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Standardization of Exposure Time of Surface Sterilants on Capitulum Explants of Gerbera (*Gerbera jamesonii* Bolus ex Hook. f.) cv. Alcatraz for *In vitro* Culture

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ABSTRACT: *In vitro* cloning of gerbera for superior quality, disease free and true-to-type plants has been preferred over conventional vegetative propagation for large scale production of plantlets. The success rate of *in vitro* cloning is positively correlated with sterilization techniques, sterilants efficacy. Sterilization is the method of making explants contamination free before establishment of culture. An experiment was conducted at Biotechnology-cum –tissue culture centre of OUAT, Bhubaneswar with the objective to establish protocol for standardization of surface sterilization exposure time for capitulum explants of Gerbera hybrid cv. Alcatraz, to obtain culture free from contamination. The MS medium was used as basal medium and supplemented with BAP and IAA was used as culture medium for establishment of explants. Proper sterilisation process was followed for maintaining aseptic environment at *in vitro* cloning. The explants (capitulum) were exposed to 0.1% $HgCl_2$ for T_2 – 2 mins, T_3 – 3 mins, T_4 – 4 mins, T_5 – 5 mins, T_6 – 6 mins, T_7 – 7 mins and T_8 – 8 mins and T_1..control (distilled water) inside laminar air flow chamber for surface sterilization. The explants capitulum treated for 6 minutes for surface sterilant of 0.1 % of $HgCl_2$ resulted in minimum fungal (0.00%) and bacterial (0.00%) contamination with maximum survival percentage (90.00%) and minimum death rate (10.00%).

Keywords: Gerbera, Alcatraz, Surface sterilization, $HgCl_2$, *In vitro* culture.

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus.) is an exceptionally beautiful flower crop belong to family Asteraceae. Gerbera is also known as Transvaal daisy or Barberton daisy. It is native to South Africa (Singh *et al*., 2017). It ranked 5th as popular cut flower because of its vibrant attractive flower colours, flower diameters, long stem length, long vase life and high yield potential. In flower industry the blooms of gerbera are commercially used as both cut flowers in flower arrangements, decoration, exhibition and bouquet preparation and decorative plants in pots, beds and borders for garden display (Patra *et al.,* 2015).

Gerbera can be propagated through both sexually and asexually. To maintain genetic uniformity and integrity gerberas are propagated through vegetatively (Division of clumps and suckers). Though division of clumps and sucker is being used as a conventional method of propagation in gerbera but the rate of propagation is also very low as commercial flower production (Aswath and Choudhary 2001). However, plants shows heterozygosity in progeny and non- uniformity in flower characteristic when propagated through seeds; resulting propagation through seed is not a desirable method (Shelahi *et al*., 2013). Some cultivars do not even sets seed. An efficient, and economically feasible propagation technique is needed for the large-scale commercial production of gerbera. To obtain large scale production of superior quality, healthy, disease-free, true-to-type gerbera plantlets in short duration of time with year round availability; propagation through tissue culture method has recently been in application (Aswath and Choudhary 2002). Propagation through tissue culture aims for proliferation and growth of tissues or organs in a sterile and controlled environment. Commercially in gerbera *in vitro* cloning young flower buds or capitulum is mostly preferred.

Odisha floral industry has been expanding. To ensure the supply of flowers according to the currents demand of consumers of Odisha, there is a need to produce large scale commercial gerbera plantlets in a simple, efficient and quicker way, which should be superior in quality, healthy, disease -free and true-to-type. The success rate of *in vitro* cloning is positively correlated with sterilization techniques, sterilants efficacy. Sterilization is the method of making explants contamination free before establishment of culture. On

an average 3-5% of losses were observed in tissue culture laboratory due to contaminations (Leifert *et al.,* 1989), mainly caused by fungus, yeast and bacteria (Leifert *et al.,* 1994; Toppo and Beura 2018). Considering the above said information into account, the objective of the present investigation was to establish a protocol for standardization of surface sterilization exposure time for capitulum explants of Gerbera hybrid cv. Alcatraz, to obtain culture free from contamination.

