Biological Forum – An International Journal **13(4): 191-195(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Surface Sterilization and *in vitro* Callusing of *Gerbera jamesonii* Bolus cv. Balance

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(Received 10 August 2021, Accepted 13 October, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Microbial contamination is one of the major setback and act as a barrier in establishing *in vitro* cultures for which the disinfection procedure of explants prior to inoculation is important and this experiment was investigated with the objective to procure disease free *in vitro* aseptic culture of *Gerbera jamesonii* Bolus cv. Balance and callus induction in supplemented MS media using quarter capitulum as explant. Explants for surface sterilization treatment were subjected to 0.1% $HgCl_2$ solution alone for different span of time and in combination with 0.5% NaOCl solution for 2 minutes along with control (Distl. water) and result revealed significant reduced rate of fungal and bacterial contamination with increased aseptic cultures when explant were soaked with 0.1% $HgCl_2$ solution for 6 min. followed by 0.5% NaOCl solution for 2 minutes. The aseptic culture obtained were transferred in MS media containing different combinations of BAP, IAA for callus induction and callus proliferation, where MS media+2.5 ppm BAP+1.0 ppm IAA showed significant earliness in callus induction, whereas higher available days for callus proliferation, and maximum callus size was observed with MS media + 2.0 ppm BAP+1.0 ppm IAA.

Keywords : gerbera, *in-vitro*, capitulum, surface sterilization, callus.

INTRODUCTION

Developing a tissue culture protocol requires the *in vivo* knowledge of the plant species and the optimization of *in vitro* chemical, physical and environmental factors for growth and multiplication (Lee, 2004). Gerbera commonly known as Transvaal daisy, Barbeton daisy or African daisy ranks among top 5 cut flower in domestic market and among top 6 cut flower in international market (Barooah and Talukdar, 2009). It is used as cut flower and the dwarf hybrids available are also well suited for potted or garden bed plants (Nomita *et al.,* 2012). With high demands of this flower due to beautiful colours available, large sized flower with better shelf life, the area under production is subsequently increasing, but due to the conventional approach of vegetative propagation through division of clumps is very slow and propagation coefficient is also very low. The vegetative propagation provides very slow and only 4 to 5 plantlets per year per plant also gives the lower quality of plant materials and cut flower (Kumar *et al.*, 2004). Propagation through *in vitro* tissue culture technique provides rapid and large scale multiplication of plants. For the *in vitro* production of gerbera plants young capitulum, leaf or petioles are used as explants for producing plant cultures from tissues. In 1974 Murashige *et al.* proposed rapid clonal multiplication of gerbera using shoot tips as explant. Use of different explants explant has been reported many workers *viz* Kadu, (2013); Son *et al.,* (2011), Akter *et al.,* (2012) used floral buds as explant, Mohanty *et al.,* (2005) used shoot tips as explant, Winarto and Yufdy (2017) used young leaves as explants from gerbera. The initial *in vitro* culture establishment requires infection free cultures from fungal and bacterial infections since, the explants taken from field carries various inherent pathogens on tissue surfaces and natural openings, the surface sterilization of explants become major challenge and most important factor for initial culture establishment free from microbial contaminations (Thokchom and Maitra, 2017). Once obtaining healthy and disease free cultures, they are needed to inoculate in different media supplemented with plant bio regulators which promotes callus induction and callus proliferation. Auxins such as IAA, IBA, NAA are used to supplement the basal media and cytokinins like BAP, kinetin are used to supplement the media are used as they play crucial role in morphogenesis of cultures. Aswath *et al.,* (2002) reported high organogenesis and callus induction for

gerbera cultivars MS media supplemented with 0.4ppm BAP, 4ppm NAA. Same trend was also reported by Paduchuri *et al.* (2010); Son *et al.* (2011); Parvin *et al.,* (2017); Shweta *et al.* (2017). Cytokinins and auxins are said to give early callus induction with increased callus size (Tyagi and Kothari, 2004).

With the expansion of floriculture industry, the rising demand of beautiful vibrant colour flowers in the market for several purposes is constantly growing which also demands the rapid multiplication of quality plants for increased flower production. Therefore, this experiment was done with the purpose to develop a standard procedure and protocol for infection free *in vitro* culture production of commercially grown cultivar of gerbera plant. With the use of quarter capitulum as explants, disease free healthy cultures are established *in vitro* after surface sterilization and aseptic cultures were then subjected to media supplemented with plant bio regulators for callus induction and callus proliferation.

