

***In vitro* Propagation in Three Varieties of Gerbera (*Gerbera jamesonii* Bolus.) from Flower Bud and Flower Stalk Explants**

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Abstract

Three types of tender leaves derived explants such as leaf tip, leaf with mid-rib and leaf blade segments as well as flower buds and flower stalks obtained from three selected varieties of gerbera (*Gerbera jamesonii* Bolus.) cultivated in Bangladesh were exploited for callus induction and *in vitro* regeneration of plantlets. Among the explants flower bud and flower stalk were suitable and superior for callus induction and subsequent regeneration of *in vitro* shoots when cultured on MS supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA. However, highest number of multiple shoots were obtained when the flower bud derived callus was subcultured on MS supplemented with 2.0 mg/l BAP. Variety with red petal showed the best response in producing multiple shoots among the three varieties. Root induction at the base of the *in vitro* regenerated shoots was tried using full and half the strengths of MS containing different concentrations of IBA. Best root induction was observed on half the strength of MS supplemented with 0.2 mg/l IBA. Following adequate acclimatization the regenerated plantlets were successfully transferred to soil where they grew to maturity and produced flowers.

Introduction

Gerbera, commonly known as African daisy, produces very attractive flowers. It is a genus of ornamental plants belongs to Asteraceae. Gerbera produces flowers of commercial interest throughout the world covering a wide range of climatic conditions and it is widely used as a decorative garden plant or as cut flowers. Gerbera ranks among the top ten cut flowers of the world (Parthasarathy and Nagaraju 1999).

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The growth of floriculture industry has taken long strides world-wide especially in the developing countries as a result of outsourcing which is due to low cost of maintenance including low labor cost (Jain 2006). For that reason gerbera has gained popularity in the past few years in many countries of the world and it is in great demand in the floral industry as cut flower as well as potted plant due to its beauty, color, long vase life (Kanwar and Kumar 2008). The market value of gerbera is high in the US, Japan, Germany and in the UK. In Europe, the Netherlands and in Asia Indonesia as well as Malaysia are the dominant gerbera suppliers world-wide. In Bangladesh, public attraction is gradually increasing particularly in urban areas about gerbera as ornamental and home decorative plants for its attractive colors and size. However, the supply of gerbera plantlets is not adequate to fulfill the local demand. It may be mentioned here that Bangladesh has a favorable climatic condition and is capable of producing a wide array of gerberas of international standard (<http://www.mdgbangla.org>).

In light of global demand, gerbera could be a major source of foreign exchange as one of the non-traditional export items in Bangladesh. In this aspect, mass commercial production of gerbera could be acted as a new dimension in Bangladesh economy. However, using conventional propagation system it is simply not possible to fulfill the demand of the export market. Seed propagation in gerbera is not always satisfactory since impurity of strain produces a great deal of variations (Schiva 1975). Moreover, traditional seed production technique is too slow for their exploitation in commercial purposes (Murashige et al. 1974). It also requires longer time to produce flower.

Vegetative propagation overcomes the problem of unpredictable characters and plants obtained by this method perform better than those developed from seeds. *In vitro* techniques of propagation have been recognized as the preferred method for multiplication of selected elite clones (Reynoird et al. 1993, Aswath and Choudhary 2002, Xi and Shi 2003, Prasanth and Sekar 2004, Kumar et al. 2004, Chakrabarty and Datta 2007 and <http://www.bari.gov.bd>).

A survey of literature indicates that tissue culture of Gerbera has been studied by various scientists using different types of explants (Barbosa et al. 1994, Parthasarathy et al. 1997, Le et al. 1999, Posada et al. 1999, Zhang et al. 2002, Modh et al. 2002, Tyagi and Kothari 2004, Aswath and Wanzeen 2004, Thakur et al. 2004, Kumar and Kanwar 2005, 2006, Sharma and Srivastava 2005 and Ray et al. 2005). However, the *in vitro* propagation systems described by various scientists in the past need to be improved further in view of their reproducibility in respect to the conditions prevail in Bangladesh. The present investigation was undertaken with a view to developing an *in vitro* plant regeneration protocol for

large scale propagation of selected Gerbera varieties cultivated in Bangladesh using suitable explants.