MATERIALS AND METHODS

The present investigation on *in vitro* culturing of Gerbera hybrid cv. Alcatraz was carried out at Biotechnology cum-Tissue Culture Centre, OUAT, Bhubaneswar. A young, healthy and disease free capitula of 2-3 days old of Gerbera cv. Alcatraz were collected from fresh plants which were grown inside the greenhouse (mother block) at the Biotechnology-cum-Tissue Culture Centre, OUAT, Bhubaneswar, for experimentation on *in vitro* culture in the laboratory.

The capitula were initially washed with running tap water for 15 minutes, followed by 15 minutes with a 1% Tween 20 solution while continuous stirring the solution of capitum, and finally with running tap water for 10 mins. To minimize infection during *in vitro* culture, explants were treated with Bavistin at 2 g/l for 20 minutes with continuous solution stirring before being washed 3-4 times with distilled water. The explants were then transferred to the laminar air flow chamber for further sterilisation. To disinfect the laminar air flow chamber, it was wiped with70% isopropanol. 0.1% $HgCl_2$ was used as treatment for surface sterilization of explants inside the laminar air flow with time exposure for T_2 – 2 mins, T_3 – 3 mins, T_4 – 4 mins, T_5 – 5 mins, T_6 – 6 mins, T_7 – 7 mins and T_8 – 8 mins; followed by rinsing 5-6 times with sterile water. And finally the explants were treated with 0.1% NaOCl solution for 5 mins to reduce amount of residual mercury, then rinsed with 4-5 times sterile water. For control (T_1) explants were only washed with distilled water 5-6 times

For *in vitro* culture of Gerbera hybrid cv. Alcatraz, MS media was used as basal media (Murashige and Skoog 1962). MS media enhanced with BAP 2.0mg/l and IAA 0.5mg/l was used as culture media for establishment of gerbera explant. After proper quantification of all the nutrients, salt, and bio regulators for preparation of culture media, the solution's pH was adjusted to 5.7+ 0.1 through 0.1 N NaOH or 0.1 N HCL. Agar (0.8% w/v) was added to the medium, cooked until completely dissolved, then poured into culture glass bottles and sealed. Bottles with culture media were autoclaved for 20 minutes at 121 at 15 psi pressure. The autoclaved medium was cooled down on a laminar air flow bench. The glassware were submerged in the detergent solution overnight and cleaned under running tap water. After cleaning with distilled water, they were oven-dried at 150 for two hours. Forceps, petridish, and scalpel were disinfected with isopropanol, wrapped with paper, and sterilised in an autoclave at 15 psi pressure and 121 for 20 minutes. Isopropanol was used to wipe the operational chamber of the laminar airflow cabinet for aseptic environment. To be certain that particles did not settle in the working area, filtered air (80-100 cft/min) was released for 5 minutes. The sterilised accessories for *in vitro* culture (excluding living tissue) were held in the chamber and subjected to UV radiation for 30 minutes.

These sterile capitulum explants were appropriately trimmed to remove brown or sterilising agent-affected areas to attain a suitable size of 1-2 cm^2. They were then cut into quarters and inoculated on MS media supplemented with BAP and IAA induce callus. After inoculation, the culture was maintained at 25±2 °C in an air-conditioned environment with 80% relative humidity and a 16-hour photoperiod provided by fluorescent tubes to meet light needs. Observation for different parameters *i.e*., fungal %, bacterial %, death % and survival% of explants were recorded at 30DAI (Days after inoculation). The treatment having the maximum survival % with minimum contamination and death % was taken as the standard time for surface sterilization of explants for further in vitro culture. Recorded data were analysed using completely randomised design (CRD) by Gomez and Gomez (1984) with 3 replications and 8 treatments. Each replications contains 10 nos. of culture bottles

RESULTS AND DISCUSSION

The relation between exposure of sterilization time of 0.1% $HgCl_2$ to the explants of capitulum and different level of contamination, death and survival percentage were presented in Table 1.

Recorded data depicted in Table 1 revealed that minimum fungal infection (0.00%) was found in T_6 – 6 mins. (Exposure of explants to 0.1% $HgCl_2$) followed by T_7 (7 mins.) and T_8 (8 mins.) with 6.66% and 13.33% respectively. Response of Bacterial infection with respect to sterilization exposure time recorded lowest value (0.00%) for the treatment (T_6)– 6 mins. exposure of explants to 0.1% $HgCl_2$ followed with equal value of 6.66% for T_7 and T_8. Death percentage (23.33%) of explants observed highest for T_8. (exposure of explants to 0.1% $HgCl_2$ for 8 mins) followed by T_7 (16.66%) and T_6 (10.00%). It is due to the harmful effect of $HgCl_2$ by browning of cultured tissue. Survival percentage was found maximum (90.00%) for T_6 (6 mins.) followed by T_7 (7mins.) (70.02%) and T_8 (8 mins.) (56.685%). However fungal infection and bacterial infection were recorded significantly highest for T_1 (Distilled water) *i.e*., 63.33% and 40.00% respectively. Survival % also showed similar response to T_1 (Distilled water) with value of 03.34%.

