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Standardization of rapid axillary shoot multiplication through *in vitro* shoot tip culture in gerbera

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ABSTRACT

Rapid axillary multiplication was standardized using *in vitro* shoot explant of two gerbera cultivars on half-strength MS medium supplemented with nine plant growth regulator combinations (BAP : 1.0, 1.5, 2.0 mg /l ; BAP + kinetin: 1.0 + 0.25, 0.5, 1.0 mg/l and kinetin: 1.0, 2.0, 3.0 mg/l). BAP: kinetin ratio of 1:4, 1:2 and 1:1 proved equally effective in inducing shoot proliferation as well as decreasing the number of days to shoot initiation. Shoot proliferation was higher on half-strength MS medium (8.12 shoots/plant) in comparison to 7.06 shoots/plant on full-strength MS medium. 100 percent rooting was achieved on MS medium supplemented with 0.75 mg/l. Root number and root length in both the cultivars improved significantly in media supplemented with IBA in comparison to NAA. Rooting response on MS solid, MS with half agar and MS liquid media was similar except that the root length in plantlets on MS liquid medium was significantly shorter. However, plantlets rooted on MS liquid and MS media with half agar exhibited a significantly improved survival (96.25%) of rooted plantlets *ex vitro* in comparison to those rooted on MS medium with full agar (71.25 and 86.25%, respectively).

Key words: Axillary shoot proliferation, gerbera, *in vitro* propagation.

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus.) has consistently figured among the top five selling cut flowers in world in the last decade. Owing to its ease of cultivation under a wide variety of conditions, assortment of available colours and demand in market, gerbera has found favour among flower growers in India. Most of gerbera grown commercially in India are raised *in vitro*. Several trials over the years have demonstrated significant improvements in flower yield in tissue culture raised gerberas over those multiplied through cuttings (Jothi *et al*., 5). A high genotype × propagation system interaction has been reported in comparisons between division and tissue culture raised gerberas (Meynet, 7). Tissue cultured gerberas can be sub-cultured indefinitely with little loss in yield over a number of sub-cultures (Farina *et al*., 4). Shoot tip culture is by far the most commonly employed *in vitro* method for commercial multiplication (Shagufta *et al*., 12). Shoot tips commence the growth more rapidly and contain more axillary buds (Xi and Shi, 15). Aswath and Choudhary (1) and Aswath *et al.* (3) have demonstrated some techniques of rapid multiplication of gerbera through axillary shoot proliferation. However, their study depended upon commercial tissue culture laboratories for initial stock cultures. The current study involved establishment of shoot tips to raise stock cultures, standardization of media for rapid axillary shoot multiplication and rooting.

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MATERIALS AND METHODS

Standardization of rapid axillary shoot regeneration in gerbera was started once enough shoots were produced *in vitro* enabling us to compare effect of different growth regulator combinations on shoot multiplication. The experiment was conducted in the Division of Floriculture, Medicinal and Aromatic Plants during the year 2009-2011. *In vitro* stock cultures of two gerbera cultivars, namely, Rejiko and South Pacific were established through aseptic shoot tips as per Nazki *et al*. (8) (Fig. 1a & b). Cultivar Rejiko is a maroon red, medium sized cut flower variety, whereas, cultivar South Pacific is a large yellow cut flower variety. Stock cultures of the two cultivars were maintained until development of sufficient number of shoots for comparing various growth regulator combinations for rapid axillary shoot proliferation and subsequent studies on rooting. Stock cultures were maintained at 23 ± 1°C at 16/8 h photoperiod. Shoot tips (0.5 cm) were excised from stock cultures and placed on half-strength MS medium and supplemented with nine growth regulator combinations (BAP: 1.0, 1.5, 2.0 mg/l; BAP + kinetin: 1.0, + 0.25, 0.5, 1.0 mg/l and kinetin: 1.0, 2.0, 3.0 mg/l). Data on percent shoot proliferation, days to shoot initiation, shoot number/explant, shoot length (cm), and clump weight were

recorded. To standardize basal medium for axillary shoot proliferation full- and half-strength Murashige Skoog media supplemented with BAP + kinetin (1.0 + 0.25 mg/l) were compared for days to shoot initiation, shoot number per explant and shoot length.