Materials and Methods

Plants of three varieties of gerbera (*Gerbera jamesonii* Bolus.) having red, yellow and white coloured petals collected from BRAC Research and Development Centre, Joydebpur, Gajipur and were used for this study. Tender leaves and 2.5 - 4.0 cm long flower buds (9 - 11 days old) along with flower stalks were collected from the field grown materials. Special treatments were applied to reduce the level of contamination since field grown materials were used for this investigation. Leaves and flower buds with stalks were first washed under running tap water for 30 min. Then the explants were washed with detergent and then the detergent was washed out completely. The explants were then immersed in 20% Savlon (v/v), an antiseptic disinfectant containing chlorhexidine gluconate and cetrimide (Novartis Consumer Health UK Ltd.) for 2 min followed by washing with distilled water. After that the explants were treated with a fungicide called bendagime (1.0 gm/500 ml) for 5 min followed by several washing with distilled water. The explants were then deepened in 70% alcohol for 30 seconds followed by washing three times with distilled water. The final surface sterilization of the explants was done with 0.1% HgCl₂ solution for 6 - 7 min inside the laminar flow cabinet. During this operation, the flask was agitated frequently and finally the explants were washed five times with sterilized distilled water.

For inoculation the surface sterilized leaf explants were cut into three pieces, namely leaf tip, leaf with mid-rib and leaf blades. One - two cm long segments of these leaves derived explants were cultured for callus induction and shoot multiplication. On the other hand the surface sterilized flower buds were dissected into 8 - 10 pieces for using them as explants (Fig. 1). About 1 - 2 cm long segments of petiole and flower stalks were also used as explants for callus induction and *in vitro* regeneration.

MS supplemented with different concentrations and combinations of BAP (1.0 - 6.0 mg/l), NAA (0.5 - 2.0 mg/l) were used for the induction of callus and development of multiple shoots from different explants of three varieties. All media contained 3% sucrose and 0.8% agar with pH 5.8, adjusted before autoclaving. For rooting 3 - 4 cm long regenerated shoots were excised and cultured on freshly prepared rooting medium containing full and half strength of MS with different combinations and concentrations of IBA and NAA. All cultures were maintained less than 16 hrs photoperiod at 25 ± 2°C. Following the development of sufficient roots, plantlets were transferred to small plastic pots

containing sterilized soil. These plantlets were acclimated and then transferred to the field and raised there till their maturity to flowering.

Results and Discussion

Various explants, namely flower bud, flower stalk, leaf tip, leaf with midrib, leaf blade, petiole were used for callus induction and *in vitro* development of plantlets. Among the different explants, flower bud exhibited the best response towards callus formation, shoot regeneration and subsequent development of plantlets in three varieties of gerbera. The age of the flower bud was found to be an important factor in callus induction and shoot proliferation. In all the varieties 7 - 9 days old flower buds were more responsive (80 - 90%) in callus induction and shoot proliferation (Table 1). There are some previous reports on using of flower bud as explants for shoot regeneration in gerbera (Pierik et al. 1975, Laliberte et al. 1985, Aswath and Choudhary 2002, Tyagi and Kothari 2004, Ray et al. 2005, Kumar and Kanwar 2006, 2007 and Nhut et al. 2007).

Table 1. Responses of the age of flower bud explants towards callus formation.

Gerbera varieties	Age of flower bud explants (days)	No. of explants inoculated	No. of explants responded to callus induction	% of responsive explants
Red variety	7	20	09	45
	8	20	13	65
	9	20	17	85
	10	20	14	70
	11	20	10	50
Yellow variety	7	20	16	80
	8	20	14	70
	9	20	11	55
	10	20	09	45
	11	20	06	30
White variety	7	20	18	90
	8	20	13	65
	9	20	13	65
	10	20	11	55
	11	20	10	50

In the present study, flower stalk was also appeared to be a good responsive explant towards callus formation and shoot regeneration. None of the previous workers used flower stalk explant for shoot regeneration.

