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Rapid clonal multiplication method of gerbera (*Gerbera jamesonii* Bolus) cv. Jallisse through *in vitro* techniques

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ABSTRACT: *A rapid clonal multiplication method was developed for* Gerbera *jamesonii Bolus cv. Jallisse through* in vitro *techniques in the tissue culture laboratory,dept.of Floriculture and Landscaping in the year 2008-09 using calli derived from immature quarter capitulum explants in modified Murashige and Skoog media. About 1/4th part of calli mass was cultured on MS medium supplemented with 2.0 mg/l BAP alongwith Ads 75 mg/l producing maximum 1290.67 number of shoots. As regards to root formation, ½ MS medium supplied with 0.3 mg/l IAA was found to be ideal for root formation with 100 % culture producing roots.*

Keywords: *In Vitro, calli, explants, BAP, IAA, Ads*

INTRODUCTION

Gerbera is one of the most internationally acclaimed commercial cut flower occupying 5th position among the top ten cut flowers of the world. It has a wide applicability in the floral industry as cut flower and potted plant. These are planted in pots, beds, border for garden display. Gerbera, the magnanimous flower is used as fresh in exhibition, flower arrangement, floral decorations, high class bouquets and also as dry flower. Its commercial cultivation is gaining momentum day by day as it provides high profit from small holdings. It can be propagated by both sexual and asexual methods. But the multiplication by these methods is too slow to be commercially practicable. And for commercialization of this plant, planting material is required on a large scale, which requires the development of an easier, quicker and economically viable method of propagation, which can only be possible by *in vitro* technique. Rapid multiplication method forms a backbone in production of huge quantity of disease free planting material in a relatively shorter period of time and small space under controlled conditions throughout the year. Rapid clonal multiplication is the need of the hour,for which the present investigation was undertaken.

MATERIALS AND METHODS

The capitula were collected from gerbera cv. Jallisse for *in vitro* study.Quarter capitula were used as explants and sterilized in 0.02% bavistin solution for 10 minutes followed by washing in sterile distilled water for 3-4 times. The explants were surface sterilized with 0.1% $HgCl_2$for 6 minute and dipped in KCl for 1minute for removal of Hg ions. The explants were inoculated in modified MS medium for callusing. The p^H of the media was adjusted to 5.7 ± 0.1 before sterilization by addition of 0.1 N NaOH or 0.1 N HCl and autoclaved for 20 minutes at 121^{o}C and 15 Psi pressure. After formation of calli,they were cultured on shoot proliferation medium. For shoot initiation and better proliferation of shoots, the MS medium was fortified with, combinations of BAP (1.5, 2.0, 2.5 mg/l) or Kinetin (2.0, 2.5 mg/l) with IAA (0.5 mg/l) and Ads (75, 100 mg/l).The calli were cultured on shoot proliferation media for 28days and subsequently subcultured twice at an interval of 22days for shoot proliferation.Each and every time single shoot excised from the multiple shoots was used.The observations were recorded on days to shoot initiation,proliferation,no of multiple shoots,length of the shoot,no of leaves/shoot & colour of leaves. For all the above cultures, three replications per treatment

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and 10 cultures per replication were used. All the cultures were carried out inside culture room maintained at 25 ± 2 °C and 16 hr photoperiod.The data recorded for

Total no of probable shoot during culture & subculture per calli mass = 4 x no of shoots during culturing x no of shoots during 1st subculturing x no of shoots during 2nd subculturing.

Total no of probable shoots/quarter capitulum explants = 4 x total no of shoots during culturing & subculturing per calli mass.

Total no of probable shoots/explants = 4 x no of shoots per1/4th quarter capitulum.

The proliferated shoots after 2nd subculturing we are taken for rooting in MS and ½ MS media fortified with various concentrations of IAA, NAA and BAP separately in cv. Jallisse for root initiation. Observations recorded up to 22 days of inoculation .Root initiation,Number of roots per plant,root length,root colour and nature of root were observed and Percentage of culture producing roots=

$$\frac{\text{Total number of cultures producing roots}}{\text{Total number of culture taken}} \times 100$$

The data recorded from all the experiments were analysed following the method of Gomez and Gomez (1984) using one way ANOVA in Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

