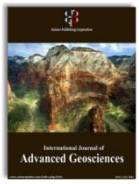
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Research paper



In vitro callus induction of gerbera from leaf explant

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Abstract

The experiment was designed to evaluate the effect of growth regulators on leaf explant of Gerbera for callus induction. Various kinds of plant growth regulators such as 6-Benzylaminopurine (BAP), α-Naphthalene acetic acid (NAA), 2, 4-Dichlorophenoxyacetic acid (2, 4-D), Indole-3-acetic acid (IAA) were used to initiate cultures. These were added to Murashige and Skoog medium in different combinations and concentrations. Leaf explants cultured on MS medium supplemented with BAP+ 2, 4-D+ IAA in T4 treatment & BAP+ 2,4-D in T5 treatment showed the best results for callus induction. On the other hand callus was induced early in the combination of BA+ 2,4-D + IAA hormone in T5, T9 & T8 treatment respectively. The rate of callus induction was very low in BA + NAA combinations but it was much earlier.

Keywords: *Callus, Gerbera, Growth Regulators, Leaf Explants, Culture.*

1. Introduction

Gerbera **(***Gerbera jamesonii* Bolus) belongs to the family Asteraceae is an herbaceous perennial flower crop, with long stalks and daisy-like flower, a native of South Africa. It was named in honor of the German botanist and naturalist Traugott Gerber. It has approximately 30 species in the wild, extending to South America, Africa and tropical Asia. Variety in color has made this flowering plant attractive for use in garden decorations and for cut flowers as it has a long vase life (Bose et al., 2003; Chung et al., 2005; Chauhan, 2004). Gerbera is very popular and is the fifth most widely used cut flower in the world after rose, carnation, chrysanthemum, and tulip. It has great potential for local as well as export market. Cultivation of flower is reported to give 3-5 times and 1.5-2.0 times more returns than obtained from rice and vegetable cultivation, respectively (Dadlani, 2003). The most commercial cultivars are propagated through vegetative means by multiplication through divisions of clumps; however, the multiplication by this method is too slow to be commercially viable as well as seedlings derived from this way is not good quality. 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. Callus induction is the first & very important step which leads to plant regeneration in large scale. Plant callus is a growing mass of unorganized plant parenchyma cell. Leaf explants are easy to sterilize than other explants & contamination rate is very low. This experiment was carried out to develop a protocol for in-vitro callus formation of gerbera for future conservation.

2. Materials and methods

The experiment was conducted in tissue culture laboratory of the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur from November 2014 to July 2015. Tender leaves of gerbera were collected from Horticultural nursery of BSMRAU, Gazipur. The leaves (4-6 mm) were washed with detergent **T**ween 20 for 4-5 min. and then sterilized with 0.1% $HgCl_2$ for 3-4 min and washed thoroughly with sterilized water to remove the chemicals. For callus induction, the explants were cultured on Murashige and Skoog **(**MS**)** medium supplemented with different concentrations and combinations of growth regulators as described below. The cultures without growth regulators served as control. The cultures were maintained at 25+2°C in dark. The data were recorded on days required to callus formation and percent explants produced callus. The Following combinations of growth regulators were used

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Combination 1

Treatment	BAP (ppm)	2,4-D (ppm)	IAA (ppm)
T_1	0	0	0
T_2	1	1.5	1.0
T_3	1	1.5	1.5
T_4	1	2.5	1.0
T_5	1	2.5	1.5
T_6	2	1.5	1.0
T_7	2	1.5	1.5
T_8	2	2.5	1.0
T_9	2	2.5	1.5

Combination 2

Treatment	BAP (ppm)	2,4-D (ppm)
T_1	0	0
T_2	0	1.5
T_3	0	2.5
T_4	1	0
T_5	1	1.5
T_6	1	2.5
T_7	2	0
T_8	2	1.5
T_9	2	2.5

Combination 3

Treatment	BA (ppm)	2,4-D (ppm)	IAA (ppm)
T_1	0	0	0
T_2	1	1.5	1.0
T_3	1	1.5	1.5
T_4	1	2.5	1.0
T_5	1	2.5	1.5
T_6	2	1.5	1.0
T_7	2	1.5	1.5
T_8	2	2.5	1.0
T_9	2	2.5	1.5

