RESEARCH PAPER | Asian Journal of Bio Science, Volume **8** | Issue 1 | April, 2013 | 15-18
Received : 16.10.2012; **Revised :** 10.01.2013; **Accepted :** 15.02.2013

In vitro micropropagation of gerbera using auxillary bud

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A protocol for rapid clonal micropropagation of gerbera was developed by auxillary bud. MS+BAP 2.0+NAA 0.5 mg/l, which was given early bud initiation (19.40 days), with higher initiation per cent (91.66%) along with longer shoot length (2.5 cm). MS+BAP 2.0+NAA 0.5+Ads 100 mg/l produced higher number of shoots (7.33 shoots/explants). The micro shoots were rooted (96%) in just 8.56 days on MS+NAA 2.0+activated charcoal 750 mg/l. *In vitro* rooted plants were acclimatized on sand + soil + FYM+ leaf mould. Plants shows 82.43 per cent survival rate during acclimatization. The plants were established in the field after acclimatization.

Key words : Gerbera, Auxillary bud, Micropropagation

How to cite this paper : Kadu, A.R. (2013). *In vitro* micropropagation of gerbera using auxillary bud. *Asian J. Bio. Sci.*, **8** (1) : 15-18.

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus), commonly known as Transvaal Daisy, is an important cut flower both in the domestic and the international markets. It ranks fifth in the international cut flower trade. *Gerbera* is one of the leading cut flowers and ranks among the top ten cut-flowers of the world (Parthasarathy and Nagaraju, 1999). The production of gerbera was approximately US$ 220 million in 2001 representing 70 million stems sold in US alone (Broek *et al.,* 2004). Micropropagation is one of the viable alternatives for large-scale multiplication of gerbera (Bhatia *et al.,* 2008). Over the years, gerbera has been propagated by direct or indirect organogenesis using various explants, including stem tips, floral buds, leaf, capitulum etc. (Kanwar and Kumar, 2008). The emergence of floriculture as an important industry in many countries has been possible due to the revolution in the propagation method of ornamentals. Micropropagation has been recognized as the most reliable, cost and labour effective method for large scale clonal propagation of elite cultivars, leading to systematic development of the floriculture industry. Cut flower trade is increasing exponentially across all the continents and the availability of micropropagated, clonal planting material in sufficient numbers has helped commercial growers to cultivate many commercial varieties for the production of cut flowers.

The drive for encouraging floriculture development in India, making it almost parallel to agriculture, is dependent on the varied agro-climatic conditions in the country, where moderate climatic control at relatively cheaper cost can deliver quality products at internationally competitive prices. Using methods of micropropagation, the commercial growers can rapidly introduce selected superior clones of ornamental plants in sufficient quantities, which would have a direct impact on the market potential. Major pot plants such as begonia, ficus, anthurium, chrysanthemum, rosa, saintpaulia and spathiphyllum are being produced in the developed countries (Anonymous, 2003). The share of the developing countries of Africa, Asia and Latin America is less than 20 per cent (Rajagopalan, 2000; Schiva, 2000) Planting material of ornamental plants is in great demand for commercial production as well as for domestic gardens and landscaping. The better quality planting material is a basic need of growers for boosting productivity. Chebet *et al.* (2003) reported the use of biotechnological approaches to improve horticultural crop production.

The floriculture industry in India after several initial setbacks, is still struggling for stabilization. Out of 70 to 80 floriculture units in the organized sector, more than half are listed companies, but very few are doing well today, while many others have closed down and presently the industry is not doing well, but managing to survive due to severe availability of genuine planting material and or availability of repetitive tissue culture protocol. Micropropagation of gerbera has been reported by several workers *viz.,* Schiva *et al.* (1982), Sharma and Srivastiva (2005), Kumar and Kanwar (2006),

Kanwar and Kumar (2008), Altaf *et al.* (2009) and Naz *et al.* (2012). Here we are reporting a repetitive protocol for micropropagation of gerbera using auxillary bud.

