

Development of *In vitro* Mass Propagation Protocol for Gerbera (*Gerbera jamesonii* Bolus) var. Orange

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Key words: Mass propagation, Gerbera, Flower bud, Hormone-free rooting, Acclimatization

Abstract

An *in vitro* mass propagation protocol was developed for Gerbera (*Gerbera jamesonii* Bolus) var. orange using flower bud explants. The young (7 days) explants were found to be suitable for culture and highest (100%) callus induction was obtained in MS medium supplemented with 5.0 mg/l BAP with 1.0 mg/l NAA. The maximum shoot regeneration (95%) and the number of shoots (10) were observed in MS medium supplemented with 6.0 mg/l BAP with 0.3 mg/l NAA. The highest shoot multiplication frequency (95%) and the number of shoots (60) were obtained in MS medium supplemented with only 2.0 mg/l BAP. The well-developed highest number of roots (5.0) per plant and average root length (5.0 cm) with 100% root induction efficiency without callusing was recorded in the half strength of MS medium devoid of any hormonal supplement. Acclimatized *in vitro* grown plantlets were successfully transferred into the field condition.

Introduction

Gerbera (*Gerbera jamesonii* Bolus) is a perennial plant belonging to the Asteraceae family known as the Transvaal daisy or African daisy (Singh et al. 2017). Gerbera is widely used as a decorative plant or as a cut flower. Gerbera daisies are versatile blooms that are often used as focal flowers in arrangements. It is used for dry and fresh flower arrangement, decoration, exhibitions, and bouquet preparation (Patra et al. 2015). It is a popular cut flower with vibrant attractive colors including crimson, red, peach, maroon, pink, orange, bicolor, etc. which makes it an economically important ornamental plant. The popularity of the Gerbera flower has grown quickly due to its high color variations and

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long shelf life. Gerbera ranked fifth most used popular cut flower behind roses, carnations, chrysanthemums, and tulips as well as including 29 to 37 species found in tropical Asia, Africa, Madagascar, and South America (Sujatha et al. 2002, Katinas and Liliana 2004, Bhatia et al. 2009 and Gao and Hind 2011).

Gerberas can propagate through sexual and asexual both. Generally, commercially developed flower cultivars are propagated vegetatively to ensure genetic uniformity and integrity. The cultivars of these species are propagated vegetatively via the division of clumps or suckers (Peper et al. 1971). As a vegetative way, multiplication by clump division is being used for decades. Through vegetative propagation, plants exhibit genetic uniformity but it is too slow to be utilized for commercial cultivation (Aswath and Choudhary 2001). However, Gerbera can propagate through seeds but as plants exhibit non-uniformity and heterozygosity in this procedure, it's not preferred for commercial cultivation purposes (Shelahi et al. 2013).

The development of commercial flower cultivation has progressed rapidly in developing countries including Bangladesh due to low maintenance costs including very low labor costs which enhance the scope of self-employment as well as contribute to an era in the national economy (Jain 2006). The micropropagation technique has no seasonal limitations which facilitate a mass number of disease-free plantlets propagation within a short period using various explants such as shoot tips, flower bud and capitulum (Kumar and Kanwar 2006, Chakrabarty and Datta 2008, Akter et al. 2012 and Naz et al. 2012). Additionally, plantlets produced through micropropagation showed genetic uniformity (Reddy and Choudhary 2002, Rashmi et al. 2018). To ensure the demand of large-scale commercial Gerbera production needs a simple, efficient, and reproducible protocol for plantlets production. The objective of the present study was to develop an efficient and reliable *in vitro* mass propagation protocol of Gerbera (*Gerbera jamesonii* Bolus) var. orange through flower bud explants.

Materials and Methods

Young flower buds (5-9 days old) of Gerbera (*Gerbera jamesonii* Bolus) var. orange were collected and used as explants from the commercial flower garden of Godkhali, Jhikargacha Upazila, Jashore district. Collected explants were placed in a conical flask and washed under running tap water for 30 minutes to remove dust and surface contaminants. A few drops of Savlon (ACI Ltd. Bangladesh) were added and shaken for 5 minutes and washed several times with distilled water. Explants were then treated with a few drops of tween-20 (Atlas Chemicals Industrial Inc. India) for 5 minutes with constant shaking and thoroughly rinsed with distilled water. After that, the explants were treated with Carbendazim fungicide powder (Autostin 50WDG, Auto Crop Care Limited, Bangladesh) 3.0 gm/l for 10 min and washed thoroughly with distilled water. The explants were taken into a laminar airflow cabinet into a sterile conical flask and final surface sterilization was done with 0.1% HgCl₂ (Merck Specialties Private Ltd.,

India) solution. Finally, explants were washed with autoclaved distilled water 5-8 times to remove the traces of HgCl₂.