For standardization of rooting, MS medium was supplemented with six auxin concentrations (IBA, 0.25, 0.5, 0.75 mg/l and NAA 0.50, 1.0, 1.50 mg/l). Data on percent rooting, days to root initiation, root number per plant, and root length was recorded. Rooting media consisting of MS medium with full agar, MS medium with half agar and MS liquid media supplemented with 0.75 mg/l IBA were compared in terms of their effect on days to root initiation, number of roots per plant and root length. Further, plantlets rooted in three different rooting media were studied for days to establishment and per cent survival *ex vitro*. Hardening of the rooted plantlets was carried out in low cost polypropylene modules as proposed by Nazki *et al.* (11) (Fig. 1g & h).

In all experiments 12 cultures per treatment were maintained each replicated thrice in completely randomised design. Data in each experiment was recorded at the end of five weeks of culture. Data recorded was subjected to statistical analysis using ANOVA.

RESULTS AND DISCUSSION

In the present study axillary shoot proliferation in gerbera was evaluated in terms of per cent shoot proliferation, days to shoot initiation, shoot number per explant, shoot length and clump weight. There was a significant improvement in per cent proliferation when BAP was used in combination with kinetin (Table 1; Fig. 1c). BAP: kinetin ratio of 1:4, 1:2 and 1:1 proved equally effective in inducing shoot

