators. Best callus growth from fully expanded leaves was reported on MS medium supplemented with 1 mg/l each of NAA, BA and IBA in *Gerbera jamesonii* (Parthasarathy et al. 1997).

Table 2: Effect of sterilants on *Gerbera*.

Treatment (T)	Sterilizing agents	Time (min)	Survival (%)
T 1	Bavistin (0.2 %)	5-6	62.22
	$HgCl_2$ (0.1 %)	1-2	52.09
T 2	Bavistin (0.2 %)	6-7	68.88
	$HgCl_2$ (0.1 %)	2-3	56.31
T 3	Bavistin (0.2 %)	7-8	87.88
	$HgCl_2$ (0.1 %)	3-4	69.92

Table 3: Callus induction using leaf explants of *Gerbera* at different concentration of growth regulators.

Medium code	Growth Regulators (mg/l)			Amount of callus	Remarks
	2,4-D	NAA	IBA		
MS 1	1.0	1.5	2.0	Medium	Poor Callus
MS 2	2.0	1.5	2.0	Poor	Very Poor
MS 3	1.0	1.0	2.0	Highest	Best callus
MS 4	1.5	2.0	1.0	Poor	Very Poor
MS 5	2.0	1.5	2.0	Medium	Poor Callus
MS 6	2.0	2.0	2.0	Poor	Very Poor
MS 7	2.0	2.5	1.0	Poor	Very Poor



Figures 1-4: Callus induction from leaf explants of *Gerbera* on MS 3 medium supplemented with 2,4-D (1.0 mg/l), NAA (1.0 mg/l), IBA (2mg/l) after 1 day (1) 7-8 days (2) 17-18 days (3) 30 days (4) of incubation.

A modified MS medium supplemented with 10 µM BA and 2.5 µM NAA were used for plant regeneration from *in vitro* leaf culture of several *Gerbera* species (Reynoird et al. 1993). Callus induction was observed from leaf and petal explants on MS basal medium supplemented with 1, 1.5 and 2 mg/dm^3 2,4-D. The calli derived from leaf explants differentiated into roots with NAA. BA and kinetin failed to induce callus from leaf and petal explants in cut flower of *Gerbera* (Kumar and Kanwar, 2006). Adventitious shoots from petiole and leaf pieces of *Gerbera* were regenerated on a medium supplemented with different concentrations of auxins and cytokinenins (Xu et al. 2002; Kumar et al. 2004). The effect of different combination of BAP (3, 5, 7 and 10 mg/l), kinetin (1, 3, 5 and 10 mg/l) and NAA (0.5, 1.0 and 2.0 mg/l) on callus induction was studied. The best callus induction was achieved on MS medium supplemented with 10 mg/l BAP and 2.0 mg/l NAA (Bhatia et al. 2008).

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