Sterilized explants (Fig. 1a) were collected in a petri dish and dissected into 4-6 pieces depending on the explant's sizes. Segmented explants were inoculated into a culture vessel containing a callus induction medium. All culture media are prepared with MS (Duchefa Biochemie, Netherlands) 4.4 mg/l (except for half strength MS media), 0.60% agar (Duchefa Biochemie, Netherlands), 3% sucrose, and medium pH (5.70). For callus induction, MS supplemented with various concentrations of BAP (KOHJIN Life Sciences Co. Ltd., Japan) 2.0-6.0 mg/l with combinations of NAA (Wako Pure Chemical Industries Ltd, Japan) 1.0 mg/l were used. The inoculated explants were incubated in a growth chamber with 23 ± 2°C temperature and 55-60% humidity in dark conditions for 3-5 weeks to induce callus. After 3-4 times sub-cultured, the callus was prepared for shoot regeneration.

Regenerative callus was transferred to shoot initiation media containing different concentrations and a combination of BAP (3.0-7.0 mg/l) and NAA (0.3 mg/l) in light condition (2000-3000 lux) and maintained at 16/8 h photoperiods. Well-regenerated initial shoots were carefully excised from calli and transferred to shoot multiplication medium supplemented with BAP (1.0-4.0 mg/l). Sub-cultured (2-3 times) 2.0-4.0 cm long multiple shoots were separated and transferred singly to rooting media containing half or full strength of MS with different concentrations of IAA, NAA, IBA (Wako Pure Chemical Industries Ltd, Japan) and hormone-free condition. Fully developed rooted plantlets were transferred to plastic black trays or pots containing autoclaved vermiculate soil. These plantlets were acclimatized for around 2-3 weeks before being transferred to the flower-growing field.

Result and Discussion

The developmental stages of explants are a critical factor for callus induction and subsequent regeneration of plants (Kanwar and Kumar 2008, Akter et al. 2012). In Gerbera, various tissue or organ such as flower bud, flower stalk, leaf, and petioles, was used as explants to the regeneration of a complete plant. The regeneration potential of Gerbera explants varied due to the varietal differences, types, and ages of explants (Akter et al. 2012). In the present study, younger flower buds (7 days) showed higher morphological potential to induce callus (90%) (Table 1). Previous studies have also shown that flower buds explants exhibited the best response for callus formation compared to the flower stalk and leaf petiole explants in Gerbera (Laliberte et al. 1985, Tyagi and Kothari 2004, Ray et al. 2005, Nhut et al. 2007, Akter et al. 2012, Shylaja et al. 2014, and Shoyeb et al. 2018). These results suggest that too younger (less than 7 days) and older (greater than 7 days) flower buds showed decreased callus induction efficiency. The results obtained in the study was presented in Fig. 1.

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

Age of flower bud explant (days)	No of explants inoculated	No of explants responded to callus induction	% of responsive explants
5	20	10	50
6	20	12	60
7	20	18	90
8	20	17	85
9	20	15	75

The flower bud explants of Gerbera orange varieties were started to induce callus from 20 days of culture on MS containing 2.0-6.0 mg/l BAP and 1.0 mg/l NAA (Table 2). The callus initiation days and percent of callus responsive depend on the increase of BAP concentration. The highest callus induction (100%) was observed in MS supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA. The efficacy of 5.0 mg/l BAP with 1.0 mg/l NAA was also recorded by Akter et al. (2012) in a related study on large-scale propagation of red var. gerbera. Even while both investigations showed a similar trend, there were significant differences in the callus induction period and percentage. The MS medium supplemented with a higher concentration of BAP (4.0-6.0 mg/l) combination with NAA (1.0 mg/l) was also found effective for callus induction in Gerbera var. red, yellow, and pink (Akter et al. 2012, Shoyeb et al. 2018). In the red, yellow, and pink varieties maximum percentage for callus induction was 73, 70 and 75% on MS medium containing 4.0 mg/l BAP and 1.0 mg/l NAA (Shoyeb et al. 2018).

