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Regeneration ability of petiole, leaf and petal explants in gerbera cut flower cultures *in vitro*

Surinder Kumar, Jitender Kumar Kanwar

Department of Biotechnology
University of Horticulture & Forestry
Solan 173 230 (H.P.) India
e-mail: skuhf@yahoo.com

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ABSTRACT

The maximum callus induction and growth in petal explant was observed on MS medium supplemented with 1, 1.5 and 2 mg l^{-1} 2,4-D and in leaf with 1.5 and 2 mg l^{-1} 2,4-D, however the callus failed to differentiate into shoots. Petiole explants did not respond to any of the treatments tried. The calli derived from leaf explant differentiated into roots with NAA in the medium. About 53% of the calli produced from petal explant developed five shoots per callus when transferred to shoot regeneration medium containing 2 mg l^{-1} BA and 0.5 mg l^{-1} IAA. The *in vitro* shoots were rooted by quick dipping the basal portion of the shoot in 2000 mg l^{-1} IBA solution. 50-60% survival of the plants was observed after 30 days of transfer to pots.

Abbreviations:
BA – 6-benzyladenine
2,4-D – 2,4-dichlorophenoxyacetic acid
IAA – indole-3-acetic acid

IBA – indole-3-butyric acid
Kin – kinetin
NAA – α-naphthalene acetic acid
TDZ – thidiazuron.

INTRODUCTION

Gerbera is a valuable ornamental species, grown for its cut flowers. The most efficient *in vivo* method for vegetative propagation is by making rhizome cuttings; however, the multiplication through this method is too slow to be commercially viable. *In vitro* propagation is commonly used now for rapid and large-scale multiplication (Palai et al. 1998, Aswath and Choudhary 2002, Zhang 2002). Adventitious shoots in gerbera have been regenerated from callus using different explants (Barbosa et al. 1994, Parthasarathy et al. 1997, Le et al. 1999, Posada et al. 1999, Kumar et al. 2004). The regeneration from floral buds or capitulum cultures has also been reported in gerbera by many workers (Pierik et al. 1975, Laliberte et al. 1985, Barbosa et al. 1994, Chen and Chen 2002). However, the percentage of explants with sprout formation with this method is low (Pierik et al. 1982). Adventitious shoots are required in mutation breeding to induce genetic variability. In the present investigation, an attempt was made to develop an efficient method to regenerate gerbera using petiole, leaf and petal as explants.

MATERIAL AND METHODS

The plants of cut flower gerbera (*Gerbera jamesonii* Bolus) were procured from the commercial nursery, Solan (H.P.) and allowed to flower under glasshouse in the Department of Biotechnology, University of Horticulture and Forestry, Solan. The gerbera used in this experiment is a cut flower cultivar with double type of flowers having red rays, transflorets and a black disc. The floral buds (0.8-1 cm diameter) were removed and dissected to excise petals from the outermost whorl of the capitulum. The fully expanded and mature leaves with petioles were removed just before anthesis. The petiole, leaf and petal explants (4-6 mm) were sterilized with 0.1% $HgCl_2$ for 3-4 min and washed thoroughly with sterilized water to remove sterilants. For callus induction, the explants were cultured on Murashige and Skoog (1962) (MS) medium supplemented with 0.8% (w/v) agar, 3% (w/v) sucrose and 1, 1.5 and 2 mg l^{-1} 2,4-D, NAA, BA and Kin. Three replications with ten explants in each were maintained for each treatment and ten explants in each treatment were evaluated. The cultures without growth regulators served as control. The cultures were maintained at 25±2°C in dark. One month old calli

(4 × 6 mm) were transferred to shoot regeneration medium comprising MS salts with cytokinins alone and in combination with auxins as described in Table 2. The data were recorded on per cent explants producing callus after 30 days and per cent calli producing shoots and number of shoots per callus after 60 days of incubation. The data were subjected to completely randomized design (Gomez and Gomez 1984). The statistical analysis based on mean values per treatment was carried out using analysis of variance (one way classification of data) technique.

In vitro shoots were rooted by quickly dipping the base of the shoot in 2000 mg l^{-1} IBA solution. The rooted plantlets were transferred to plastic pots (10 cm) containing a mixture of Farmyard manure (FYM) : sand (1:1). The hardening and acclimatization procedures were followed as described by Kumar et al. (2004). The per cent survival of the plants was recorded 30 days after their transfer to pots.