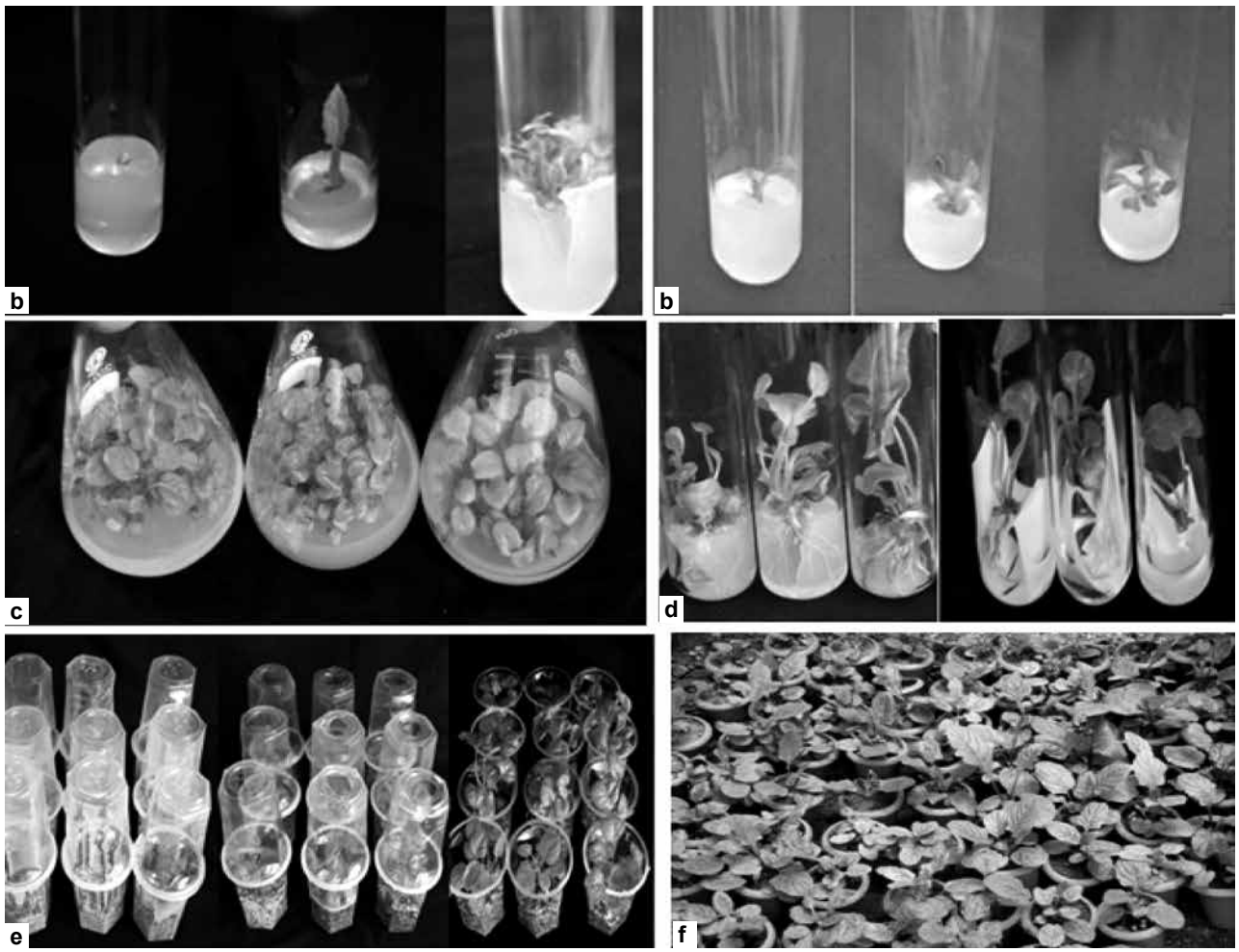

Fig. 1. Micropropagation stages in gerbera. a. Establishment of shoot tip without leaves; b. Establishment of shoot tip with leaves; c. Mass multiplication of gerbera shoots under BAP 1.0 mg/l + kin. 0.25 mg/l; d. Rooting of plantlets in solid and; e. liquid MS medium; f. Various stages in hardening of plantlets

Table 1. Influence of growth regulators on axillary shoot proliferation in gerbera.

½ MS + PGR	conc. (mg/l)	Shoot proliferation (%)		Days to shoot initiation		Shoots No./ explant		Shoot length (cm)		Clump weight (mg)	
		C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2	C_1	C_2
BAP	1.00	84.66 (66.99)	92.66 (74.35)	8.66	7.83	5.16	6.00	2.41	2.83	63.26	72.60
BAP	1.50	87.03 (69.07)	93.51 (75.6)	9.00	7.83	5.83	7.16	2.50	3.00	63.40	73.26
BAP	2.00	85.17 (67.63)	90.73 (72.31)	9.00	8.66	5.50	6.83	2.83	2.83	68.16	71.50
BAP + kinetin	1.00 + 0.25	98.14 (83.60)	100.00 (90.00)	7.33	7.16	8.16	8.83	3.66	4.00	64.80	69.53
BAP + kinetin	1.00 + 0.50	97.22 (80.42)	98.14 (83.60)	7.66	7.66	8.16	8.66	3.66	3.83	64.60	70.60
BAP + kinetin	1.00 + 1.00	93.51 (75.31)	97.22 (84.40)	7.66	7.66	8.16	8.16	3.50	3.66	69.26	72.86
Kinetin	1.00	81.84 (64.84)	91.73 (73.48)	9.66	9.33	4.50	6.50	3.66	3.83	63.40	72.06
Kinetin	2.00	81.47 (64.55)	90.73 (72.31)	10.66	9.83	6.16	6.33	3.16	3.50	70.40	75.20
Kinetin	3.00	79.62 (63.24)	88.88 (70.61)	11.33	10.33	5.50	6.00	2.66	2.83	70.60	75.66
CD (P = 0.05)		5.36	5.57	0.77	1.13	1.85	1.43	0.68	0.75	7.16	NS

C_1 = Rejiko, C_2 = South Pacific, Data in parenthesis are the Arc Sin transformed values.

