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Surface sterilization and *in vitro* callusing of gerbera (*Gerbera jamesonii* Bolus) cv. Intense

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Abstract
The present experiment was carried out in the laboratory of bio technology cum tissue culture centre at O.U.A.T, Bhubaneswar with the objective to obtain disease free aseptic culture of *Gerbera jamesonii* Bolus cv. Intense using quarter capitulum as explant. Treatments taken for surface sterilization were $HgCl_2$ 0.1% solution with different span of time (4, 5, 6 & 7 minutes) alone and in combination with NaOCl 0.5% solutions for 2 minutes along with control (distl. water). Lower rate of infection (6.33%), maximum percentage of asceptic cultures (93.67%) and higher survival rate (84.67%) were recorded when explants were treated with $HgCl_2$ 0.1% solution for 7 min. followed by NaOCl 0.5% solution for 2 min. After completion of this experiment, the quarter capitulum sterilized with the mentioned best sterilants the cultures were subjected in MS media containing different concentrations of BAP, IAA in combination for callus induction. MS media supplemented with 2.5 ppm BAP and 0.5 ppm IAA showed significantly earliness in callus induction (16.97 days), higher available days for callus proliferation (27.83 days) and maximum callus size (2.50 cm).

Keywords: Gerbera, surface Sterilant, callus, *in-vitro,* capitulum

Introduction
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 plants with quality flower production. 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) [2]. 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) [8]. 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) [6]. 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. 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) [13]. Once obtaining healthy and disease free cultures, they are needed to inoculate in different media supplemented with plant bio regulators of different concentration which promotes formation of callus. Cytokinins and auxins are said to give better callus induction (Tyagi and Kothari, 2004) [14].
Therefore, this experiment was done with the purpose to develop a 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 were obtained after surface sterilization and cultures were subjected plant bio regulator supplemented media for callus induction.

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 importantly grown cut flower cultivar cv. Intense with large sized red colour bloom is taken for study. The explants taken for *in vitro* culture production were young capitula of 3-4 days. Explants were taken from the plants grown under poly house condition. The capitula were first washed with running tap water for 10 minutes and then were washed with liquid detergent solution Teepol @ 10 ml/l for 15 minutes by continuously stirring the solution with capitula. 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 or 30 minutes by continuously stirring to reduce the chances of fungal infection. After the treatment with fungicide solution explants were rinsed with distilled water 3-4 times.

To establish *in-vitro* cultures the basal media used was MS media given by Murashige and Skoog in 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, salpel, 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 0.1% of $HgCl_2$ and 0.5% NaOCl was used alone and in combination for different span of time. Explants were cut in horizontal sections using sterilized forced and scalpel and quarter capitulum explant were inoculated to culture media tubes. These cultures were kept under 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 (%), asceptic 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. Intense with the best sterilant treatment they were subjected to callusing media for callus induction. MS media used for callus induction 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, size of callus, 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 result obtained from this experiment showed significant difference in rate of fungal infection, bacterial infection, asceptic cultures procured and rate of survival of cultures when treated with different treatments of surface sterilization. According to Table 1, gerbera cv. Intense showed lowest rate of fungal infection (6.33%) when treated with $HgCl_2$ 0.1% for 7 minutes followed by NaOCl 0.5% for 2 minutes (T9), which showed at par effect with T8 (11.33%). T2 ($HgCl_2$ 0.1% for 5 minutes) and T5 ($HgCl_2$ 0.1% for 7 minutes) gave similar response for lowest rate of bacterial infection (3.33%) having at par effect with T3 (6.67%), lowest percentage of death of cultures were observed with T3 ($HgCl_2$ 0.1% for 7 minutes). Total asceptic cultures procured (93.67%) and survival percentage of cultures (84.67%) was highest with T_9 ($HgCl_2$ 0.1% for 7 minutes followed by NaOCl 0.5% for 2 minutes) followed by T8 (88.67% and 80.67% respectively). Kour *et al* in 1999 surface sterilized the flower bud with 0.1% $HgCl_2$ solution for 2-3 minutes. Beura *et al* (2007) [4] surface sterilized the explants of dracaena, Warar *et al* (2008) [15] surface sterilized gerbera explants of cv. Sciella with 0.1% $HgCl_2$ solution for 5 minutes. Beura *et al* (2003) [3] reported the lower rate of infection and higher rate of survival in explants of gladiolus when surface sterilized with 0.1% $HgCl_2$. Thokchom and Maitra (2017) [13] 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. Over sterilization increases the tissue mortality of explant causing the death of cultures (Majid *et al*, 2014) [7].