The quantity and time of surface sterilization chosen for explants characteristically depends on the type of explants and plant species (Rezadost *et al.,* 2013). Different plants morphological characters like softness and hardness of the tissue might influence the sterilization time exposure (Sharma *et al*., 2014). Higher amount of $HgCl_2$ is phytotoxic in nature due to heavy metal contamination of mercury to the plant tissue affecting survival of plants. However, lower concentration shows satisfactory results (Mamun *et al*.,

2004). $HgCl_2$ significantly reduces contamination percentage with increase in its exposure time due to its anti-bacterial property (Gochhayat *et al.,* 2017). Similar results were recorded with Beura *et al.* (2007) surface sterilized the explants of dracaena, Warar *et al.* (2008) surface sterilized gerbera explants of cv. Sciella with 0.1% $HgCl_2$ solution for 5 minutes. Sharma and Beura (2021) recorded similar results for *Gerbera jamesonii* cv. Balance. Beura *et al.* (2003) reported minimun % of infection and maximum survival % in explants of gladiolus with 0.1% $HgCl_2$ as surface sterilant. Thokchom and Maitra (2017) found explants of gerbera treated with 0.1% $HgCl_2$ solution followed by NaOCl @ 1.5% for 10 minutes showed significantly higher % of survival of cultures and lower % of contamination.

Table 1 : Study on surface sterilization exposure time of 0.1% $HgCl_2$ to the explants of capitulum of Gerbera hybridcv. Alcatraz at 30 DAI.

Treatment No.	Treatment Details ($HgCl_2$ 0.1 %)	Fungal Infection (%)	Bacterial Infection (%)	Death (%)	Survival (%)
T_1	Control (Distilled water)	63.33 (52.71)	40.00 (39.22)	**03.33 (10.51)**	03.34 (10.53)
T_2	2 Mins.	56.66 (48.89)	30.00 (33.18)	03.33 (10.16)	10.01 (18.38)
T_3	3 Mins.	50.00 (44.98)	20.00 (26.44)	03.33 (10.51)	26.67 (31.06)
T_4	4 Mins.	40.00 (39.29)	16.66 (24.08)	06.66 (14.92)	36.68 (37.26)
T_5	5 Mins.	16.66 (24.08)	10.00 (18.43)	06.66 (14.95)	66.68 (54.73)
T_6	6 Mins.	**00.00 (0.00)**	**00.00 (0.00)**	10.00 (18.43)	**90.00 (71.54)**
T_7	7 Mins	06.66 (14.95)	06.66 (14.95)	16.66 (24.06)	70.02 (56.78)
T_8	8 Mins	13.33 (21.41)	06.66 (14.95)	23.33 (28.87)	56.68 (48.82)
S.E(m) ±		**0.69**	**0.83**	**0.79**	**0.61**
CD (5%)		**2.08**	**2.51**	**2.40**	**1.85**

(Values in the parenthesis are Arc sign transformed values)

CONCLUSIONS

The present study on standardization of surface sterilization exposure time on capitulum of Gerbera hybrid cv. Alcatraz concluded that there is a positive correlation between sterilant efficacy and success rate of *in vitro* cloning. The explants capitulum treated for 6 minutes for surface sterilant of 0.1 % of $HgCl_2$ resulted in minimum fungal (0.00%) and bacterial (0.00%) contamination with maximum survival percentage (90.00%) and minimum death rate (10.00%).

FUTURE SCOPE

This experiment on surface sterilization exposure time and survival % of Gerbera hybrid cv. Alcatraz will facilitate large scale production of disease free, true-to-type gerbera plantlets which will ultimately solve the supply demand of flowers of Odisha state. This also provides findings for conducting further researches on *In vitro* culturing of gerbera.

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Conflict of Interest. None.

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