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IN- VITRO REGENERATION OF *GERBERA JAMESONII* FROM LEAF EXPLANT

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ABSTRACT

Gerbera is one of the most important ornamental cut flower and is in great demand all over the world. To commercialize G. jamesonii tissue culture techniques are used to meet the growing demand for the planting material. In the present investigation callus induction and organogenesis were optimized with the leaf explants by manipulating the growth regulators. IBA, NAA and 2,4-D were used in different concentrations and combinations in MS medium to obtain callus from leaf explants.1.5 mg/L of 2.4-D was found suitable for best callus induction and 2.5mg/Lof BAP and 0.5mg/L of IAA induces best shooting whereas 2.5mg/L of BAP and 2.0 mg/L of NAA exhibited best result in inducing more no. of shoots per explant and length of shoot. 0.5-2.0mg of NAA as well as IAA were found best for rooting.

KEYWORDS : *culture techniques , organogenesis , growth regulators.*

INTRODUCTION

The plants of *Gerbera jamesonii* are important commercial cut flower which is one of the top ten cut flowers in the world. Due to slow vegetative growth and other conventional methods of propagation being inadequate for the production of large no. of plants, tissue culture technique using different explants have been attempted by different workers (Chu1992; Hutterman *et al.* 1993; Mantell *et al.,*1985;Pierik *et al.,* 1987)

In the present study, experiments were conducted to investigate organogenesis from leaf explants of *G.jamesonii* . The effect of various concentrations of growth regulators on the initiation ,multiplication, root induction were examined.

MATERIALS AND METHODS

Leaves (1.5-2.8cm)were taken as explants from potted Gerbera grown in a nursery . Young leaves were rinsed in running tap water for 30 mins and then washed with detergent followed by washing in running tap water. The leaves were surface sterilized by putting it in 0.1 %(w/v) HgCl2 solution for 10 mins and finally washed thrice with sterilized distilled water.

The nutrient media used for these studies were Murashige and Skoog(1962) Medium with 30gm/l sucrose and semi solid with 5-8 gm/l agar. The pH of medium was adjusted to 5.8 before autoclaving .Explant cultured without growth regulators served as the control.

Different growth regulators like IBA (Indole 3-butyric acid), NAA (Naphthalene Acetic Acid) and 2,4-D (dichloro phenoxy acetic acid) were added in the culture media in the concentration of 0.5,1.0,1,5,2.0,2.5 and 3.0mg/L. Each treatment was applied in three replications of 10 explants each.The explants were cultured in a growth room at 25*C in the dark to encourage the formation and growth of callus. Culture obtained from explants were regularly subcultured for maintenance and multiplication of shoots. One month old culture were transferred to a growth chamber with 16 hr photoperiod. For shoot and root regeneration

MS medium supplemented with different growth regulators were used. For shoot regeneration BAP, NAA and IAA were used in different combination and concentrations. For rooting MS medium supplemented with IAA in different concentrations were used.

RESULT

The leaf explant has been used for callus induction as well as regeneration of shoot and root formation . Different types of auxin and cytokinin were used in order to obtain complete regeneration of *Gerbera jamesonii* Bolus.in vitro.

When explants were inoculated in plain MS medium calli did not form but it formed on medium supplemented with any of the growth regulators tested (Table 1). Amongst all the growth regulator, best result was obtained with 1.5 mg/L of 2,4-D.which is about 95.8% and the callus is friable type. As regards relative efficacy 1.0 mg/L of NAA exhibited best result while the effect of IBA was not found significant.

The calli differentiated into shoot after 6-8 weeks of growth in the MS medium containing different combination of growth regulators. Best result of shooting was obtained with 2.5mg/L of BAP and 0.5 mg/L of IAA. Similarly MS medium supplemented with 2.5 mg/Lof BAP and 2.0 mg/L of NAA exhibited best results as regards no. of shoots per explant and length of shoot (Table 2 and fig 6)

Rooting was obtained with MS medium supplemented with NAA as well as IAA. In both cases 0.5 to 2.0 mg/L concentration were found best for rooting percentage, root length and types of root (Table 3).

DISCUSSION

Plant hormones and types of explants plays a very important role in determining the regeneration of *Gerbera jamesonii* in vitro.

In the present investigation percentage of explant producing callus was found best with 1.5 mg/L of 2,4-D . Parthasarthy *et al* (1995) found !.0 mg/L IBA.NAA or BA was found best in inducing higher percentage of callus induction from leaf explants.Prasad (2014) also found 1.5mg/L of 2,4-D was most effective in inducing best callus induction from leaf explants.

