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Impact of Plant Bioregulators on *In Vitro* Callogenesis of Gerbera Hybrid cv. Alcatraz

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ABSTRACT: In vitro cloning of gerbera for true-to-type and disease free plants has been preferred over conventional vegetative propagation for large scale production of plantlets. An experiment was conducted at Biotechnology-cum –tissue culture centre of OUAT, Bhubaneswar with the objective to establish protocol for callusing in gerbera hybrid cv. Alcatraz through in vitro cloning using capitulum as an explant. The MS medium was used as the base and was added with varying concentrations of BAP, IAA, and NAA in various combinations to create callus emergence media. Proper sterilisation process was followed for maintaining aseptic environment at in vitro cloning. The experiment revealed that T_6 (MS + BAP 2.0mg/l + IAA 0.5mg/l) produced callus earlier (18.20 days) with longer callus development days (27.10 days) and larger callus spread (4.03cm^2) of compact and greenish white callus after 45 days of inoculation. However, T_2 (MS + BAP 1.0mg/l) recorded the lowest values for all parameters. After 1st sub-culturing T_7 (MS + BAP 3.0mg/l + IAA 0.5mg/l) was found best for all the parameters which was also showed at par effect with T_6 (MS + BAP 2.0mg/l + IAA 0.5mg/l).

Keywords: Gerbera, Alcatraz, Callus formation, *In vitro* culture.

INTRODUCTION

Gerbera (*Gerbera jamesonii* Bolus.) commonly known as Transvaal daisy or Barbeton daisy is an exceptionally beautiful flower belong to Asteraceae family which is native to South Africa (Singh *et al*., 2017). Gerbera name was given in the honour of German naturalist Traugott Gerber. 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). 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.

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 production of flower (Aswath and Choudhary 2001). However, plants shows heterozygosity and non-uniformity if propagated through seeds (Shelahi *et al.,* 2013). Some cultivars do not even sets seed. A more straightforward, efficient, and economically feasible propagation technique is needed for the large-scale commercial production of gerbera. Large-scale multiplication of disease-free, true-to-type gerbera plantlets has been successfully achieved by in vitro cloning (Aswath and Choudhary 2002).

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 are disease free and true-to-type. Taking the aforementioned into account, the current study was conducted to produce plantlets using in vitro cloning. Establishing a methodology for callusing in gerbera hybrids was one of the goals of this study.

MATERIALS AND METHODS

The present investigation was carried out at Biotechnology cum-Tissue Culture Centre, OUAT, Bhubaneswar.

A. Source of Explants and its Surface Sterilization

A young, healthy and disease free capitula of 2-3 days old of Gerberacv. Alcatraz were collected at early morning from fresh plant grown inside the greenhouse (mother block) in the Biotechnology-cum-Tissue Culture Centre, OUAT, Bhubaneswar, for experimenting in vitroculture in the laboratory.

The capitula were washed with running tap water for 15 minutes, then with a 1% Tween 20 solution for 15 minutes while continuously stirring the solution with the capitula, and finally with running tap water for 10 minutes. To reduce infection during in vitro culture, explants were treated with Bavistin at 2 g/l for 20 minutes with continuous solution stirring and rinsed 3-4 times with distilled water. The explants were then transferred to the laminar air flow chamber for further sterilisation. 70% isopropanol was used to wipe the laminar air flow chamber. Inside the laminar explants, 0.1% $HgCl_2$ was applied to explants for varying amounts of time before being rinsed 5-6 times with sterile water. The explants were rinsed with 0.1% NaOCl solution for 5 mins for dissolving the residual mercury as it is toxic to plant and again rinsed 4-5 times with sterile water.

B. Stock solution, Media preparation and Sterilization

The stock solution for micro and macro nutrients were prepared separately by dissolving required quantity of salts in sterile distilled water and MS media (Murashige and Skoog 1962) was prepared and utilised as basal media throughout the investigation. The stock solutions for micro and macro nutrients were made separately by dissolving the necessary salts in sterile distilled water, and MS media (Murashige and Skoog 1962) was created and used as the basal medium throughout the experiment. To prepare culture media, the needed quantities of macronutrients, micronutrients, FeEDTA, vitamins, and plant bioregulators were obtained from the stock solution, and the required amount of sucrose dissolved in distilled water was added fresh to the medium. Using 0.1 N NaOH or 0.1 N HCL, the solution's pH was adjusted to 5.7+ 0.1. The initial volume was then increased to 1 L by adding distilled water. Agar (0.8% w/v) was added to the medium, cooked until completely dissolved, then poured into culture glass bottles and sealed.