Table 1: Effect of surface sterilant and timing of surface sterilization of capitulum explants of *Gerbera jamesonii* cv. Intense Basal medium – MS Duration – 28 Days

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	-	83.33 (65.91)	3.33 (10.52)	13.33 (21.42)	0.00 (2.50)	13.33 (21.42)
T_3	5 min	-	70.00 (56.79)	6.67 [a] (14.96)	23.33 (28.88)	3.33 (18.43)	20.00 (26.57)
T_4	6 min	-	46.67 (43.09)	10.00 (18.43)	43.33 (41.17)	0.00 (2.50)	43.33 (41.17)
T_5	7 min	-	36.67 (37.27)	3.33 (10.52)	60.00 (50.77)	0.00 (2.50)	60.00 (50.77)
T_6:	4 min	2 min	19.33 (26.08)	0.00 (2.50)	80.67 (63.92)	11.67 (36.27)	69.00 (56.17)
T_7:	5 min	2 min	14.33 (22.25)	0.00 (2.50)	85.67 (67.76)	12.33 (37.46)	73.33 (58.91)
T_8	6 min	2 min	11.33[a] (19.67)	0.00 (2.50)	88.67 (70.33)	8.00 (29.33)	80.67[a] (63.92)
T_9	7 min	2 min	6.33 (14.58)	0.00 (2.50)	93.67 (75.44)	9.00 (31.31)	84.67 (66.95)
SE(m) ±			2.39	1.57	1.73	1.30	1.99
CD (0.05)			6.15	4.04	4.45	3.33	5.12

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). The cultures were maintained at controlled asceptic culture room with 24±1 c˚ and white fluorescent lights for 16 hour photoperiod. Days to callus initiation was significantly earlier (16.97 days) with cultures inoculated in MS basal media supplemented with 2.5 ppm BAP and 0.5 ppm IAA (T_6) showing at par effect with T_{11} (MS + 2.5 ppm BAP + 0.5 ppm IAA) (17.16 days), whereas maximum days taken for callus initiation (26.29 days) was with plain MS media as control (T_1). Days to callus proliferation was observed early (27.83 days) with both T5 (MS + 2.0 ppm BAP + 0.5 ppm IAA) and T6 (MS + 2.5 ppm BAP + 0.5 ppm IAA). MS media supplemented with 2.5 ppm BAP and 0.5 ppm IAA (T_6) provided significantly maximum days available (28.03) to callus development and recorded maximum callus size (2.5 cm) and recording at par effect with T11 and T10. Cytokinins

play an important role in cell morphogenesis of *in vitro* cultures (Parvin *et al*, 2017) [11]. The nature 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). Parvin *et al* in 2017 [1, 11] showed earliness in callus induction using capitulum as explant inoculated in media supplemented with 3 mg/l BAP and 0.01 mg/l NAA. Similar findings were also observed by Paduchuri *et al* in 2010, Bhatia *et al* in 2008 and Patnaik and Beura in 2008 [9, 12].

Table 2: Effect of plant bio regulators on callus initiaton, callus development, callus proliferation, nature of callus, size and colour of callus of *Gerbera jamesonii* cv. Intense

Treatment Details			Days to callus initiation	Days to callus proliferation	Available days for callus development	Nature of callus	Callus spread (cm)	Colour of callus
Tr. No.	BAP	IAA						
T_1	MS (Control)		26.29	39.20	18.71	Compact	1.27	Greenish white
T_2	0.5 ppm	0.5 ppm	23.13	35.80	21.87	Compact	1.39	Greenish white
T_3	1.00 ppm	0.5 ppm	22.97	34.72	22.03	Compact	1.77	Greenish white
T_4	1.5 ppm	0.5 ppm	22.27	34.36	22.73	Compact	2.26	Greenish white
T_5	2.00 ppm	0.5 ppm	19.97	27.83	25.03	Compact	2.43	Greenish white
T_6	2.5 ppm	0.5 ppm	16.97	27.83	28.03	Compact	2.50	Greenish white
T_7	0.5 ppm	1 ppm	23.38	34.67	21.62	Compact	1.50	Greenish white
T_8	1.00 ppm	1 ppm	21.67	33.67	23.33	Compact	1.67	Greenish white
T_9	1.5 ppm	1 ppm	21.33	31.33	23.67	Compact	1.87	Greenish white
T_{10}	2.00 ppm	1 ppm	19.06	29.33 [a]	25.94 [a]	Compact	2.21 [a]	Greenish white
T_{11}	2.5 ppm	1 ppm	17.16[a]	28.33 [a]	27.84 [a]	Compact	2.43 [a]	Greenish white
SE(m) ±			0.66	0.79	0.83	_	0.13	_
CD (0.05)			1.68	2.01	2.47	_	0.32	_

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. This cultures were subjected to MS media supplement with cytokinin and auxin which helped in organogenesis of the cells leading to early callus induction and maximum size of callus.

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