Table 2. Response of flower bud explant towards callus formation in the MS containing BAP and NAA.

Hormonal supplements (mg/l)		No of explants inoculated	Days to callus induction	No of responsive explants	% of responsive explants
BAP	NAA				
2.0	1.0	40	30-40	20	50
3.0	1.0	40	20-30	34	85
4.0	1.0	40	20-30	36	90
5.0	1.0	40	20-25	40	100
6.0	1.0	40	20-30	38	95

The regenerative calli were sub-cultured on MS medium containing different concentrations of BAP (3.0-7.0 mg/l) and NAA (0.3 mg/l) for shoot regeneration. The addition of a higher concentration of cytokinin and combination with lower auxin is

essential for shoot induction of Gerbera (Husbullah et al. 2008). Pierik et al. (1973) showed that strong auxin (NAA) with BAP induced better shoots than weak auxin (IAA). The highest shoot regeneration response (95%) was found when the medium was supplemented with 6.0 mg/l BAP and 0.3 mg/l NAA after 20-25 days of sub-culture and the mean number of shoots was 10 (Table 3). Regenerated shoots remain healthy and green until transferring into root initiation media.

Table 3. Effects of different hormonal combinations in MS for shoot regeneration from flower bud derived callus.

Hormonal supplements (mg/l)		No of subculturing callus	Days to shoot initiation	No of responsive callus	% of responsive explant	Mean no of shoots
BAP	NAA					
3.0	0.3	40	32-38	26	65	4.0
4.0	0.3	40	30-35	28	70	6.0
5.0	0.3	40	22-28	34	85	8.0
6.0	0.3	40	20-25	38	95	10
7.0	0.3	40	25-30	32	80	7.0

The best response towards shoot formation by using 3.0 mg/l BAP and 0.5 mg/l IAA was reported after 60 days (Warar et al. 2008) and 70% of shoots were found in the combination of 3.0 mg/l BAP and 0.3 mg/l NAA (Rashmi et al. 2018). Bhatia et al. (2012) demonstrated that a modified MS medium supplemented with 10 mg/l BAP and 1.0 mg/l IAA provided the optimum establishment of immature flower bud explants. However, in this present study, we found higher regeneration efficiency within short periods in an optimum hormonal concentration.

The effects of various concentrations of BAP (1.0-4.0 mg/l) were observed in regenerating multiple shoots (Table 4). Maximum multiple shoot regeneration was obtained when single shoots were cultured on MS supplemented with 2.0 mg/l BAP. Chakrabarty and Datta (2008) noted that BAP was superior for promoting shoot multiplication to other cytokinin, such as TDZ and Kin, and additionally demonstrated that the best multiplication rate was observed in 2.0 mg/l BAP which was justified by our experiment. However, according to the present study percentages of responsive clumps (95%) as well as mean no of shoots (60) was found to be best whereas Akter et al. (2012) recorded the highest mean no of shoots 28.6 and Mishra et al. (2014) recorded 8.7-10.5 shoots/clumps on same hormonal concentration. After 120 days and 5 subculture cycles, the highest length (4.0 cm) of shoots was found in the present investigation. Although Naz et al. (2012) recorded a 7.0 cm shoot length while using a higher concentration of BAP (10 mg/l).

Table 4. Effects of sub-culturing with different concentrations of BAP hormone in MS on multiple shoot formation.

Hormonal supplements (mg/l)	No of subculturing clumps	% of responsive clumps	Mean no of shoots/ subculturing clumps	Mean length of shoot (cm) after 120 days
BAP				
1.0	40	60	20	2.0
2.0	40	95	60	4.0
3.0	40	80	35	3.0
4.0	40	75	30	2.5

