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INFLUENCE OF SOME PHYTOHORMONES BASED CULTURE MEDIUM ON *IN VITRO* MULTIPLICATION OF GERBERA (*Gerbera jamesonii*)

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ABSTRACT : The experiment was conducted during 2009-10 at Plant Tissue Culture Laboratory of Uttar Pradesh Horticulture Department, Lucknow and Bundelkhand University, Brahmanand Mahavidyalaya, Rath, U.P. to study the *in vitro* multiplication of gerbera cultivars (Tamara and Panama) from shoot bud and capitulum bud culture explants using MS medium supplemented with phytohormones i.e. BAP (0.5, 1, 2, 3 mg/l), Kinetin (0.5, 1, 2, 3 mg/l) and IAA (0.5, 1.0 mg/l). MS medium supplemented with BAP 2.0 mg/l + IAA 1.0 mg/l gave the highest culture regeneration, number of multiple shoots, number of leaves per plant, shoot length and took least days to bud break in both-shoot bud and capitulum bud culture taken as explant.

Keywords : *Gerbera, in vitro, phytohormone, shoot bud culture, capitulum bud culture.*

Gerbera is amongst the ten most important commercially grown flower crops in the world. Gerbera can contribute largely to the floriculture industry by virtue of its yield potential and long vase life. Gerbera (Asteraceae family), native to South Africa and Asia, is one of the most famous cut flowers in the world (Parthasarthy and Nagaraju, 5). It is grown commercially in India for export and the domestic market. Very high quality cut flower and millions of tissue cultured gerbera plants produced by India every year. Tropical Floritech Pvt. Ltd, Bangalore is the leading player in commercial cultivation of gerbera in India (Choudhary and Prasad, 2). The tissue culture work in gerbera was taken up because of numerous significant benefits over traditional propagation method. The much faster rates of multiplication can be achieved in *in vitro* than by traditional means. Thus, mass propagation through tissue culture is needed for research and development of the gerbera industry (Kaur *et al.* 3). Cost effective micropropagation would facilitate commercialization of the technology. Phytohormones play significant role in shoot multiplication at faster rate; therefore, the present experiment was carried out to study the influence of kinetin, BAP and IAA on *in vitro* multiplication of gerbera to develop a rapid and low-cost protocol for micropropagation of gerbera.

MATERIALS AND METHODS

The experiment was conducted at Plant Tissue Culture Laboratory of Uttar Pradesh Horticulture Department, Lucknow and Bundelkhand University, Brahmanand Mahavidyalaya, Rath, U.P. during 2009-10.

Preparation of Explants

Two years old plants of gerbera cultivars (Tamara-V_1 and Panama-V_2) were taken as a source of explants from nursery of Uttar Pradesh Horticulture Department, Lucknow. Few selected plants in nursery were severely treated with 0.1% Carbendazim (Bavistin) to obtain the healthy shoots and capitulum. 15-20 cm long shoots and capitulum having 5-8 cm diameter were excised from the elite donor plant and brought to the laboratory in an aqueous solution of ascorbic acid 100 mg/l and citric acid 100 mg/l. Shoots were defoliated and three different sub experiments were undertaken to optimize ideal explants. The hard, brown region of the capitulum and shoots were rejected due to the problem of phenol leaching and in borne contamination.

Sterilization of Explants

Surface sterilization of nodal stem segments of the shoots were first treated with Tween 20 (2% v/v) for 30 minutes and then washed thoroughly under running tap water. Again these explants were kept in a solution containing 0.1% Carbendazim (Bavistin) + 25 mg/l Rifampicin (0.1% Streptomycin Sulphate) per 100 mg/l of distilled water and 2-3 drops of Tween-20 for one hour. The segments were washed 3-4 times using sterilized double distilled water. After thorough washing, the explants were then rinsed with sterile double distilled water and brought under laminar airflow hood for further surface sterilization treatments. The explants were treated with different sterilizing

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agents (70% ethyl alcohol, 0.1% $HgCl_2$ and NaOCl) for different durations (4, 6 and 8 minutes). After treating with different surface sterilizents, the explants were washed with sterilized distilled water (3-4 times) and dried on pre sterilized filter paper sheets.