proliferation as well as decreasing the number of days to shoot initiation. Shoot number per explant was significantly higher in media where BAP and kinetin were used in combination. Cytokinins at appropriate levels are known to suppress terminal bud elongation and promote axillary shoot proliferation. Therefore, arriving at a right cytokinin level for optimum shoot production is a pre-requisite for mass propagation of gerbera through shoot tip culture. A number of workers since classical experiments of Murashige have tried various growth regulator combinations with diverse results in *Gerbera jamesonii*. Parthasarathy *et al*. (11) reported best results at 0.75 mg/l BAP in terms of culture weight, shoot number and length. In the current study, highest shoot length and clump weight in both cultivars were recorded in media supplemented with BAP and kinetin as against BAP or kinetin alone. Earlier, Aswath and Choudhary (2) reported highest shoot proliferation on 1.5 mg/l BAP supplemented medium.

In another experiment full- and half-strength MS medium were tried to study shoot proliferation in both the cultivars (Table 2). Shoot number explant^{-1} and shoot length improved significantly in explants incubated on half-strength MS medium. Results also revealed significant differences in two cultivars in response to change in media in terms of shoot number explant^{-1} and shoot length. Several workers have studied influence of changes in basic media composition on shoot proliferation. Aswath and Choudhary (1) reported better shoot number and length on half-strength MS medium, which is in conformity with results obtained in the present investigation.

Highest per cent rooting in both cultivars was recorded in medium supplemented with 0.75 mg/l IBA (Table 2). In both cultivars, IBA 0.50 and 0.75 mg/l significantly improved rooting percent in comparison to rooting in NAA supplemented media. Gerbera shoots are known to root without addition of any extraneous

Table 2. Influence of media and cultivars on axillary shoot proliferation in gerbera.

Medium	Days to shoot initiation			Shoot No./ explant			Shoot length (cm)		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
MS	7.62	7.12	7.37	7.75	6.37	7.06	3.65	2.82	3.23
½ MS	6.75	6.00	6.37	9.00	7.25	8.12	4.05	3.10	3.57
Mean	7.18	6.56	-	8.37	6.81	-	3.85	2.96	-
CD (P = 0.05)									
Medium		NS			0.60			0.32	
Cultivar		NS			0.60			0.32	
Medium × Cultivar		NS			NS			NS	

C_1 = Rejiko, C_2 = South Pacific

auxins (Mandal and Datta, 6). However, rooting per cent and quality are known to improve significantly with the use of auxins particularly IBA. In the current study also root number and root length in both the cultivars improved significantly in media supplemented with IBA in comparison to NAA (Table 3). Szulc and Rogozinska (13) reported that number of roots was higher in *G. jamesonii* cv. Moanco with increasing IBA concentration. Aswath and Choudhary (2) reported best rooting on IBA 1.75 mg/l, while, Nisha and Anupam (10) reported maximum roots per shoot in medium supplemented with IBA 1.0 mg/l. Udayalaxmi *et al*. (14) reported maximum rooting on 1.0 or 2.0 mg/l NAA.

Perusal of data regarding the effect of media on rooting behaviour of gerbera cvs. Rejiko and South Pacific (Table 4; Fig. 1d & e) reveal no significant influence on days to root initiation and root number per shoot. However, varietal differences in terms of days to root initiation and root number per shoot were significant. Since the three root media differed only in the quantity of gelling agent agar (full, half and no agar), days to root initiation and root number as expected did not vary significantly. However, roots in MS liquid medium were significantly shorter. Nutrients in liquid media are soaked up the paper bridge and hence become available to the plant through capillary action. The roots have to probe less depth of nutrient medium for nutrients and hence remain short. No reports regarding rooting of gerbera on media with altered agar levels were found. Gerbera roots bear abundant root hair due to which solid agar medium tends to stick to them and it is impossible to remove it without damaging the roots. Agar media clinging to roots was found to encourage disease development during the hardening procedure. To overcome this problem, experiment involving influence of *in vitro* rooting media on establishment and survival of two gerbera cultivars was taken up in the current study. Results reveal significantly less number of days to establishment taken by plantlets previously rooted in liquid media. The data also reveal improved *ex vitro* survival of plantlets rooted in MS liquid and MS medium with half agar (Fig. 2). The difference could be because of less damage to the roots on transition from *in vitro* to *ex vitro* conditions. No reports regarding the effect of *in vitro* rooting medium on *ex vitro* establishment and survival of rooted plantlets in gerbera.