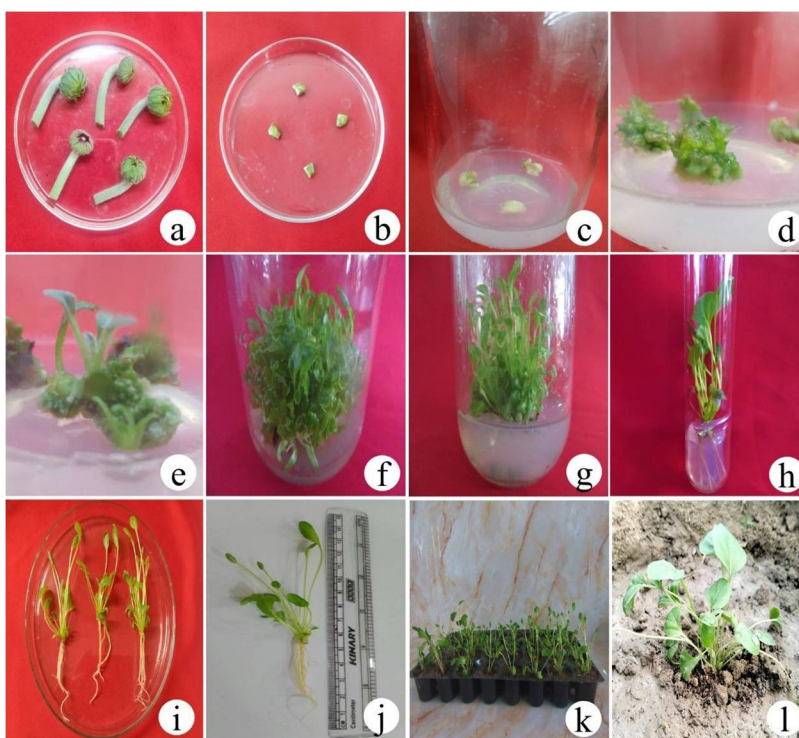


Fig. 1. Different stages of micropropagation of *Gerbera jamesonii* (a) Seven days flower buds used as explants for *in vitro* regeneration. (b) Segmentation of flower bud into 4-6 pieces (c) Induction of callus from explants in dark condition on MS supplemented with 5.0 mg/l BAP and 1.0 mg/l NAA (d) Sub-culturing callus on shoot initiation medium supplemented with 6.0 mg/l BAP and 0.3 mg/l NAA after 20-25 days of sub-culture in light condition. (e) Shoot elongation from flower bud-derived callus following sub-culturing on MS supplemented with the same medium. (f) Multiplication of shoots in only 2.0 mg/l BAP hormone-containing medium (g) Proliferation of shoots after 4th sub-culturing in the same medium (h) Formation of the roots within 2-3 weeks in half and full-strength hormone-free MS medium. (i) and (j) Showing the length of roots and shoots of plantlets. (k) Acclimatization of regenerated plantlets in soil conditions. (l) Successfully transferred *in vitro* grown plantlets into the field condition after two weeks.

Table 5. Effects of IAA, IBA, NAA, and hormone-free containing full and half MS medium on root induction from regenerated shoots.

Plant growth regulators (mg/l)	% of root formation	Days to root initiation	Average no. of roots	Average length of roots (cm)	*Intensity of callus formation	
Half MS	IAA No callus					
	0.5	100	15-25	3.8	4.5	-
	1.0	100	14-20	4.0	5.0	-
	IBA callus later					
	0.5	100	16-22	4.0	4.5	+
	1.0	100	15-20	4.5	5.0	++
	NAA callus fast					
	0.5	100	20-25	4.8	3.5	+++
	1.0	100	16-20	5.0	4.0	+++
	Hormone-free	100	15-30	5.0	5.0	-
Full MS	IAA					
	0.5	100	14-20	5.5	4.0	-
	1.0	100	13-18	6.0	5.0	-
	IBA					
	0.5	100	15-20	3.5	4.5	+
	1.0	100	14-18	5.0	5.0	++
	NAA					
	0.5	100	18-25	4.5	4.0	+++
	1.0	100	15-20	5.0	5.0	+++
	Hormone-free	100	15-25	5.0	5.0	-

*Intensity of callusing: (-) No callusing, (+) Slight callusing, (++) Considerable callusing, and (+++) Intensive callusing.