In the present investigation friable callus was found in all the concentration of 2,4-D as reported earlier by Prasad (2014) also .Compact and nodular callus from *Gerbera jamesonii* leaf are produced with BAP (Kumar *et al* 2004).

In the present investigation an average of 4.5 shoot were produced with the combination of growth regulators BAP and IAA. In an earlier report by Tyagi *et al* (2004), an average of 5-8 shoots were formed by calli from *Gerbera jamesonii* leaf explant which was induced by 4mg/L of Kinetin and 0.1 mg/L of IAA. Prasad (2014) found an average of 9-12 shoots formed by calli from *Gerbera* jamesonii leaf explant that was induced in MS medium supplemented with 1mg/L BAP. In the present case maximum rooting was found in the MS medium containing NAA or IAA (0.5, 1.0 and 2.0mg/L)

It is concluded that plant regenerated from leaf explant via adventitious shoot formation may be very useful in mutation breeding. Hence this regeneration protocol could be used to obtain large number of plantlets from this useful ornamental plant.

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Table-I

Growth Regulator mg/l	Concentration	% of explant producing Callus	Callus growth	Callus type
IBA	0.5	10.5	+	F
	1.0	12.6	++	F
	1.5	18.8	+++	F
	2.0	16.5	++	F
	2.5	15.0	++	F
	3.0	12.5	++	F
NAA	0.5	60.5	++	
	1.0	85.2	+++	F&N
	1.5	80.5	+++	F&N
	2.0	68.5	++	
	2.5	65.2	++	
	3.0	60.0	+	
2,4D	0.5	92.2	+++	Friable
	1.0	93.5	+++	Friable
	1.5	95.8	+++	Friable
	2.0	92.5	+++	Friable
	2.5	90.5	+++	Friable
	3.0	85.0	+++	Friable

Effect of Growth regulators on shooting

Table-2

Growth Regulator mg/L		Days taken for shoot formation	no of shoots/ explant	Length in cm
BAP	IAA			
0.5	0.5	60-63	3.0	1.75
1.0	0.5	61-62	3.25	1.65
1.5	0.5	61-63	3.55	2.05
2.0	0.5	60-61	3.85	2.25
2.5	0.5	63-64	4.5	2.52
	NAA			
0.5	0	-	-	-
1.0	.5	67.68	5.85	1.6
1.5	1	64.65	3.25	2.0

2.0	1.5	63-64	3.82	2.6
2.5	2.0	63-64	4.56	3.1

Effect of Growth regulator on rooting

Table-3

Growth hormone mg/L		Rooting %	Root length	Type of root
NAA	0.5	100	2.5	Multiple adventitious root without secondary roots
	1.0	100	2.0	
	2.0	100	1.52	
	3.0	85	1.05	
	4.0	80	0.5	
IAA	0.5	100	3.7	Multiple adventitious root without secondary roots
	1.0	100	3.2	
	2.0	100	3.7	
	3.0	80	2.58	
	4.0	70	2.16	

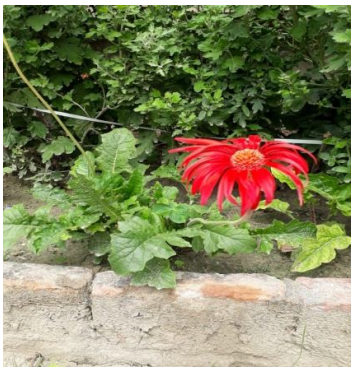

Fig1. Gerbera growing in border

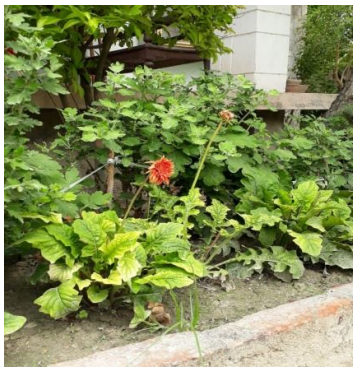

Fig2. Potted plant of Gerbera

Fig. 3 Leaf explant of Gerbera cultured on MS medium supplemented with growth regulators

Fig.4 Callus initiation from cut ends of the explant

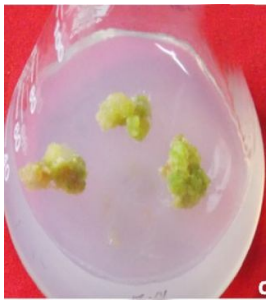

Fig 5 Callus inuction after 6 weeks in MS medium supplemented with 1.5mg/L 2,4-D

Fig 6 In Vitro shoot bud differentiation from callus in MS+ BAP +IAA

Fig 7 Rooting in MS medium supplemented with IAA 2mg/L

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