MATERIAL AND METHODS

The present study was carried out in Bio-tech cum Tissue Culture Centre of Odisha University of Agriculture and Technology, Bhubaneswar, Orissa. Here the commercially grown cut flower cultivar Balance with white coloured large sized bloom is taken for study. The explants taken for *in vitro* culture production were young capitula of 3-4 days grown under poly house condition. The capitulum were first washed with running tap water for 10 minutes and then washed with liquid detergent solution Teepol @ 10 ml/l for 15 minutes by continuously stirring the solution. Later on they were again cleaned with running tap water for 10 minutes. Explants were then treated with fungicide solution of bavistin @ 2 g/l for next 20 to 30 minutes by continuously stirring the solution to reduce the chances of fungal infection. After the treatment with fungicide solution explants were then rinsed thoroughly with distilled water to remove the traces of chemical.

To establish *in-vitro* cultures the basal media used was MS media given by Murashige and Skoog, (1962). The MS media was prepared and supplemented with 2 pm BAP and 1 ppm IAA and autoclave sterilized at 121°C for 20 minutes. All the glassware required in the experiment like petri plates and forcep, scalpel, tissue paper, absorbant cotton were steam sterilized at 121°C for 20 minutes in autoclave. The laminar air flow was wiped with 70% rubbing alcohol prior to use. The prepared media in culture tubes and sterilized glaswares and equipments, sterilized distilled water glass jars were kept inside laminar air flow and were UV sterilized for 20 minutes. For surface sterilization of explants the treatments comprised of 0.1% $HgCl_2$ solution treatment for different span of time (4, 5, 6 & 7 minutes) alone and in combination with 0.5% NaOCl solutions for 2 minutes along with control (Distl. water). After the surface sterilization explants were cut in horizontal sections using sterilized forced and scalpel and quarter capitulum explant were inoculated to culture media tubes in MS media supplemented with 1ppm BAP and 0.1ppm IAA. These cultures were kept under temperature controlled air conditioned room for further observation for 28 days. Data was recorded for rate of fungal infection (%), rate of bacterial infection (%), death of cultures (%), aseptic cultures obtained (%) and survival of cultures (%) and analysed following the CRD design with 3 replication in each treatment and 10 cultures per replication.

After 1st inoculation and obtaining the healthy disease free cultures of cv. Balance with the best sterilant treatment they were subjected to callusing media for callus induction and callus proliferation. MS media used for callus induction was fortified with BAP (cytokinin) and IAA (auxin) plant bio regulators in different concentration and in combination. The data was recorded for days to callus initiation, days to callus proliferation, available days for callus development, nature of callus, and size of callus and colour of callus. This experiment was conducted using Complete Randomized Design with 3 replications for each treatment and 10 cultures for each replication.

RESULT AND DISCUSSION

The first stage of establishing *in vitro* cultures is extraction of explants from *in vivo* condition and there sterilization for elimination of micro organisms (Chawla, 2002). The result obtained from this experiment showed significant difference in rate of fungal infection, bacterial infection, aseptic cultures procured and rate of survival of the *in vitro* cultures of gerbera cv. Balance when treated with different treatments for surface sterilization. According to Table 1, capitulum explants of gerbera cv. Balance recorded significant minimum rate of fungal infection (5.33%) when surface sterilized with 0.1% $HgCl_2$ for 6 minutes followed by 0.5% NaOCl for 2 minutes (T_8), recording at par effect with T_9 (8.33%) and T_7 (8.67%), whereas T_1 (Control) recorded the maximum rate of fungal infection of the cultures (100%). T_7, T_8 and T_9 have not recorded any bacterial infection but T_3 and T_4 recorded lower rate of bacterial infection (10.00%) having at par effect with T_6 (13.33%). T_8 (0.1% $HgCl_2$ for 6 minutes followed by 0.5% NaOCl for 2 minutes) significantly recorded the maximum aseptic cultures (94.67%) followed by T_9 and T_7. Capitulum explants when surface sterilized with 0.1% $HgCl_2$ for 6 minutes followed by 0.5% NaOCl for 2 minutes (T_8) also recorded the minimum rate of death (6.00) among the *in vitro* cultures, where explants surface sterilized with increased period of time of 0.1% $HgCl_2$ followed by 0.5% NaOCl for 2 minutes increased the death rate (10.33%) among the cultures. Maximum survival percentage of cultures (88.67%) was also observed with T_8 (0.1% $HgCl_2$ for 6 minutes followed by 0.5% NaOCl for 2 minutes) having at par effect with T_7 (84.33%). Warar *et al.,* (2008) surface sterilized gerbera explants of cv. Sciella with 0.1% $HgCl_2$ solution for 5 minutes. Beura *et al.,* (2003) reported the lower rate of infection