RESULTS AND DISCUSSION

The explants failed to produce callus on MS medium without growth regulators. The callus was observed in leaf and petal explants only when MS medium was supplemented with auxin (2,4-D or NAA) or cytokinin (BA or Kin). The maximum callus induction and growth was recorded with 1, 1.5 and 2 mg l^{-1} 2,4-D in petal explant and with 1.5 and 2 mg l^{-1} 2,4-D in leaf explant (Table 1). In petal explant, the friable and brownish callus produced with 2,4-D, turned dark brown after 2-3 subcultures and died. The cytokinins (BA and Kin) failed to induce callus on leaf explant. On the other hand, petiole explant did not respond to any of the treatments tried. This variation in callus induction/differentiation in different explants might have resulted due to the maturity of petiole and leaf explants in response to different growth regulators, since the explants (leaf and petiole) were collected just before anthesis. Bonga (1987) reported that the type of explant used for induction of callus mainly depends upon the juvenility of the explants. Jerzy and Lubomski (1991) reported adventitious shoot formation at the base of leaf petiole with 3 mg l^{-1} BA whereas leaf blade form only callus. However, Reynoird et al. (1993) obtained adventitious shoots from mature leaves of gerbera with 0.67 uM TDZ. The difference in the results may be due to the difference in genotype and cytokinin used. Compact, nodular and creamish callus was produced with all the concentrations of NAA and Kin on the medium in petal explant (Fig. 1A). The calli derived from Kin medium did not differentiate while the calli derived from NAA containing medium differentiated into shoots after six weeks of incubation in shoot regeneration medium (Fig. 1B).

Table 1. Number of *Gerbera* explants producing calli and callus growth in response to growth regulators

Treatment (mg l^{-1})	Callus induction (%)		Callus growth		Type of callus	
	Leaf	Petal	Leaf	Petal	Leaf	Petal
Control	0	0	0	0	0	0
2,4-D, 1	7.5 (13.5)	84.0 (66.5)	+	++	Friable	Friable
2,4-D, 1.5	10.2 (18.7)	85.5 (68.0)	+	++	Friable	Friable
2,4-D, 2	11.7 (21.5)	87.2 (69.4)	+	++	Friable	Friable
NAA, 1	4.5 (09.2)	76.0 (60.9)	+	+	Friable	Nodular
NAA, 1.5	7.5 (13.5)	77.5 (62.0)	+	+	Friable	Nodular
NAA, 2	8.7 (14.7)	79.5 (63.4)	+	+	Friable	Nodular
BA, 1	0	4.9 (10.1)	0	+	0	Friable
BA, 1.5	0	6.5 (12.0)	0	+	0	Friable
BA, 2	0	9.2 (16.8)	0	+	0	Friable
Kin, 1	0	2.7 (07.8)	0	+	0	Nodular
Kin, 1.5	0	2.7 (07.8)	0	+	0	Nodular
Kin, 2	0	3.0 (08.1)	0	+	0	Nodular
$LSD_{0.05}$	1.9	2.1				

Figures in brackets are transformed arc sine values
+ moderate callus growth ++ good callus growth

Table 2. Number of *Gerbera* calli producing shoots and number of shoots per callus in response to growth regulators in petal explant

Growth regulators applied to the MS medium	Concentration of growth regulator (mg l^{-1})	Calli producing shoots (%)	Mean number of shoots per callus
BA	1	0	0
	2	0	0
Kin	3	0	0
	5	0	0
BA : IAA	1 : 0.1	46.0(42.6)	3.5
BA : IAA	2 : 0.5	53.0(46.7)	5.0
BA : NAA	1 : 0.25	25.0(29.9)	2.7
BA : NAA	1 : 0.25	32.0(34.4)	3.5
Kin : IAA	3 : 0.1	0	0
Kin : IAA	3 : 0.25	0	0
Kin : NAA	5 : 0.5	0	0
$LSD_{0.05}$		2.7	1.1