Shoot Initiation

Organogenesis can be induced from the callus, was reported by Chawla, 2002. In this experiment, calli derived from capitulum explants were used for shoot multiplication study during culturing with different combinations of BAP or Kinetin with Ads and BAP with IAA &Ads. In cv. Jallisse, BAP 2.0 mg/l or Kinetin 2.0 mg/l with Ads 75 mg/l reduced the days for shoot initiation, produced maximum number of multiple shoots per calli mass and number of leaves per multiple shoots. Increased concentration of BAP or Kinetin from 2.0-2.5 mg/l increased the days to shoot initiation. BAP 2.0 mg/l or 2.5 mg/l increased the days to shoot initiation. BAP 2.0 mg/l or 2.5 mg/l with Ads 75.0 mg/l or 100 mg/l produced longer shoots. The result obtained in present study were similar to the result by Aswath and Choudhary, 2001, where, shoot organogenic capacity was higher with BAP and Ads. The above treatment was also found to be effective for shoot initiation and multiplication. Kinetin 2.0 mg/l or 2.5 mg/l with Ads 75.0 mg/l or 100.0 mg/l produced longer shoots. However, increased concentration of BAP decreased the shoot length. BAP and Kinetin combinations produced light coloured leaves during culturing of capitulum explant.

During 1st subculturing, Kinetin 2.0 mg/l with Ads 75.0 mg/l and BAP 2.0 mg/l with Ads 100.0 mg/l produced maximum number of multiple shoots per single shoot in cv. Jallisse. longer shoots were recorded with Kinetin 2.0 mg/l or 2.5 mg/l with Ads 75.0 mg/l or 100.0 mg/l. Number of leaves per shoot was maximum in Kinetin 2.0 mg/l with Ads 75.0 mg/l in cv. Jallisse. So, during 1st subculturing for the cv. Jallisse, Kinetin 2.0 mg/l with Ads 75.0 mg/l was most effective combination for production of maximum number of multiple shoots and leaves followed by BAP 2.0 mg/l with Ads 75.0 mg/l or 100.0 mg/l.

During 2nd subculturing, BAP 2.0 mg/l or 2.5 mg/l with Ads 75.0 mg/l or 100.0 mg/l and BAP 2.0 mg/l or 2.5 mg/l along with IAA 0.5 mg/l and Ads 100.0 mg/l produced maximum number of shoots with more number of leaves in cv. Jallisse. . However MS medium fortified with 2.0 mg/l or 2.5 mg/l Kinetin with Ads 75.0 mg/l or 100.0 mg/l produced longer shoots. The result corroborated with the findings of Aswath and Choudhary (2001).

Total number of shoots during culturing and subculturing was higher (80.67) with MS medium fortified with BAP 2.0 mg/l alongwith Ads 75 mg/l in cv. Jallisse. Number of approximate probable shoots per quarter capitulum explant were 322.67 in Jallisse. However, total number of probable shoots per capitulum were 1290.67 in cv. Jallisse. MS medium fortified with BAP 2.0 mg/l in combination with Ads 75 mg/l for cv. Jallisse was the best combinations for production of maximum number of shoots per explant. BAP, a potent cytokinin along with Adjuvant Ads was most effective combination for shoot proliferation in Gerbera.

Root Formation

The proliferated shoots after 2nd subculturing we are taken for rooting in MS and ½ MS media fortified with various concentrations of IAA, NAA and BAP separately in cv. Jallisse. Early root initiation, maximum number of roots per plant, longer roots and percentage of culture producing roots were maximum in ½ MS medium fortified with 0.3 mg/l IAA followed by ½ MS supplemented with 0.5 mg/l IAA and MS medium supplemented with 0.3 mg/l IAA in both the cv. Jallisse. Rooting in cv.Jallisse was obtained by ½ MS medium supplemented with 0.3 mg/l IAA

Table 1
Effect of BAP, Kinetin, IAA and Ads on Multiple Shoot Production during 1st Sub-culturing of Quarter Capitulum Explants of var. Jallisse