and higher rate of survival in explants of gladiolus when surface sterilized with 0.1% $HgCl_2$. Imtiyaz *et al* in 2012 used 6 different combination of carbendazim and $HgCl_2$ for different time for surface sterilization of gerbera cv. Rejiko and South pecific. Kadu, (2013) surface the auxillary buds of gerbera with 0.1% $HgCl_2$ for 6 min. Thokchom and Maitra, (2017) observed the low rate of infection when explants of gerbera were treated with 0.1% $HgCl_2$ solution followed by NaOCl @ 1.5% for 10 minutes and showed high rate of survival of cultures. Toppo and Beura, (2018) surface sterilized the leaf explant of *Anthurium andreanum* cv. Fire using $HgCl_2$ 0.1% for different span of time. Over sterilization increases the tissue mortality of explant causing the death of cultures (Majid *et al.*, 2014).

Table 1: Effect of surface sterilant and timing of surface sterilization of capitulum explants of *Gerbera jamesonii* cv. Balance.

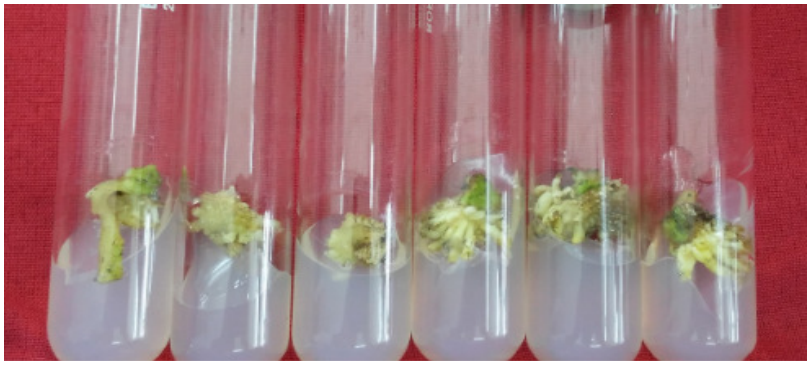
Treatment Details			Fungal Infection (%)	Bacterial Infection (%)	Aseptic Culture (%)	Death (%)	Survival (%)
Tr. No.	$HgCl_2$ 0.1%	NaOCl 0.5%					
T_1	Control (Distl water)		100.00 (87.50)	0.00 (2.50)	0.00 (2.50)	0.00 (2.50)	0.00 (2.50)
T_2	4 min	-	70.00 (56.79)	16.67 (24.09)	13.33 (21.42)	0.00 (2.50)	13.33 (21.42)
T_3	5 min	-	66.67 (54.74)	**10.00 (18.43)**	23.33 (28.88)	0.00 (2.50)	23.33 (28.88)
T_4	6 min	-	53.33 (46.91)	**10.00 (18.43)**	36.67 (37.27)	0.00 (2.50)	36.67 (37.27)
T_5	7 min	-	36.67 (37.27)	16.67 (24.09)	46.67 (43.09)	0.00 (2.50)	46.67 (43.09)
T_6:	4 min	2 min	23.33 (28.88)	13.33 [a] (21.42)	63.33 (52.73)	0.00 (2.50)	63.33 (52.73)
T_7:	5 min	2 min	8.67 [a] (17.12)	0.00 (2.50)	91.33 [a] (72.88)	7.00 [a] (1534)	84.33 [a] (66.68)
T_8	6 min	2 min	**5.33 (13.35)**	0.00 (2.50)	**94.67 (76.65)**	**6.00 (14.18)**	**88.67 (70.33)**
T_9	7 min	2 min	8.33 [a] (16.78)	0.00 (2.50)	91.66 [a] (73.21)	10.33 (18.75)	81.33 (64.40)
SE(m) ±			3.71	2.22	2.69	0.59	2.72
CD (0.05)			9.55	5.72	6.93	1.53	6.99

The sterilized cultures obtained were carefully transferred to the treatment media containing the plant bio regulators *viz.* BAP and IAA in combination of different concentrations (Table 2).

Table 2: Effect of plant bio regulators on callus initiation, callus development, callus proliferation, nature of callus, size and colour of callus of *Gerbera jamesonii* cv. Balance Basal medium – MS Duration – 45 Days.