Bottles containing 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 was soaked in the detergent solution overnight and cleaned under running tap water. After being cleaned with distilled water, they were dried in an oven at 150℃ for two hours. Forceps, petridish, and scalpel were washed with isopropanol, wrapped with paper, and sterilised in an autoclave at 15 pressure and 121℃ for 20 minutes. Isopropanol was used to clean off the operating chamber of the laminar air flow cabinet. To guarantee that particles did not settle in the working area, filtered air (80-100 cft/min) was released for 5 minutes. The sterilised items for usage (excluding living tissue) were held in the chamber and subjected to UV radiation for 30 minutes.

Inoculation. These sterile capitulum explants were appropriately trimmed to remove brown or sterilising agent-affected areas to achieve a suitable size of 1-2cm^2. They were then cut into quarters and inoculated on MS media supplemented with BAP (cytokinin) and plant bio regulators NAA and IAA (auxin) at varying concentrations and in combination to induce callus. After proper callus development, those callus were cut into quarters placed further proliferation in callus development media. Observation for different parameters *i.e.,* days to callus initiation, days to callus proliferation, callus bulking, callus development, nature of callus, size of callus, colour of callus of explants were recorded 45 DAI (Days after inoculation). The treatment with early callus initiation and larger callus size and compact in nature with infection and death were taken as the protocol for further in vitro propagation.

Establishment of culture. 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.

Statistical analysis. Three replications, each with ten culture bottles, were used in the completely randomised design (CRD) experiment. The experimental observations' raw data were statistically analysed using the methodology outlined by Gomez and Gomez (1984). The "F" variance ratio test was used to determine the significance and non-significance of the treatment effect. At the 5% level of significance, the calculated "F" value was compared to the "F" value in the table.

RESULTS ANND DISCUSSION

The sterilised capitula explants were inoculated to different treatments of callus emergence media containing various concentration of plant bio regulators in combination. After 45 days of inoculation of explants the results were recorded in Table 1.

The callogenesis experiment depicted in Table 1 revealed that MS media boosted with BAP 3.0mg/l + IAA 0.5mg/l (T_7) significantly produced callus earlier in 17.43 days followed by T_6 (MS media + BAP 2.0mg/l + IAA 0.5mg/l) and T_9 ((MS media + BAP 2.0mg/l + NAA 0.5mg/l) in 18.20 days and 21.10 days respectively. T_6 and T_9 stood at par with T_7 also. However, T_2 (MS media + BAP 2.0mg/l) developed callus lately in 28.32 days. Treatment (T_6) MS media + BAP 2.0mg/l + IAA 0.5mg/l showed maximum days for callus development (27.10 days) which stood at par treatments T_7 (MS media + BAP 3.0mg/l + IAA 0.5mg/l) and T_{10} ((MS media + BAP 3.0mg/l + NAA 0.5mg/l) in 26.75 days and 23.11 days respectively. For callus spread T_6- MS media + BAP 2.0mg/l + IAA 0.5mg/l delivered maximum growth and spread

(4.03cm^2) and stood at par with T_7, T_9 and T_{10} with 3.86 cm^2, 3.14cm^2 and 3.51 cm^2. However, T_2 (MS media + BAP 2.0mg/l) revealed minimum days to callus development (16.53 days) and lower callus spread 1.97cm^2). MS media supplied with different concentration of BAP produced friable and greenish callus while MS media supplemented with IAA and NAA of different concentration showed compact, greenish-white callus.

Table 1: Impact of plant bioregulators on quarter capitulum explant of Gerbera cv. Alcatraz callus initiation, development, spread, nature, and colour.

Basal Medium- MS				Culturing duration- 45 Days				
Treatment No.	Treatment Details			Days to callus initiation	Days to callus development	Callus spread (cm^2)	Nature of the callus	Colour of the callus
	BAP	IAA	NAA					
T_1	MS Media (Control)			-	-	-	-	-
T_2	1	-	-	28.32	16.53	1.97	Friable	Greenish
T_3	2	-	-	26.54	17.97	2.39	Friable	Greenish
T_4	3	-	-	26.47	18.32	2.58	Friable	Greenish
T_5	1	0.5	-	21.60	22.55	2.91	Compact	Greenish white
T_6	2	0.5	-	18.20	27.10	4.03	Compact	Greenish white
T_7	3	0.5	-	17.43	26.75	3.86	Compact	Greenish white
T_8	1	-	0.5	24.63	20.07	2.89	Compact	Greenish white
T_9	2	-	0.5	21.10	20.33	3.14	Compact	Greenish white
T_{10}	3	-	0.5	23.85	23.11	3.51	Compact	Greenish white
S.E(m) ±				1.3	0.9	0.4	-	-
CD (5%)				3.9	2.7	1.1	-	-