In case of red variety, all the leaf derived explants induced callus within 18 - 20 days on MS with 6.0 mg/l BAP and 1.0 mg/l NAA depending on the nature of

the explant. In case of yellow and white varieties all the explants induced callus within 15 - 20 days on MS supplemented with either 5.0 or 6.0 mg/l BAP with 1.0 mg/l NAA, respectively. The induced calli were subcultured initially on the same medium having the identical hormonal supplements for shoot regeneration.

Following such subculture the induced calli were found to increase their volume, but failed to induce shoots irrespective of variety as well as explants used. However, a few reports are available about *in vitro* shoot regeneration from different parts of leaf including petiole (Orlikowska et al. 1999).

Table 2. Effect of different concentrations of BAP and NAA on shoot regeneration via callus formation from flower bud and flower stalk explants for red variety.

Hormonal supplements (mg/l)		No. of explants inoculated	Days to callus induction	No. of responsive explants	% of responsive explants	Days to shoot initiation	Mean no. of shoots per explant
BAP	NAA						
Flower bud							
1.0	0.5	30	35	Only callus	-	-	-
2.0	0.5	30	35 - 45	"	-	-	-
3.0	1.0	30	35 - 45	06	20	30 - 35	3.16
4.0	1.0	30	35 - 45	12	40	25 - 30	5.17
5.0	1.0	30	35 - 45	22	73	22 - 25	9.5
6.0	1.0	30	35 - 45	10	33	28 - 30	2.3
Flower stalk							
1.0	0.5	30	45 - 60	Only callus	-	-	-
2.0	0.5	30	45 - 60	"	-	-	-
3.0	1.0	30	45 - 60	"	-	-	-
4.0	1.0	30	45 - 60	06	20	25 - 30	4.1
5.0	1.0	30	45 - 60	16	54	20 - 25	7.37
6.0	1.0	30	45 - 60	11	37	25 - 30	3.23

In all the three varieties of gerbera the best response regarding the induction of callus and proliferation of multiple shoots was obtained when flower bud explants were cultured on MS supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA. Initiation of callus occurred within three weeks after inoculation. The percentage of responsive explants in respect of shoot regeneration was around 75 - 83 and mean number of shoots per explant varied from 9 - 12 in all the varieties. Initiation of shoots from proliferated callus is presented in Fig. 2.

In case of flower stalk explant, higher number of multiple shoots in all the three varieties of gerbera was also observed on MS supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA. The percentage of responsive explants regarding shoot

regeneration was around 54 - 68 and mean number of shoots per explant was 8 - 10 in all the varieties. Multiple shoots initiated in MS with 5.0 mg/l BAP and 1.0 mg/l NAA from the flower stalk explants (Fig. 3) were green and healthy.

Table 3. Effect of subculturing on MS with different concentrations of BAP for multiple shoot regeneration in red variety.

Hormonal supplement BAP (mg/l)	Explants	No. of subculturing clumps	% of responsive clumps	Mean no. of shoots/ subculturing clumps	Mean length of shoot (cm) after 120 days
01	FB	30	80.50	20.50	2.5
	FS	30	78.23	15.25	2.8
02	FB	30	98.25	28.64	4.6
	FS	30	94.86	18.15	4.1
03	FB	30	75.78	14.80	3.7
	FS	30	72.57	08.75	2.9
04	FB	30	72.32	11.08	2.4
	FS	30	70.44	07.55	2.6

FB = Flower bud, FS = Flower stalk.