Table 3. Influence of auxins on *in vitro* rooting in gerbera.

Auxin	Conc. (mg/l)	Rooting (%)		Days to root initiation		Root No. per shoot		Root length (cm)	
		C_1	C2	C_1	C2	C_1	C2	C_1	C2
IBA	0.25	93.33 (77.71)	96.67 (83.85)	15.33	12.13	4.13	4.20	2.53	2.66
IBA	0.50	96.67 (83.85)	100.00 (90.00)	16.20	12.36	4.53	4.53	3.06	3.53
IBA	0.75	100.00 (90.00)	100.00 (90.00)	15.40	11.40	5.13	4.86	3.66	3.73
NAA	0.50	83.33 (66.14)	86.67 (68.85)	22.93	16.00	2.26	2.40	1.80	1.93
NAA	1.00	93.33 (77.71)	96.67 (83.85)	20.33	14.86	3.20	4.26	2.06	3.53
NAA	1.50	93.33 (77.71)	96.67 (83.85)	17.200	13.13	3.20	4.80	2.26	3.73
CD (P = 0.05)		10.14	8.12	4.09	3.62	1.61	1.04	0.54	0.49

C_1 = Rejiko, C_2 = South Pacific; Data in parenthesis are the Arc Sin transformed value

Table 4. Influence of media and cultivars on rooting in gerbera.

Medium	Days to root initiation			No. of roots per shoot			Root length (cm)		
	C_1	C_2	Mean	C_1	C_2	Mean	C_1	C_2	Mean
M_1	15.26	12.06	13.66	4.60	4.80	4.70	3.46	3.66	3.56
M_2	14.93	11.73	13.33	4.66	4.9	4.80	3.66	3.8	3.76
M_3	13.80	9.66	11.73	4.73	4.93	4.83	3.06	3.13	3.10
Mean	14.66	11.15		4.66	4.88		3.40	3.55	
CD (P=0.05)									
Medium		NS			NS			0.25	
Cultivar		2.48			NS			NS	
Medium × cultivar		NS			NS			NS	

M_1 = Murashige and Skoog (MS) medium, M_2 = MS medium with half agar, M_3 = MS liquid medium; C_1 = Rejiko, C_2 = South Pacific

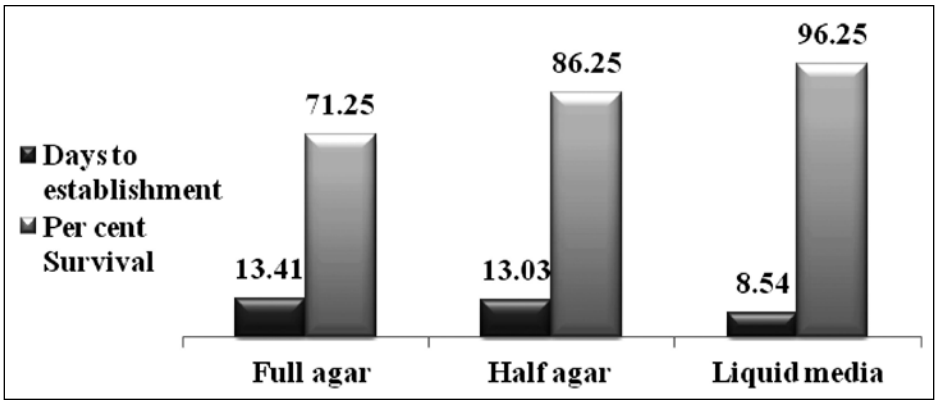

Fig. 2. Effect of quantity of agar in rooting medium on survival of rooted gerbera plantlets.

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