There is no significant change in morphology of capitula explant but later significant capitulum growth was observed with swelling and greening of explant. This could be the result of auxin-supplemented conditions causing the development of meristematic tissues in an immature capitulum segment (Mandal *et al.,* 2010; Rashmi *et al.,* 2018). In an attempt to induce organogenesis in gerbera, several cytokinin and auxin combinations have been tested (Murashige *et al.,* 1974). Cytokininauxin ratio plays critical rolein plant *in vitro* culture to enhance cell division, cell enlargement and cell plasticity. Thus, it is crucial for the *in vitro* culture's cell morphogenesis (Parvin *et al*., 2017). Similar outcomes on callogenesis were reported by by Patnaik and Beura (2008); Paduchuri *et al.* (2010); Bhatia *et al.* (2012); Sharma and Beuran (2021).

The callus developed from capitula explants were subcultured in callus emergence media containing various concentration of plant bio regulators in combination. After 60 days of inoculation of callus the results were recorded in Table 2.

The callus subculturing in callus emergence media experiment depicted in Table 2 revealed that MS media enhanced with BAP 3.0mg/l + IAA 0.5mg/l (T_7) callusprolification was earlier significantly in 09.74 days followed by T_{10} (MS media + BAP 3.0mg/l + NAA 0.5mg/l) and T_6 ((MS media + BAP 2.0mg/l + IAA 0.5mg/l) in 11.43 days and 11.52 days respectively. $T_{10}$and T_6 stood at par with T_7 also. However, T_2 (MS media + BAP 2.0mg/l) proliferated callus lately in 15.17 days. Treatment (T_7) MS media + BAP 3.0mg/l + IAA 0.5mg/l showed maximum days for callus bulking (20.26 days) which stood at par treatments T_5 ((MS media + BAP 1.0mg/l + IAA 0.5mg/l),T_6 (MS media + BAP 2.0mg/l + IAA 0.5mg/l) and T_{10} (MS media + BAP 3.0mg/l + NAA 0.5mg/l) in 18.40days, 18.48 days and 18.57days respectively. For callus spread T_7- MS media + BAP 3.0mg/l + IAA 0.5mg/l delivered maximum growth and spread (5.13cm^2) and stood at par with T_6 and T_{10} with 4.92cm^2 and 5.01 cm^2. However, T_2 (MS media + BAP 2.0mg/l) revealed minimum days to callus bulking (14.83 days) and lower callus spread 3.06cm^2). MS media supplied with different concentration of BAP in combination with IAA and NAA produced compact and greenish white callus excluding only MS supplied with only BAP during 1stsubculturing.Cytokininauxin ratio plays critical role in plant *in vitro* culture to enhance cell division, cell enlargement and cell plasticity. Thus, it is crucial for the *in vitro* culture's cell morphogenesis (Parvin *et al*., 2017). Similar outcomes on callogenesis were reported by Patnaik and Beura (2008); Paduchuri *et al.* (2010); Bhatia *et al.* (2012).

Table 2: Impact of plant bioregulators on callus proliferation, callus bulking, callus spread, nature of callus and colour of callus during 1st subculturing of callus from quarter capitulum explant of Gerbera cv. Alcatraz.

Basal Medium- MS				Subculturing duration- 30 Days				
Treatment No.	Treatment Details			Days to callus proliferation	Days to callus bulking	Callus spread (cm^2)	Nature of the callus	Colour of the callus
	BAP	IAA	NAA					
T_1	MS Media (Control)			-	-		-	-
T_2	1	-	-	15.17	14.83	3.06	Compact	Greenish
T_3	2	-	-	14.91	15.09	3.41	Compact	Greenish
T_4	3	-	-	13.64	16.36	3.20	Compact	Greenish
T_5	1	0.5	-	11.60	18.40	4.17	Compact	Greenish white
T_6	2	0.5	-	11.52	18.48	4.92	Compact	Greenish white
T_7	3	0.5	-	09.74	20.26	5.13	Compact	Greenish white
T_8	1	-	0.5	13.11	16.89	3.45	Compact	Greenish white
T_9	2	-	0.5	12.88	17.12	4.22	Compact	Greenish white
T_{10}	3	-	0.5	11.43	18.57	5.01	Compact	Greenish white
S.E(m) ±				0.6	0.7	0.2	-	-
CD (5%)				1.8	2.1	0.6	-	-

CONCLUSIONS

The experiment on callogenesis of Gerera cv. Alcatraz concluded that quarter of capitum can be used to produce callus in vitro which could produce healthy plantlets on large scale in less time. MS media enhanced with BAP 2.0mg/l and IAA 0.5mg/l resulted best for callus formation and development.

FUTURE SCOPE

This experiment on callogenesis of Gerbera hybrid cv. Alcatraz will facilitate large scale production of true-to-type healthy 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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