Treatment Details (mg/l)			Days to callus initiation	Days to callus proliferation	Available days to callus development	Nature of callus	Callus spread (cm^2)	Colour of callus
Treat. No.	BAP	IAA						
T_1	MS (Control)		27.71	39.80	17.29	Compact	1.37	Greenish white
T_2	0.5 ppm	0.5 ppm	25.83	38.00	19.17	Compact	1.53	Greenish white
T_3	1.00 ppm	0.5 ppm	24.42	37.31	20.58	Compact	1.62	Greenish white
T_4	1.5 ppm	0.5 ppm	23.34	36.00	21.66	Compact	1.77	Greenish white
T_5	2.00 ppm	0.5 ppm	20.68	33.71 [a]	24.32	Compact	1.97	Greenish white
T_6	2.5 ppm	0.5 ppm	20.32	33.00 [a]	24.68	Compact	2.33[a]	Greenish white
T_7	0.5 ppm	1 ppm	23.71	36.61	21.29	Compact	1.60	Greenish white
T_8	1.00 ppm	1 ppm	22.60	35.63	22.40	Compact	1.77	Greenish white
T_9	1.5 ppm	1 ppm	21.33 [a]	33.62 [a]	23.67 [a]	Compact	2.03	Greenish white
T_{10}	2.00 ppm	1 ppm	21.01 [a]	**30.53**	**26.00**	Compact	**2.40**	Greenish white
T_{11}	2.5 ppm	1 ppm	**19.00**	34.00	23.99 [a]	Compact	2.10	Greenish white
SE(m) ±			0.98	1.33	0.91	—	0.08	—
CD (0.05)			2.50	3.34	2.67	—	0.21	—

The cultures were maintained at temperature controlled aseptic culture room with 24±1°C and white fluorescent lights with 16 hour photoperiod. Earliness to callus initiation (19.00 days) was significantly recorded with cultures inoculated in MS basal media supplemented with 2.5 ppm BAP and 1.0 ppm IAA (T_{11}) showing at par effect with T_{10} (21.01 days) and T_9 (21.33 days), whereas maximum days taken for callus initiation (26.29 days) was observed with plain MS media as control (T_1) which also resulted in the delayed callus proliferation (39.80 days). Days to callus proliferation were observed early (30.53 days) with T_{10} (MS + 2.0 ppm BAP + 1.0 ppm IAA) which also provided significantly maximum days available to callus development (26.00 days) and maximum callus size (2.40 cm^2). Media optimization is an essential parameter for growth and dedifferentiation of plant cell tissues. Cytokinins play an important role in cell morphogenesis of *in vitro* cultures (Parvin *et al.,* 2017). The natures of callus observed in the cultures were compact with all treatment and colour callus were greenish white. The organogenesis for callus induction requires both cytokinin and auxin (Aswath and Choudhary, 2002). Similar findings were also observed by, Bhatia *et al.,* (2008); Patnaik and Beura (2008); Paduchuri *et al.,* (2010). Son *et al.,* (2011) observed MS media supplemented with BAP @ 3mg/l and IAA @ 0.1 mg/l for culture establishment and callus formation and same trend was observed by Parvin *et al.,* (2017), Shylaja *et al.,* (2014); Hasbullah *et al.,* (2015). Kumar *et al.,* (2019) reported earliness in callus induction in media supplemented with 2ppm 2, 4-D and 1.5 ppm Kinetin. Shweta *et al.,* (2017) studied the response of capitulum explants of gerbera for micropropagation and reported MS media fortified with 3 mg/l BAP and 0.1 mg/l IAA to be the best medium for culture establishment and primordial emergence (14.66 days) due to Cytokinins in plant cell culture regulate cell division, stimulate axillary and adventitious shoot proliferation.



Aseptic culture establishment of capitulum explant in cv. Balance

Callus induction and callus proliferation in cv. Balance

CONCLUSION

The result of this experiment clearly showed that surface sterilization of explants is important to reduce rate of contamination and in increasing the percentage of survival of cultures. These cultures when subjected to MS media supplement with BAP and IAA plant bio regulator helped in organogenesis of the cells leading to early callus induction and maximum size of callus. The present investigation will provide a rapid commercial multiplication protocol for gerbera for large scale production. In light of the global demand this commercial augmentation for gerbera plant multiplication will be efficient and profitable.

Acknowledgment. The authors are thankful to the faculty and members of Department of Floriculture and Landscaping and Biotechnology cum Tissue Culture Centre, College of Agriculture, Odisha University of Agriculture and Technology for the help, guidance and necessary facilities provided for the conduct of this experiment.

Conflict of Interest. None.

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How to cite this article: Sharma, R. and Beura, S. (2021). Surface Sterilization and *in vitro* Callusing of *Gerbera jamesonii* Bolus cv. Balance. *Biological Forum – An International Journal*, *13*(4): 191-195.