Research Methodology

The present investigation was conducted during 2006-2008 in Plant Tissue culture Laboratory, Mahabeej Biotechnology Centre, Nagpur. 0.5 cm long auxillary buds were collected from field grown plant. The explants were washed thoroughly with 2-3 drops of tween-20 (a liquid detergent) and were rinsed under running tap water for 30 min. To control the bacterial and fungal contamination, explants were treated with 100 mg/l streptomycin+0.1 per cent carbendazime in 100 ml of distilled water and 2-3 drop of tween-20 for one hour. The segments were washed 3-4 times using sterile distilled water. The explants were then rinsed with sterile double distilled water and brought under laminar air flow hood for surface sterilization. The explants were treated with 0.1 per cent $HgCl_2$ for 6 minutes and washed (3-4 times) with sterile distilled water and blotted dry on pre sterilized filter paper sheets. Thereafter, the explants were transferred on MS medium (Murashige and Skoog, 1962) fortified with various concentration of BAP (0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/l) and NAA (0.25, 0.5, 0.75 and 1.0 mg/l) for initiation and proliferation. All the media were added with 0.8 per cent (w/v) agar and 3 per cent (w/v) sucrose. The pH of the medium was kept 5.8 prior to autoclaving. Observations were recorded periodically. *In vitro* developed microshoots were subjected to various concentrations of NAA (1.0 and 2.0) and different amount of activated charcoal (250, 500, 750 and 1000 mg/l) for rooting. All the media were added with 0.8 per cent (w/v) agar and 3 per cent (w/v) sucrose. All cultures were incubated at 25±2^{0}C and were exposed to a photoperiod of 16/8 hours light and dark cycling under 2000 Lux intensity provided by cool day light fluorescent tubes (40 W, Philips, India) with 70 per cent ± 5 per cent relative humidity maintained in the culture room. Sub-culturing was carried out after every 4 weeks interval.

For acclimatization, two months old rooted plantlets were transferred into autoclaved, sand + soil + FYM (1:1:1), sand + soil + FYM+ leaf mould (1:1:1:1), soilrite and sand + soil + FYM+ soilrite containing potting mixture. After that these pots were shifted to green house condition provided with misting. Once the plants develop 4-6 leaves, they were shifted to field. The growth parameters and survival was recorded periodically.

A completely randomized design with five replicates of each treatment was used for the experiment. The data for each parameter were subjected to analysis of variance (ANOVA) using OP Sheron Statistical software for agriculture research. The mean values were compared with the least significant difference test at 0.05 per cent level.

Research Findings and Analysis

The exprimental findings of the present study have been presented in the following sub heads:

Shoot initiation and establishment:

The effect of different concentration of cytokinin and auxin were tested on initiation and establishment of *in vitro* culture. It was observed that the optimum concentration of cytokinin and auxin for culture initiation of auxillary bud was MS+BAP 2.0+NAA 0.5 mg/l, which gave early bud initiation (19.40 days), with higher initiation per cent (91.66%) along with longer shoot length (2.5 cm). This treatment was followed by MS+BAP 2.5+NAA 0.25 mg/l, which gave 83 per cent shoot initiation and 1.633 cm long shoots in 21.5 days (Table 1).

Barbosa *et al.* (1994) and Posada *et al.* (1999) also got good response with BAP 1 and 3 mg/l for shoot initiation, while Naz *et al.* (2012) got 90 per cent shoot initiation with BAP 10 mg/l.

Shoot proliferation:

Well established microshoots were transferred to proliferation medium to get more number of shoot. The data clearly reveled that (Table 2), when the microshoots were transferred to the proliferation medium containing MS+BAP

Table 1: Effect of different concentrations of BAP and NAA on shoot initiation through axillary bud in gerbera after 4 weeks

Treatments (mg/l)	Days taken for axilarry bud initiation	% initiation	Shoot length (cm)
BAP 0.0+NAA 0.0	26.000	39.500	0.333
BAP 0.5+NAA 0.25	24.833	50.333	0.433
BAP 1.0+NAA 0.50	24.333	65.333	1.133
BAP 1.5+NAA 0.25	22.500	80.667	1.500
BAP 2.0+NAA 0.50	19.400	91.667	2.500
BAP 2.5+NAA 0.25	21.500	83.000	1.633
BAP 3.0+NAA 0.50	25.000	70.667	1.400
C.D. (P=0.05)	1.285	1.141	0.362
S.E. ±	0.420	0.373	0.118

Table 2: Effect of different concentrations of BAP, NAA and Ads on shoot proliferation in gerbera after 4 weeks

Treatments (mg/l)	% multiplication	No. of shoots/explant	Shoot length (cm)
BAP 2.0+NAA 0.50+Ads 50	80.167	6.367	4.433
BAP 2.0+NAA 0.50+Ads 100	91.500	7.333	6.333
BAP 2.0+NAA 0.50+Ads 150	85.333	5.917	5.233
BAP 2.0+NAA 0.50+Ads 200	73.500	4.833	4.600
C.D. (P=0.05)	1.138	0.645	0.634
S.E. ±	0.344	0.195	0.191