Callus initiation during root formation can inhibit the plant growth and development of *in vitro* raised plantlets when transferred to field conditions. The callus-free root is desirable for the proper physiological activities of plantlets. Auxin-free medium is suitable for root induction without callusing (Constantinovici and Sandu 1995, Posada et al. 1999). In the present study, root induction was also investigated on half and full-strength MS media without hormones. Half hormone-free media showed 100% root within 15-30 days with an average number of roots of 5.0 and length 5.0 cm and full-strength hormone-free medium also showed 100% rooting within 15-25 days with an average number of roots 5.0 and length 5.0 cm without callus initiation. Well-rooted plantlets were transferred to autoclaved vermiculate soil with a mixture of coco dust where the ratio of vermicompost, soil, and coco dust is 1:2:1 for proper acclimatization. The highest survival rate (95%) of the acclimatized plantlets was recorded on pot condition. The acclimatized plantlets were successfully transferred to the soil of the flower garden.

The present study established an effective and reproducible protocol for rapid *in vitro* mass propagation of Gerbera plantlets using the young flower bud explants of Gerbera (*Gerbera jamesonii* Bolus) var. orange using flower bud explants. The information from this study will provide details potential information related to callus induction, regeneration, shoot multiplication, *in vitro* rooting, and acclimatization of Gerbera plantlets which might be helpful for *Agrobacterium*-mediated genetic transformation as well as mass propagation of Gerbera plantlets.

Acknowledgments

The authors are highly grateful to the Research Cell, Dr. M. A. Wazed Miah Institute of Advance Studies and Research, Jashore University of Science and Technology, Jashore 7408, Bangladesh for providing the research grant # JUST/Research Cell/147 to carrying out the research and also like thanks to the Department of Genetic Engineering and Biotechnology, Jashore University of Science and Technology, Jashore 7408, Bangladesh for providing the laboratory facilities for this work.

References

- Akter N, Hoque MI and Sarker RH** (2012) *In vitro* propagation in three varieties of gerbera (*Gerbera jamesonii* Bolus.) from flower bud and flower stalk explants. Plant Tissue Cult. Biotech. **22**(2): 143-52.
- Aswath C and Choudhary ML** (2001) Effect of cytokinin on the proliferation of multiple shoots in gerbera. Indian J. Hort. **58**(4): 383-385.
- Bhatia R, Singh KP, Jhang T and Sharma TR** (2009) Assessment of clonal fidelity of micropropagated gerbera plants by ISSR markers. Sci. Hortic. **119**(2): 208-11.
- Bhatia R, Singh KP and Singh MC** (2012) *In vitro* mass multiplication of gerbera (*Gerbera jamesonii*) using capitulum explants. Indian J. Agri. Sci. **82**(9): 768-774.
- Chakrabarty D and Datta SK** (2008) Micropropagation of gerbera: lipid peroxidation and antioxidant enzyme activities during acclimatization process. Acta Physiol. Plant. **30**: 325-31.
- Constantinovic D and Sandu C** (1995) Studies on the regenerative capacity of different gerbera explants for an *in vitro* effective multiplication. Cercet. agron. Mold. **3-4**(104): 149-52.
- Gao T and Hind DJN** (2011) Gerbera Linnaeus. Fl. China **20**: 13-21.
- Hasbullah NA, Taha RM and Awal A** (2008) Growth optimization and organogenesis of *Gerbera jamesonii* Bolus ex Hook f. *in vitro*. Pak. J. Biol. Sci. **11**(11): 1449-1454.
- Jain SM** (2006) Mutation-assisted breeding for improving ornamental plants. ISHS Acta Horticulturae. **714**(1): 85-98.
- Katinas L** (2004) The Gerbera complex (Asteraceae: Mutisieae): to split or not to split. SIDA, Contributions to Botany **21**(2): 935-40.
- Kumar S and Kanwar JK** (2006) Regeneration ability of petiole, leaf and petal explants in gerbera cut flower cultures *in vitro*. Folia Horticulturae. **18**(2): 57-64.
- Kanwar JK and Kumar S** (2008) *In vitro* propagation of Gerbera- A Review, Hort. Sci. (Prague) **35**(1): 35-44.