Basal medium- MS **Duration – 22 days**

Treat. No.	*Treatments (mg/l)*				*Multiple shoots/ single shoot*	*Length of the shoots (cm)*	*No. of leaves*	*Colour of the leaves*
	BAP	*Kinetin*	*IAA*	*Ads*				
T_1	2.0	-	-	75.0	4.33	2.60	13.33	Light Green
T_2	2.5	-	-	75.0	5.00	2.47	11.67	Light Green
T_3	-	2.0	-	75.0	5.67	2.83	13.67	Light Green
T_4	-	2.5	-	75.0	5.00	2.93	10.33	Light Green
T_5	2.0	-	-	100.0	5.67	2.20	11.67	Light Green
T_6	2.5	-	-	100.0	5.33	2.17	10.67	Light Green
T_7	2.0	-	0.5	100.0	3.67	2.47	9.67	Light Green
T_8	2.5	-	0.5	100.0	4.00	2.40	12.67	Light Green
T_9	-	2.0	-	100.0	3.67	3.03	9.67	Light Green
T_{10}	-	2.5	-	100.0	3.33	2.97	10.00	Light Green
	SE(m)				0.45	0.08	1.36	
	CD (5 %)				1.33	0.25	3.95	

Table 2
Effect of BAP, Kinetin, IAA and Ads on Multiple Shoot Production during 2nd Sub-culturing of Quarter Capitulum Explants of var. Jallisse

Basal medium- MS **Duration – 22 days**

Treat. No.	*Treatments(mg/l)*				*Multiple shoots/ single shoot*	*Length of the shoots(cm)*	*No.of leaves*	*Colour of the leaves*
	BAP	*Kinetin*	*IAA*	*Ads*				
T_1	2.0	-	-	75.0	2.33	4.33	12.33	Light Green
T_2	2.5	-	-	75.0	2.00	4.40	9.00	Light Green
T_3	-	2.0	-	75.0	1.00	4.97	5.67	Light Green
T_4	-	2.5	-	75.0	1.33	5.00	7.67	Light Green
T_5	2.0	-	-	100.0	1.67	4.07	8.67	Light Green
T_6	2.5	-	-	100.0	2.33	4.37	10.67	Light Green
T_7	2.0	-	0.5	100.0	2.33	4.07	10.00	Light Green
T_8	2.5	-	0.5	100.0	2.00	4.53	9.33	Light Green
T_9	-	2.0	-	100.0	1.00	5.10	5.67	Light Green
T_{10}	-	2.5	-	100.0	1.00	5.03	6.33	Light Green
	SE(m)				0.29	0.14	1.19	
	CD(5%)				0.86	0.40	3.45	

Table 3
Effect of Plant Bioregulators on Multiple Shoot Regeneration of Capitulum Explants in cv. Jallisse

Treat. No.	*Treatments(mg/l)*				*Total number of shoots during culturing and subculturing*	*Probable number of shoots/ 1/4th captilum*	*Probable number of shoots/ capitulum*
	BAP	*Kinetin*	*IAA*	*Ads*			
T_1	2.0	-	-	75.0	80.67	322.67	1290.67
T_2	2.5	-	-	75.0	45.33	181.33	725.33
T_3	-	2.0	-	75.0	41.33	165.33	661.33
T_4	-	2.5	-	75.0	40.33	161.33	645.33
T_5	2.0	-	-	100.0	71.67	286.67	1146.67
T_6	2.5	-	-	100.0	42.67	170.67	682.67
T_7	2.0	-	0.5	100.0	40.67	162.67	650.67
T_8	2.5	-	0.5	100.0	42.00	168.00	672.00
T_9	-	2.0	-	100.0	13.00	52.00	208.00
T_{10}	-	2.5	-	100.0	14.33	57.33	229.33
	SE(m)				11.41	45.66	182.65
	CD(5%)				33.01	132.04	528.16

