RESEARCH ARTICLE

EFFECT OF *IN VITRO* SUBCULTURES ON VIGOUR OF GERBERA (*GERBERA JAMESONII* BOLUS EX.)

Rashmi R[1]*., M.V. Dhananjaya[2], C. Aswath[3] and Satish R. Patil[4]

[1]Horticulture, ICAR-KVK, Dakshina Kannada (KVAFSU, Bidar), Mangaluru, Karnataka, India
[2]Division of Vegetable Crops, ICAR-IIHR, Bengaluru, Karnataka, India
[3]Division of Floriculture and Medicinal Crops, ICAR-IIHR, Bengaluru, Karnataka, India
[4]Department of FLA, Kittur Rani Chennamma College of Horticulture, Arabhavi (UHS, Bagalkot) Karnataka, India
Email: rashmi.hortico@gmail.com

Received-08.03.2020, Revised-28.03.2020

Abstract: Gerbera is commercially multiplied through tissue culture using shoot tip explant and sub culturing frequently. This method allows for obtaining large amounts of healthy homogenous plants. However, frequent *in vitro* subculture leads to vitrification and reduced vigour in some populations. A study was therefore undertaken to investigate the effect of *in vitro* subcultures on vigour and confirmed that fifteen subcultures can be continuously performed from shoot tip without losing vigour with a span of 45 weeks. If this procedure is followed from 100 shoot tips, an approximately 47000 ready to plantlets can be obtained in a span of 45 weeks without losing vigour and quality. Quality planting material can be obtained at cheaper rate in the range of Rs.10-15 save the grower by 70% on planting material cost.

Keywords: Gerbera, Tissue culture, Shoot tip, Sub culture, Vigor

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus) is considered as one of the nature's beautiful creations because of having excellent flowers of exquisite shape, size and bewitching color. It is native to South Africa, popularly known as 'Transval daisy' or 'Barbeton daisy'. Gerbera ranks fifth in the global cut flower trade. It is a popular cut flower in Holland, Germany and USA. Gerbera is ideal for beds, borders, pots and rock gardens. The flowers are of various colors, suit very well in different floral arrangements and decorations.

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 India, Commercial production of gerbera is centered around Pune and Bengaluru, Parts of Sikkim, Nagaland, Meghalaya and Uttarkhand, from where flowers are being sent to local and international market. In light of global demand, gerbera could be a major source of foreign exchange. In this aspect, mass commercial production of gerbera could be acted as a new dimension. However, using conventional propagation system it is simply not possible to fulfill the demand of the export market. The non-availability of good quality planting material of commercially important variety is a major constraint for widespread and commercial cultivation in India.

In vitro propagation is now commercially used to quickly increase cultivar selections for both cut and potted flowering plants. Moreover, the plants produced through tissue culture are genetically pure and free from diseases. To commercialize this crop and to meet the growing demand for planting material, tissue and organ culture techniques are being used as alternative methods for propagation in many countries. This method enables a million fold expansions per year of a desired plant (Murashige *et al.*, 1974; Aswath and Choudhary, 2001; Aswath and Choudhary, 2002).

From the literature, it is evident that, Gerbera are highly amenable to *in vitro* studies, as various explants such as shoot tip, leaf, petiole, and other parts of the plant were found to favorably respond to different culture media with different types and concentrations of growth hormones.

Gerbera is commercially multiplied through tissue culture using shoot tip explant and sub culturing frequently. This method allows for obtaining large amounts of healthy homogenous plants. However, frequent subculture in cytokinin and auxin media leads to vitrification, instances of occurrence of abnormal plants with changed morphology and reduced vigour have been observed in some populations due to the repeated subculture of the *in vitro* cultures. Potential hazards of tissue culture have been reported (Daniells 1997). A study was therefore undertaken to investigate the effect of number of subcultures on micro propagation of gerbera.