- Laliberte S, Chretien L and Vieth J** (1985) *In vitro* plantlet production from young capitulum explants of *Gerbera jamesonii*. Hort. Sci. **20**(1): 137-139.
- Mishra SJ, Chandra R, Prasad L and Patel RK** (2014). Influence of some phytohormones base culture medium on *in vitro* multiplication of gerbera (*Gerbera jamesonii*). Hort Flora Res. Spectrum **3**(1): 65-68.
- Murashige T and Skoog F** (1962). A revised medium for rapid growth and bioassays with tobacco cultures. Physiologia Plantarum. **15**: 473-497.
- Naz S, Naz F, Tariq A, Aslam F, Ali A and Athar M** (2012) Effect of different explants on *in vitro* propagation of gerbera (*Gerbera jamesonii*) Afr. J. Biotechnol. **11**(37): 9048-53.
- Nhut DT, An TTT, Huong NTD, Don NT, Hai NT, Thien NQ and Vu NH** (2007) Effect of genotype, explant size, position and culture medium on shoot generation of *Gerbera jamesonii* by receptacle transverse thin cell layer culture. Scientia Horticulturae **111**(2): 146-151.
- Patra SK, Beura S and Shasani T** (2015) Efficacy of GA₃ on growth and flowering regulation of *in vitro* raised hybrid gerbera under shade net. Res. J. Agric. Sci. **35**(3): 173-77.
- Peper H, Brandis AV and Dopke H** (1971) Clonal propagation of gerberas can be profitable. Result from Ahlem on the culture and clonal propagation of gerberas. TASPO **105**: 7-11.
- Pierik R, Steegmans HHM and Marelis JJ** (1973) Gerbera plantlets from *in vitro* cultivated Capitulum explants. Scientia Horticulturae **1**(1): 117-119.
- Posada M, Ballesteros N, Obando W and Angarita A** (1999) Micropropagation of gerbera from floral buds. Acta Hort. **482**: 329-332.
- Rashmi R, Aswath C, Dhananjaya MV and Patil SR** (2018) Commercial Multiplication of Gerbera (*Gerbera jamesonii* Bolus ex. Hooker F.) from young capitulum explants. Int. J. Curr. Microbiol. App. Sci. **7**(11): 2524-2537.
- Ray T, Saha P and Roy SC** (2005) *In vitro* plant regeneration from young capitulum explants of *Gerbera jamesonii* Plant Cell Biotechnol. Mol. Biol. **6**(1-2): 35-40.
- Reddy A and Choudhary M** (2002) Rapid plant regeneration from *Gerbera jamesonii* Bolus cultures. Acta Bot. Croat. **61**(2): 125-134
- Shelahi SA, Majeed DM, and Hassan SM** (2013) Propagation of Gerbera (*Gerbera Jasmonii* Bolus) by using a tissue culture technique. J. Biotechnol. Res. Cent. **7**(1): 50-8.
- Shabanpour KA, Sharifi A, Bagheri A and Moshtaghi N** (2011) Effect of genotypes and culture medium on shoot regeneration and proliferation of *Gerbera jamesonii*. Afr. J. Biotechnol. **10**(57): 12211-12217.
- Shoyeb M, Hossain MS, Sarkar MAR and Rahman SM** (2018) Determination of varietal effect on efficient *in vitro* micropropagation of two varieties of gerbera (*Gerbera jamesonii* Bolus) through callus induction. Plant Cell Biotechnol Mol Biol. **19**(3-4): 118-33.
- Shylaja MR, Sashna P, Chinjusha V and Nazeem PA** (2014) An efficient micropropagation protocol for *Gerbera Jamesonii* Bolus from Flower buds. Int. J. Plant Animal Env. Sci. **4**(3): 641-643.
- Singh VK, Prasad VM, Kumari S, Rajoria P and Misra P** (2017) Identification of the suitable hardening protocol and hardening medium in micropropagation of gerbera (*Gerbera jamesonii* Bolus). Int. J. Curr. Microbiol. App. Sci. **6**(7): 2476-84.
- Sujatha K, Gowda JVN and Khan MM** (2002) Effects of different fertigation levels on gerbera under low-cost greenhouse. Ornam. Hortic. **5**(1): 54-59.

Tyagi P and **Kothari SS** (2004) Rapid in vitro regeneration of *Gerbera jamesonii* (H. Bolus ex Hook f.) from different explants. *Indian J. Biotech.* **3**: 584-588.

Warar MH, Kulkarni BS, Jagadeesha RC and **Reddy BS** (2008) Effect of cytokinins with auxin on proliferation of multiple shoots in gerbera (*Gerbera jamesonii* B.) variety Sciella. *J. Agric. Sci. Technol.* **21**(4): 597-599.

(Manuscript received on 25 November, 2022; revised on 30 November, 2022)