Figures in brackets are transformed arc sine values

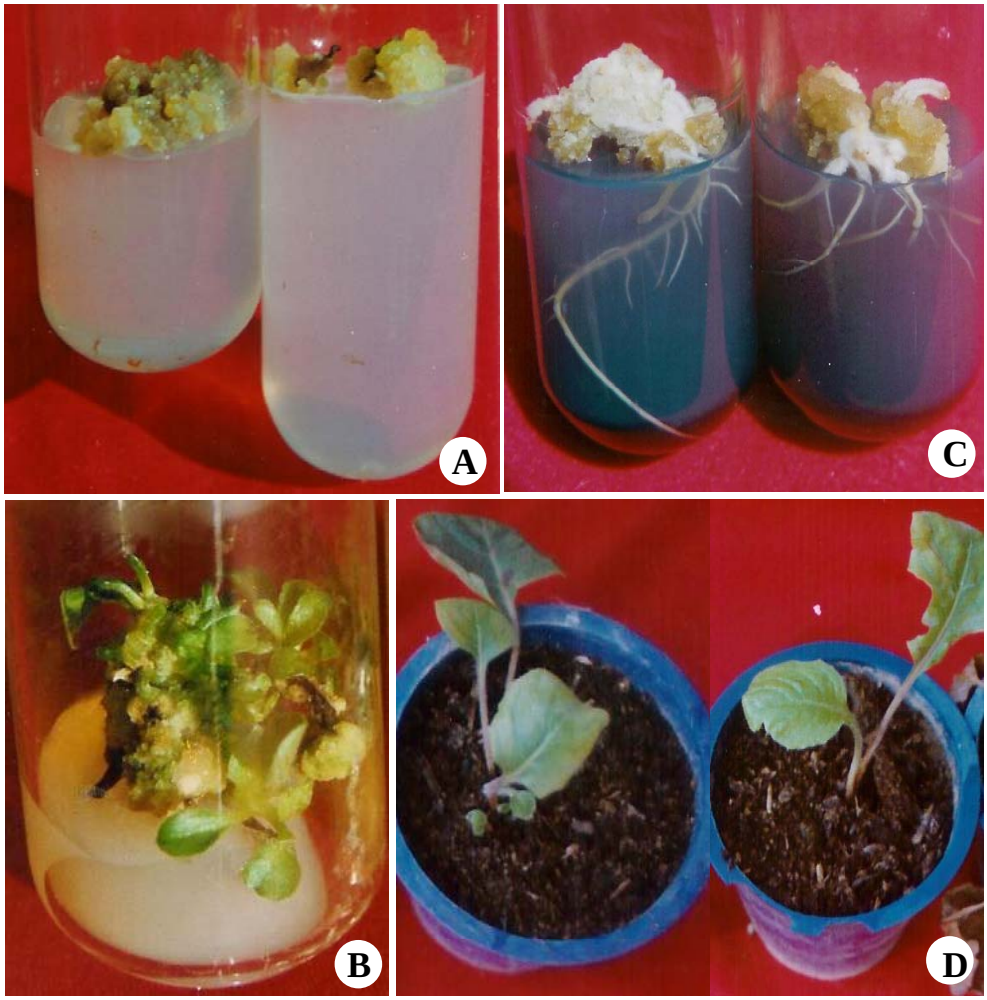

Fig. 1 A-D – A. Callus induction on MS medium supplemented with 2 mg l^{-1} NAA in petal explant after one month of culture. B. Differentiation of callus into shoots on medium with 2 mg l^{-1} BA + 0.5 mg l^{-1} IAA in petal explant. C. Root formation from callus on medium with 1 mg l^{-1} NAA in leaf explant. D. Two months old hardened plants of gerbera in plastic pots (10 cm dia) containing FYM : sand mixed in the ratio 1:1.

About 53% of the calli produced shoots on MS medium supplemented with 2 mg l^{-1} BA + 0.5 mg l^{-1} IAA followed by 46% with 1 mg l^{-1} BA + 0.1 mg l^{-1} IAA in the medium. The mean number of five shoots per callus was observed with 2 mg l^{-1} BA + 0.5 mg l^{-1} IAA. The minimum number of 2.7 shoots per callus was observed in medium with 1 mg l^{-1} BA + 0.25 mg l^{-1} NAA (Table 2). The calli produced from the leaf explant with auxin (2,4-D or NAA) differentiated only into roots (Fig. 1C).

The regeneration efficiency of the calli depends significantly on the type of explant and medium components. Vardja and Vardja (2001) inferred that all cells of the plants normally carry the same genetic information, but the morphogenetic responses vary according to the spatial and temporal distribution of the cells and their physiological and developmental stages. The genetic make-up, varied endogenous concentrations of growth hormones and response of the genotype to different concentration of growth hormones play a key role.

In vitro produced shoots were separated and rooted with 2000 mg l^{-1} IBA solution by quick dip method. The shoots were transferred to pots (10 cm), hardened and acclimatized with 50-60 per cent success (Fig. 1D).

From the above discussion it may be concluded that the plants regenerated from petal explant *via* adventitious shoot formation may be very useful for mutation breeding as the chances of mutant arising from adventitious shoots are very high (Kumar et al. 2004). Also, with the technique described in this paper, each petal produced on an average five shoots per callus and as there are numerous petals in a floral bud, a large number of shoots can be obtained initially, which can be multiplied to achieve a commercial goal.

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ZDOLNOŚĆ REGENERACYJNA *IN VITRO* EKSPLANTANTÓW Z PŁATKÓW, LIŚCI I OGONKÓW LIŚCIOWYCH GERBERY

Streszczenie: Największa indukcja kallusa i jego wzrostu z eksplantantów z płatków była obserwowana na pożywce MS z dodatkiem 1,0; 1,5 i 2,0 mg l^{-1} 2,4-D a z eksplantantów liściowych na pożywce z dodatkiem 1,5 i 2,0 mg l^{-1} 2,4-D, jednak nie zaobserwowano różnicowania pędów. Nie zaobserwowano żadnej reakcji w testowanych kombinacjach dla eksplantantów z płatków. Kallus pochodzący z eksplantantów liściowych różnicował korzenie na pożywce z NAA. Około 53% kallusa z ogonków liściowych wykształciło po 5 pędów po przeniesieniu na pożywkę do regeneracji pędów z 2,0 mg l^{-1} BA i 0,5 mg l^{-1} IAA. Otrzymane *in vitro* pędy ukorzeniano przez krótkie zanurzenie dolnej części łodygi w roztworze 2000 mg l^{-1} IBA. Przeżywalność roślin po 30 dniach od przesadzenia do doniczek wynosiła 50-60%.