Preparation of Culture Media

Preparation of stock solutions of analytical grade chemicals of high purity and standard make e.g. Qualigens fine Chemical Company (Glaxosmithkline), Ranbaxy Laboratories Ltd., and E. Merck (India) Ltd., Sigma Chemical Company, USA, Hi- Mida Laboratories Pvt. Ltd., India were used. The stock solution of macro and micro nutrients were made by dissolving the required amount in measured volume of glass double distilled water and stored in refrigerator at 4°C. The stock solutions of growth hormones were made by dissolving it in respective solvent i.e. the auxin (IBA, IAA) stocks were prepared by dissolving first in with few drops of absolute alcohol and made the volume by using glass double distilled water and stock solution of cytokinins (BAP, Kinetin) were prepared by dissolving chemicals in 0.5-1.0 ml of 0.1 NaOH and gentle shaking the final volume was made by using glass double distilled water. Final concentrations of both auxin and cytokinin were kept 1mg/ml. All stock solution was stored in refrigerator. Murashige and Skoog (4) medium was used for inoculation of shoot and capitulum segments of gerbera. Plant growth regulators like BAP (0.5, 1, 2, 3 mg/l), Kinetin (0.5, 1, 2, 3 mg/l) and IAA (0.5, 1.0 mg/l) were incorporated in culture medium.

The experiment was laid out in RBD with three replication comprising 17 treatments. The data recorded under experiment were statistically analysed using software.

RESULTS AND DISCUSSION

In vitro micro shoot proliferation of shoot bud and capitulum bud culture of gerbera genotypes-Tamara (V_1) and Panama (V_2) were cultured having MS basal medium supplemented with different concentration of BAP, Kinetin (Kin.) and IAA (Table 1). The cultured shoot buds showed multiple shoot differentiation mostly from the base of rebuts. Combination of BAP 2.0 mg/l + IAA 1.0 mg/l gave maximum culture regeneration (V_1-88.00 and V_2-93.33%), no of multiple shoot/explants (V_1-8.69 and V_2-10.53), no of leaves per explants (V_1-5.98 and V_2-4.79) and took minimum days for bud break (V_1-5.20 and V_2-6.24 days) in both the cultivars. Length of shoot (Fig. 1) revealed that BAP 2.0 mg/l + IAA 1.0 mg/l in combination also exhibited the highest shoot length (V_1-5.31 cm and V_2-5.35 cm) in both the cultivars. However, treatment containing only MS medium (Control) showed the poor performance for almost all characters except culture per cent which was observed least in Kin 1.0 + IAA 0.5 mg/l in both the cultivars.

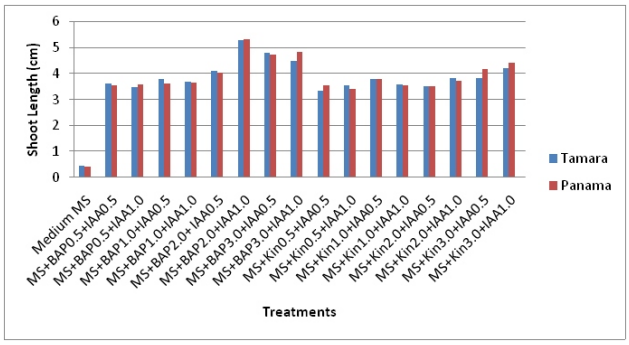

Figure 1: Effect of phytohormones on shoot length of *in vitro* gerbera from shoot bud culture.