Table 3: Effect of different concentrations of NAA and activated charcoal on rooting in gerbera after 4 weeks

Treatments (mg/l)	Days taken for root initiation	% rooting	Root length (cm)
NAA 1.0+Activated charcoal 250	24.333	55.333	0.400
NAA 2.0+Activated charcoal 250	23.000	61.000	0.467
NAA 1.0+Activated charcoal 500	19.167	75.667	1.100
NAA 2.0+Activated charcoal 500	17.500	86.333	1.700
NAA 1.0+Activated charcoal 750	13.500	90.833	2.167
NAA 2.0+Activated charcoal 750	8.567	96.000	3.500
NAA 1.0+Activated charcoal 1000	10.833	89.167	3.100
NAA 2.0+Activated charcoal 1000	19.667	81.000	2.600
C.D. (P=0.05)	1.234	1.817	0.182
S.E. ±	0.408	0.601	0.060

Table 4: Effect of carriers substrate on acclimatization of micropropagated plants of gerbera after 4 weeks

Treatments	Plant height (cm)	No. of leaves/plant	No. of roots/plant	Survival (%)
Sand + Soil + FYM	3.467	2.833	1.467	47.167
Sand + Soil + FYM+ leaf mould	4.600	7.667	3.467	82.433
Soilrite	2.500	4.000	1.667	51.250
Sand + Soil + FYM+Soilrite	4.133	5.233	2.267	63.333
C.D. (P=0.05)	0.340	1.163	0.398	3.253
S.E. ±	0.103	0.351	0.120	0.982

2.0+NAA 0.5+Ads 100 mg/l. It produced higher number of shoots (7.33 shoots/explant), longer shoot length (6.33 cm) with higher multiplication rate (91.50%). This result was followed by MS+BAP 2.0+NAA 0.5+Ads 150 mg/l. which produced comparative low number of shoots (5.917 shoots/explant) with low multiplication ratio (85.33%).

Meyer and Staden (1988) also got good shoot proliferation using BAP in gerbera aurantica. Thakur *et al.* (2004) found maximum number of shoots using MS+BAP 2.0 and 2.5 mg/l in two cultivars of gerbera namely Kazak and Gold disk, respectively.

In vitro rooting of shoots:

3-5 cm long well established plantlets were transferred to rooting medium. When the plantlets were transferred to rooting medium (Table 3) fortified with MS+NAA 2.0+activated charcoal 750mg/l. It produced 96.0 per cent rooting, with longest root (3.50 cm) in just 8.56 days. While least rooting was produced in MS+NAA 2.0+Activated charcoal 250 mg/l containing culture medium. This produced only 55.33 per cent rooting, less root length (0.40 cm) in more number of days (24.33 days). Reynoird *et al.* (1993) rooted *in vitro* microshoots with ½ MS medium containing 0.25 μM NAA. Naz *et al.* (2012) found 80 per cent rooting response with NAA 10 mg/l in 11 days. Reynoird *et al.* (1993) achieved rooting in gerbera with IAA 3 mg/l.

Hardening of *in vitro* plantlets:

It is observed that the maximum mortality is observed during hardening (transfer from *in vitro* to *ex vitro* condition) because it faced extreme change in their environmental and physiological condition. To overcome from this problem, acclimatization is very necessary before planting them in to field condition. Plants were tested for suitable carrier substrates *viz.,* sand + soil + FYM, sand + soil + FYM+ leaf mould, soilrite and sand + soil + FYM + soilrite. The suitable

carrer substrate not only improve plants survival but also its physiological parameters/conditions. The data presented in Table 4 clearly reveled that, when the plants were transfer to sand + soil + FYM+ leaf mould, plants showed higher survival rate (82.43%), longer plants (4.6 cm), more number of leaves/ plant (7.66) with more number of roots/plant (3.467). while least per cent survival was observed in sand + soil + FYM.

Similar results were also observed by Parthasarthy and Nagraju (1996), who achieved 90-100 per cent success in polybags containing equal amount of sand+soil+FYM. While Aswath and Chaudhary (2002) found 100 per cent survival in cocopeat + soil + sand (3:1:1) in *G. jamesonii* cv. 101.

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