*Corresponding Author

MATERIALS AND METHODS

The present experiment was carried out in the Division of Floriculture and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru. Shoot tip of variety Arka Ashwa was taken from the Capitulum regenerated *in vitro* plant and inoculated in MS medium supplemented with BAP 3 mg/l and NAA 0.3 mg/l continuosly for 5 sub cultures in a span of 30 days each. *In vitro* generated shoots from 5^{th} subculture was sub cultured for further multiple shoot proliferation in a sustainable manner. Ten subcultures were carried out in the MS medium supplemented with different growth hormones at an interval of one month, on the whole over a period of 10-12 months. This continuous procedure for shoot production would facilitate the steady supply of propagules, helps to identify the best performing, multiple shoot proliferation medium for sustainable medium and helps to fix the number of sub cultures in gerbera.

The tissue cultured plantlets measuring 5-6 cm after repeated sub culturing from 12^{th} to 15^{th} subcultures on MS medium with different plant hormones was taken followed by washing with distilled water to remove the adhering agar. The plantlets were treated with 0.1 per cent Bavistin for five minutes and later, the plants were transferred to coco peat and hardened under poly tunnels. The acclimatized plants of 12^{th} to 15^{th} sub culture, which were in healthy condition were evaluated under *in vivo to know* effect of *in vitro* subcultures on vigour of gerbera.

The experiment was laid out in a completely randomized design in a naturally ventilated poly house of 560 m^2 area. Healthy tissue cultured plants were planted at monthly interval of 12^{th} to 15^{th} *in vitro* subculture plants with spacing of 30 x 30 cm. Shallow planting was done in the beds along with the coco peat media. The beds were irrigated thoroughly to maintain the optimum soil moisture condition immediately after planting.

RESULTS AND DISCUSSION

The performance of four sub-cultures i.e the 12^{th}, 13^{th}, 14^{th} and $l5^{th}$ were assessed on the basis of morphological, flowering, flower quality and yield parameters to know effect of different *in vitro* subcultures on vigour and also to check carry over effect of hormones. Vegetative growth is measured as a better way in terms of number of leaves, plant spread, leaf length, leaf width, days to sucker production and number of suckers per plant are very much important as they play a key role in deciding the ultimate crop yield. These parameters differed within the different sub-cultures of gerbera studied (Table 1.).

Number of leaves of different subcultures did not varied significantly. Maximum number of leaves was recorded in the plants of 12^{th} subculture. The data on leaf length and leaf width at different sub cultures did not varied significantly. Days to sucker production of different subcultures did not show any significant difference. 14^{th} Subculture plants was found to be late which took 156.60 days for sucker production followed by 15^{th} sub culture plants. Whereas subculture 12 took minimum number of days (152.80). Number of suckers varied significantly among the four different sub cultures. The maximum (2.48) number of suckers was produced in 12th sub culture plants, which was statistically on par with 13^{th} sub culture plants. Whereas minimum number of sucker production (1.88) was registered in 14^{th} sub culture plants. Leaves are the prime important functional units for photosynthesis, which greatly influence the growth and flower yield, which ultimately might have increased the dry matter production per plant. The results are in agreement with the findings of Nair *et al*. (2002) and Amreen *et al.* (2013). Naik *et al.* (2003) reported that morphogenetic efficiency continued to remain unaltered with sustained subculturing in Gerbera.

The data pertaining to floral characters like days to bud burst, days to first flower opening and days taken to flower senescence in plants are presented in table 2. Significant differences were observed among different sub cultures for the days to bud burst. 15^{th} sub-culture plants was found to be early for flower bud emergence which took 64.34 days and whereas 12^{th} sub-culture took maximum number of days (67.72 days) to produce visible flower bud. Among the different sub cultures studied, 15^{th} subculture plants took minimum (85.86) days to first flower opening and was statistically on par with 14^{th} sub-culture plants (86.78), whereas the 12^{th} sub-culture plants took maximum (88.34) number of days to first flower opening. Days taken to flower senescence in plants of different sub cultures did not show any significant difference. 15^{th} sub culture plants took 19.80 days for flower senescence in Plants. However, 12^{th} sub culture plants took only 17.80 days for the flower senescence in plant. Vardja and Vardja (2001) inferred that all cells of the plants normally carry the same genetic information, but the morphogenetic responses vary according to the spatial and temporal distribution of the cells and their physiological and developmental stages. The genetic make-up, varied endogenous concentrations of growth hormones and response of the genotype to different concentration of growth hormones play a key role.