Multiple shoot differentiation from capitulum bud culture in Tamara and Panama is presented in Table 2. The cultured capitulum buds showed multiple shoot differentiation mostly from the base of rebuts. The MS medium containing BAP 2.0 mg/l + IAA 1.0 mg/l showed the highest culture regeneration (V_1-90.66 and V_2-97.33%), no of multiple shoot/ explants (V_1-11.32 and V_2-10.63), number of leaves per explants (V_1-6.02 and V_2-5.01) and lowest days taken for bud break (V_1-7.66 and V_2-5.40 days) in both the cultivars. The longest shoot length (V_1-4.55 and V_2-4.13 cm) was also noticed in MS medium containing BAP 2.0 mg/l + IAA 1.0 mg/l (Fig. 2). The lowest culture regeneration (V_1-18.66 and V_2-21.33%) was observed in medium containing Kin 1.0 + IAA 1.0 mg/l. Whereas, the minimum number of multiple shoots per explants (V_1-2.32 and V_2-3.97), number of leaves per explants (V_1-3.97 and V_2-4.08), shoot length (V_1-3.12 and V_2-4.10) and maximum days for bud break (V_1-10.32 and V_2-12.66) were recorded in MS medium without hormones.

The better performance of MS medium supplemented with BAP 2.0 mg/l + IAA 1.0 mg/l as compared to MS medium (Control) alone for multiple shoot differentiation from shoot bud and capitulum bud culture in both the cultivars of gerbera may be due to growth promoting action of phytohormones. The similar findings were also reported by Barbosa *et al.* (1) Reynoird *et al.* (6), and Sabanpour *et al.* (7) in gerbera.

Table 1: Effect of phytohormones on multiple shoot differentiation from shoot bud culture in gerbera.

Treatments	Culture (%)		Days taken for bud break		No. of multiple shoots/ explant		No. of leaves/explant	
	Cultivar		Cultivar		Cultivar		Cultivar	
	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2
Medium MS (Control)	21.33	20.00	12.23	13.86	1.36	1.32	1.29	1.38
MS+BAP0.5+IAA0.5	26.66	26.66	7.98	7.48	1.48	2.09	2.52	2.39
MS+BAP0.5+IAA1.0	25.33	29.33	7.69	7.56	1.57	2.17	4.10	4.08
MS+BAP1.0+IAA0.5	26.66	22.66	7.62	8.27	2.81	3.34	5.20	5.62
MS+BAP1.0+IAA1.0	32.00	26.66	7.21	8.32	1.86	1.26	4.38	4.59
MS+BAP2.0+ IAA0.5	28.00	26.66	7.43	9.22	1.99	2.15	4.23	3.77
MS+BAP2.0+IAA1.0	88.00	93.33	5.20	6.24	8.69	10.53	5.98	4.79
MS+BAP3.0+IAA0.5	33.33	26.66	6.72	7.45	2.71	3.82	4.09	3.84
MS+BAP3.0+IAA1.0	24.00	24.00	6.54	9.49	2.48	3.02	3.67	3.66
MS+Kin0.5+IAA0.5	22.66	21.33	7.47	10.07	1.98	1.43	3.48	4.02
MS+Kin0.5+IAA1.0	22.66	22.66	7.02	7.39	2.51	2.99	4.20	4.32
MS+Kin1.0+IAA0.5	18.66	20.00	7.21	8.20	1.90	1.66	4.00	4.66
MS+Kin1.0+IAA1.0	25.33	25.33	6.51	6.67	3.05	3.88	4.11	5.34
MS+Kin2.0+IAA0.5	22.66	24.00	6.46	7.47	2.82	3.32	4.20	3.66
120MS+Kin2.0+IAA1.0	28.00	28.00	6.61	6.61	2.66	2.28	3.32	4.66
MS+Kin3.0+IAA0.5	24.00	24.00	6.33	7.49	3.80	4.09	2.74	3.32
MS+Kin3.0+IAA1.0	42.66	40.00	6.50	8.85	6.84	8.22	4.66	3.66
CD (P=0.05)	5.95	6.71	0.67	1.11	0.54	0.64	0.85	0.61

*Cultivars : V_1-Tamara, V_2-Panama

Table2: Effect of phytohormones on multiple shoot differentiation from capitulum bud culture.