Table 4
Effect of IAA, NAA and BAP on Rooting of var.Jallisae

Basal medium- MS **Duration – 22 days**

	Treat. No.Treatments(mg/l)				*Days to root initiation*	*No.of roots/ plant*	*Length of the roots/ plant(cm)*	*Nature of root*	*Colour of root*	*Percentage of culture producing root*
	MS	*IAA*	*NAA*	*BAP*						
T_1	Full	-	-	-	-	-	-	-	-	-
T_2	MS	0.3	-	-	9.00	4.67	4.23	Normal	Off white	100.00(87.50)
T_3	MS	0.5	-	-	9.33	3.67	4.33	Normal	Off white	96.66(82.19)
T_4	MS	0.3	-	1.0	-	-	-	-	-	-
T_5	MS	0.5	-	1.0	-	-	-	-	-	-
T_6	MS	-	1.0	-	15.00	4.33	3.33	Thin wiry	Off white	86.66(69.02)
T_7	MS	-	1.0	1.0	-	-	-	-	-	-
T_8	½ MS	-	-	-	-	-	-	-	-	-
T_9	½ MS	0.3	-	-	7.33	4.00	5.00	Normal	Off white	100.00(87.50)
T_{10}	½ MS	0.5	-	-	8.33	4.33	4.90	Normal	Off white	96.66(82.19)
T_{11}	½ MS	0.3	-	1.0	-	-	-	-	-	-
T_{12}	½ MS	0.5	-	1.0	-	-	-	-	-	-
T_{13}	½ MS	-	1.0	-	11.67	3.67	3.90	Normal	Off white	86.66(69.02)
T_{14}	½ MS	-	1.0	1.0	-	-	-	-	-	-
	SE(m)				0.28	0.29	0.05			2.23
	CD(5%)				0.78	0.82	0.16			6.17

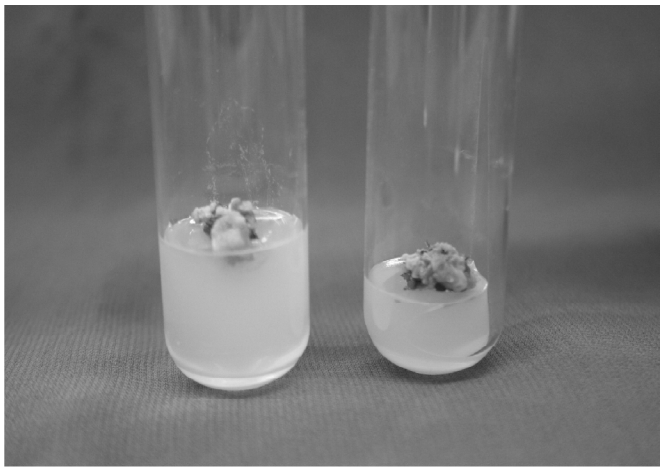

Plate 1: Greenish callus mass of quarter capitulum explant of cv. Jallisse

(a) MS + BAP 2.0 mg/l + IAA 1.0 mg/l
(b) MS + BAP 2.5 mg/l + IAA 1.0 mg/l

Plate 3: Root formation from capitulum explants of gerbera cv. Jallisse with,

(a) ½ MS + IAA 0.3 mg/l
(b) ½ MS + IAA 0.5 mg/l
(c) MS + IAA 0.3 mg/l

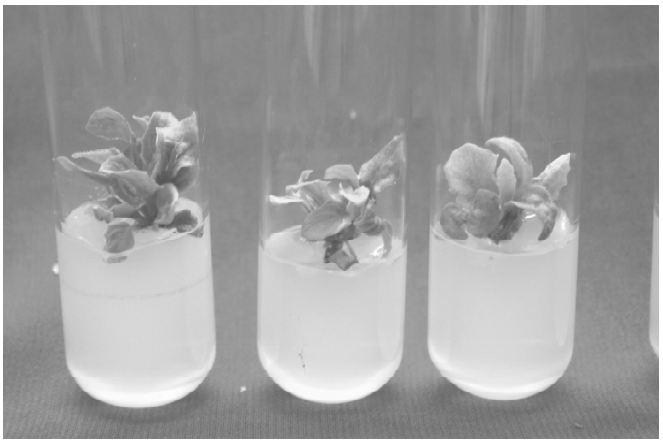

Plate 2: Multiple shoot regeneration from callus derived from quarter capitulum explants of gerbera cv. Jallisse (1st sub-culturing)

(a) MS + Kinetin 2.0 mg/l + Ads 75 mg/l
(b) MS + BAP 2.0 mg/l + Ads 100 mg/l
(c) MS + kinetin 2.5 mg/l + Ads 100 mg/l

corroborated with the findings of Pattnaik (2007) in cv. Common Red. Preference of IAA for rooting in gerbera has been reported by Laliberte *et al*. (1985), who obtained cent per cent rooting in both the cultivars Pastourelle and Nardi of gerbera. Root production by ½ MS medium supplied with 0.5-1.0 mg/l NAA was reported by Aswath and Choudhary (2002) reported that MS medium supplied with 0.5 mg/l IAA or 1.0 mg/l IAA with 100 mg/l Ads were ideal combinations for root formation in gerbera.

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