Flower yield and its quality parameters decide the significance of the particular variety, which is suitable for commercial cultivation. The increase in flower yield might be attributed by more number of leaves per plant and would have resulted in production and accumulation of maximum photosynthates and their utilization for buildup of new cells, thereby increasing the production of more number of flowers with bigger size. The data on quality and yield characters such as flower stalk

length, flower diameter, length of ray florets, width of ray florets, vase life and number of flowers per plant are presented in table 3.

Significant differences were observed among the different sub cultures. Length of flower stalk was more in 14th sub culture plants (54.32 cm), which was significantly on par with the 15th sub culture plants. While the shorter stalk length was recorded in 12th sub culture plants (51.94 cm). Flower head diameter did not show any significant difference among four different sub cultures. Length of ray florets significantly differed between the sub cultures and ranged from 3.18 cm (12th sub culture) to 3.55 cm (15th sub culture). Maximum length of ray floret was registered in 15th sub culture (3.55 cm) followed by 13th sub culture (3.41cm), and was found to be significantly on par with other sub cultures. The width of ray floret was more in 15th sub culture plants (0.85 cm) followed by 14th sub culture plants (0.80 cm), whereas minimum was noticed in 12th sub culture plants (0.74 cm). The 15th sub culture plants extended its vase life up to a maximum of 10.940 days, whereas the least number of days was recorded in 12th sub culture plants (10.30 days). Significantly highest (23.60) number of flowers was recorded in 12th sub culture plants, whereas 14th sub culture plants registered minimum number of flowers per plant (19.60). Naik *et al.* (2003) reported that morphogenetic efficiency continued to remain unaltered with sustained sub culturing in Gerbera. In contrast, observed a decrease in performance with an increased passage of sub culturing in *Gamelina arborea*. In the sustainable culture, there are two distinct advantages. First, as there was no need to start with a fresh explant, the time lag for the initial culture was curtailed by about 6 weeks. Second, each subculture resulted in an exponential increase in the number of shoots (Ray *et al.* 2006). The performance index revealed no significant variation from the first to the fifteenth subculture. Morphogenetic efficiency continued to remain unaltered with sustained sub culturing. It is clear from this study that the *in vitro*-generated Gerbera performed better. The reason of such superiority *in vitro* generated plants would be the complete expression of morphogenetic potential from the disease-free, favorable environment in which they were exposed during *in vitro* propagation.

Table 1. Effect of *in vitro* subcultures on morphological characters of gerbera

Sl. No.	Treatments	Parameters				
		Number of leaves	Leaf length (cm)	Leaf width (cm)	Days to sucker production	Number of suckers per plant
T_1	**Subculture 12**	22.72	41.20	13.20	152.80	2.48
T_2	**Subculture 13**	21.70	43.20	11.10	155.20	2.36
T_3	**Subculture 14**	20.30	41.20	12.80	156.60	1.88
T_4	**Subculture 15**	22.20	42.60	13.50	156.40	2.00
S.Em±		**0.786**	**0.651**	**1.04**	**1.025**	**0.075**
CD @ 1%		**NS**	**NS**	**NS**	**NS**	**0.309**

Table 2. Effect of different *in vitro* subcultures on floral characteristics of gerbera