Treatments	Culture%		Days taken for bud break		No of multiple shoots/explant		No of leaves/explant	
	Cultivar		Cultivar		Cultivar		Cultivar	
	V_1	V_2	V_1	V_2	V_1	V_2	V_1	V_2
Medium MS (Control)	21.33	22.66	10.32	12.66	1.32	1.02	2.32	1.03
MS+BAP0.5 +IAA05	24.00	26.66	7.32	7.66	1.66	1.37	1.31	2.98
MS+BAP0.5+ IAA1.0	28.00	29.33	6.32	8.66	3.66	2.00	4.01	3.98
MS+BAP1.0+IAA0.5	26.66	26.66	7.66	7.32	2.00	1.66	5.05	5.05
MS+BAP1.0+IAA1.0	18.66	22.66	8.30	5.32	1.02	2.61	5.64	4.34
MS+BAP2.0+IAA0.5	24.00	24.00	6.31	7.39	2.00	2.32	4.34	4.01
MS+BAP2.0+IAA1.0	90.66	97.33	7.66	5.40	11.32	10.63	6.02	5.01
MS+BAP3.0+IAA0.5	29.33	30.66	12.31	7.66	2.00	2.00	4.00	3.00
MS+BAP3.0+IAA1.0	22.66	25.33	5.00	8.66	1.65	1.67	4.01	4.68
MS+Kin0.5+IAA0.5	29.33	25.33	8.31	9.32	2.00	2.32	4.33	5.66
MS+Kin0.5+IAA1.0	20.00	23.66	12.32	8.32	2.66	3.66	4.00	4.59
MS+Kin1.0+IAA5.0	34.66	33.66	8.32	7.32	3.00	5.00	5.00	4.12
MS+Kin1.0+IAA1.0	18.66	21.33	7.66	8.66	2.32	3.97	3.97	4.08
MS+Kin2.0+IAA5.0	28.33	25.33	7.32	7.66	1.32	3.66	3.66	4.69
MS+Kin2.0+IAA1.0	29.33	24.00	7.66	8.66	2.66	3.94	3.94	3.49
MS+Kin3.0+IAA0.5	20.00	25.33	8.32	7.32	1.32	2.79	2.79	3.19
MS+Kin3.0+IAA1.0	37.3	36.00	7.70	4.00	3.32	4.00	4.00	4.32
CD (P=0.05)	4.60	5.82	0.21	0.18	0.27	0.17	0.07	0.16

*Cultivars : V_1- Tamara, V_2- Panama

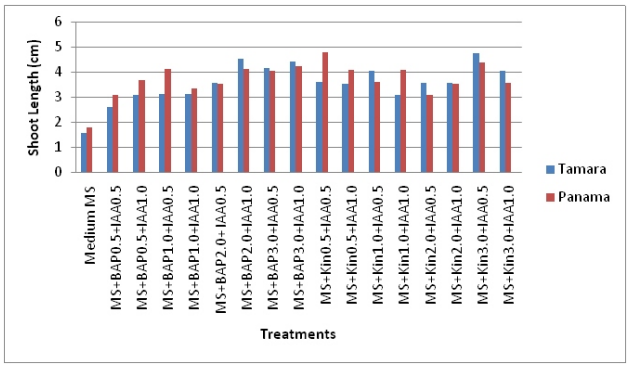

Figure 2 : Effect of phytohormones on shoot length of *in-vitro* gerbera from capitulum bud culture.

Conclusion

Two varieties of gerbera *i.e.* Panama and Tamara can be successfully multiplied in in vitro through both shoot bud and capitulum bud culture. MS medium supplemented with BAP 2.0 mg/l + IAA 1.0 mg/l gave the highest culture regeneration, number of multiple shoots, number of leaves per plant, shoot length and took least days to bud break in both shoot bud and capitulum bud culture.

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