Response of callus induction and shoot proliferation was significantly influenced by the concentrations and combinations of cytokinins and auxins. Profuse shoot proliferation occurred in all the three varieties of gerbera when MS was supplemented with BAP and NAA. Hasbullah et al. (2008) also reported that addition of auxins along with cytokinins was essential for shoot induction in gerbera. When BAP was supplemented with IAA callus was induced but no shoot proliferated. On the other hand when BAP was supplemented with NAA, in some combinations callus induction as well as shoot proliferation occurred. Pierik et al. (1973) also reported that addition of strong auxin (NAA) with BAP promoted better shoot formation compared to weak auxin (IAA).

It was noticed that when the regenerated shoots were cultured and maintained on MS supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA for longer duration for further shoot multiplication, the previously regenerated shoots became stunted in growth and vitrified. To overcome this problem shoots were cultured on MS containing only BAP. Chakrabarty and Datta (2008) obtained best shoot multiplication on MS containing only BAP, Kn and TDZ.

During this study different concentrations of BAP (1.0 - 4.0 mg/l) were tested for shoot multiplication. Best response (Table 3) towards multiple shoot regeneration from callus obtained from flower bud was observed on MS with 2.0 mg/l BAP in all the three varieties of gerbera (Figs 4 and 5). In this hormonal supplement the mean number of shoots in flower bud and flower stalk explants were 28.64 and 18.15, respectively.



Figs 1-9: 1. Transverse section of flower bud used as explant for callus induction and shoot regeneration. 2. Induction of shoots from the flower bud derived callus. 3. Regeneration of multiple shoots from the flower stalk explant. 4. Induction of shoot from flower bud derived callus following subculturing. 5. Same as Fig. 4 but showing the proliferation of shoots. 6. Elongated *in vitro* roots developed at the base of the regenerated shoot. 7. Regenerated plantlets transferred to plastic pots. 8. *In vitro* propagated plants. 9. Flower developed from tissue cultured derived plant.

Induction of healthy root system from the regenerated shoots is an essential part for successful development of plantlets. During the present study it was noticed that several roots developed spontaneously from the *in vitro* grown shoots but the spontaneously developed roots were found to be inadequate for transplantation of the *in vitro* grown shoots to the soil. Therefore, separate root induction was necessary.

For root induction, regenerated shoots were cultured on half and full strength of MS supplemented with IBA, IAA and NAA. It was observed that half strength of MS with 0.2 mg/l IBA showed 95 - 100% for root induction in the red,

yellow and white varieties (Fig. 6). Kanwar and Kumar (2006) also reported that IBA was the best medium for root induction in Gerbera. When NAA was used at 0.1 and 0.2 mg/l with half the strength of MS, only callus was induced. Efforts were made to establish plantlets in pots with proper root systems (Fig. 7). Plants with sufficient roots were transplanted to soil (Fig. 8) and their survival rate was 100% in the field condition. These tissue culture derived plants flowered (Fig. 9) very much identical to that of the control plants.

Table 4. Effect of different auxins on root formation in red variety.

Growth regulators (mg/l)	% of shoots forming roots	Days to root initiation	Days to root development	Nature of the roots
IAA 0.1	27	20 - 25	40 - 45	Long & thin
IAA 0.2	38	20 - 25	40 - 45	"
IBA 0.1	62	15 - 20	35 - 40	"
IBA 0.2	100	15 - 20	35 - 40	"
NAA 0.1	Only callus	-	-	-
NAA 0.2	"	-	-	-

All the three Gerbera varieties used in this study showed almost identical responses towards *in vitro* regeneration. The overall responses of the red petal variety were found to be relatively better compared to the other two varieties of local gerbera.

Through this investigation it has been possible to develop an efficient and reproducible *in vitro* mass propagation system from different explants of gerbera. Based on the results it may be concluded that the *in vitro* mass propagation protocol developed in this investigation could profitably be explored for the commercial cultivation of gerbera. The present protocol of propagation may help in obtaining adequate number of plantlets for cultivation in fulfilling the requirements of the local market of gerbera.

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Website Consultation

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