Sl. No.	Treatments	Parameters		
		Days to bud burst	Days to first flower opening	Days taken to flower senescence in plants
T_1	**Subculture 12**	67.72	88.34	17.80
T_2	**Subculture 13**	66.42	87.18	19.20
T_3	**Subculture 14**	66.14	86.78	18.40
T_4	**Subculture 15**	64.34	85.86	19.80
S.Em±		**0.230**	**0.278**	**0.628**
CD @ 1%		**0.964**	**1.150**	**NS**

Table 3. Effect of *in vitro* subcultures on flower quality and yield parameters of gerbera

Sl. No.	Treatments	Parameters					
		Stalk length (cm)	Flower diameter (cm)	Length of ray florets (cm)	Width of ray florets (cm)	Vase life (days)	No. of flowers per plant
T_1	**Subculture 12**	51.94	9.39	3.18	0.74	10.30	23.60
T_2	**Subculture 13**	52.96	9.57	3.41	0.79	10.80	20.80
T_3	**Subculture 14**	54.32	9.67	3.34	0.80	9.90	19.60
T_4	**Subculture 15**	53.40	9.93	3.55	0.85	10.90	21.20
S.Em±		**0.383**	**0.178**	**0.05**	**0.01**	**0.474**	**0.83**
CD @ 1%		**1.618**	**NS**	**0.198**	**0.04**	**NS**	**2.53**

From the present study it is confirmed that fifteen subcultures can be continuously performed from shoot tip without loosing vigor with a span of 45 weeks. If this procedure is followed from 100 shoot tips, an approximately 47000 ready to plantlets can be obtained in a span of 45 weeks without loosing vigour and quality of flower. Quality planting material can be obtained at cheaper rate in the range of Rs.10-15 save the grower by 70% on planting material cost. Continuous sub cultures up to 15 may bring down the cost of plant material to farmers.

REFERENCES

Amreen, T., Naik, H. B. and Sanathkumar, V. B. (2013). Performance of Gerbera genotypes under protected cultivation. *Plant Archives.,* 13(2): 757-761.

Aswath, C. and Choudhary, M. L. (2001). Effect of cytokinin on the proliferation of multiple shoots in gerbera. *Indian J. Hort.,* 58(4): 383-385.

Aswath, C. and Choudhary, M.L. (2002). Rapid plant regeneration f rom callus culture of *Gerbera jamesonii*. *Acta Bot. Croat*, 61(2): 125-134.

Daniells, J. (1997). Beware the potential hazards of tissue culture. Info *Musa*, 6(2): 17-18.

Jain, S. M. (2006). Mutation assisted breeding for improving ornamental plants. *ISHS Acta Horticulturae*, 714(1).

Kanwar, J. K. and Kumar, S. (2008). *In vitro* propagation of *Gerbera*- A Review. *Hort. Sci. (Prague)*, 35(1): 35-44.

Murashige, T., Sepra, M. and Jones, J.B. (1974). Clonal multiplication of gerbera through tissue culture. *Hort. Sci*. 9: 175-180.

Naik, D., Vartak, V. and Bhargava, S. (2003). Provenance and subculture dependant variation during micropropagation of *Gmelina arborea*. *Plant Cell Tissue Organ Cult*., 73: 189-195.

Nair, A., Sujatha and Medhi, R.P. (2002). Performance of Gerbera cultivars in the Bay Islands. *Indian Journal of Horticulture*, 59(3): 322- 325.

Ray, T., Dutta, I., Saha, P., Das, S., Roy, S. C. (2006). Genetic stability of three economically important micropropagated banana (*Musa spp.)* cultivars of Indo- Ganjetic plains, as assessed by RAPD and ISSR markers. *Plant Cell Tissue Organ Cult.,* 85: 11–21.

Vardja, R. and Vardja, T. (2001). The effect of cytokinin type and concentration and the number of subcultures on the multiplication rate of some decorative plants. *Proc. Estonian Academy Sci. Biol. Ecology*, 50(1): 22-32.