Combination 4

Treatment	BA (ppm)	NAA (ppm)
T_1	0	0
T_2	4	1
T_3	5	1
T_4	6	1
T_5	4	2
T_6	5	2
T_7	6	2

3. Results and discussion

3.1. BAP, 2, 4-D and IAA

The result of callus induction in response of different concentration of growth hormone (BAP, 2,4-D, IAA) are presented in Table **1**. Among the nine treatments, callus induction days ranges from 47-59.67and Percentages of callus initiation ranges from (49-86%). Higher days were required in treatment T_9 (59.5) and T_8 (59.5) and the lower were in T_5 (47) and T_9 (50.8). Percentages of callus formation are higher in T_4 (85.5) and lower in T_5 (49.65) and T_6 (50.8). Paduchuri et al. (2010) reported that highly efficient and reproducible callus induction protocol for *Gerbera jamesonii* has been developed using leaves as explant source. The optimal callus was developed on Murashige and Skoog (MS) basal medium supplemented with BAP 2mg/L + Kinetin 1 mg/L + NAA 2mg/L + 2,4- D 2.5mg/L.

Table 1: Effect of Different Concentration of BAP, 2, 4-D and IAA on Callus Induction

Treatment	Callus initiation (Days)	Callus initiation (%)
T_1	-	-
T_2	59	75.25
T_3	55	65
T_4	50.8	85.5
T_5	47	49.65
T_6	50	50.8
T_7	57.5	60.57
T_8	59.5	60
T_9	59.67	70.25
%CV	9.14%	18.80%

3.2. BAP, 2, 4-D

The number of days required for callus induction ranges from (33-39) days and induction percentage ranges from 40-100 **in (**Table **2)**. Maximum number of days were required in T_4 (39) followed by T_6 (35.75) and minimum were in T_8 (33.67). Percentages of callus initiation were higher in T_5 (86) and lower in T_9 (46.68). Hasbullah et al., (2008) observed that leaf explants cultured on MS medium supplemented with 1.0 mg L^{-1} BAP and 2.0 mg L^{-1} 2, 4-D showed the best results for callus induction.

Table 2: Effect of Different Concentration of BAP, 2, 4-D on Callus Induction

Treatment	Callus initiation (Days)	Callus initiation (%)
T_1	-	-
T_2	-	-
T_3	-	-
T_4	39	61
T_5	34.8	86
T_6	35.75	64.67
T_7	34.5	65.1
T_8	33.67	59.72
T_9	35	46.68
%CV	5.25%	19.91%

3.3. BA, 2, 4-D and IAA

Among various treatment (Table 3), the number of days required for callus induction ranges from (28.75-394.6) days and percentages of callus initiation ranges from (22.22-55.56) %. Explant treated with T_2 requires maximum days (34.6 days) for callus induction and minimum were in T_3 (28.35 days). Callus induction percent were higher in T_2 (55.56) and T_7 (55.56) respectively and it was lower in T_9 (33.35 %). Satyavani et al. (2011) reported that maximum number of callus are induced from stem explants on MS medium enriched with 0.5 mg L^{-1} IAA, 2,4-D and 1 ppm of 6-BA in case of bitter Apple Tissue culture.

Table 3: Effect of Different Concentration of BA, 2, 4-D and IAA on Callus Induction

Treatment	Callus initiation (Days)	Callus initiation (%)
T_1	-	-
T_2	34.6	55.56
T_3	28.75	44.44
T_4	34.25	44.44
T_5	29.25	44.44
T_6	-	-
T_7	32.2	55.56
T_8	-	-
T_9	30.5	33.35
CV%	7.91%	18.06%

3.4. BA and NAA

The rate of callus induction in BA and NAA was very low. Among the seven treatments (Table **4**), only T_3 andT_4 produces callus. Day's requirement of callus induction is 24 and 25.5 respectively and percentage was only 33.33 and 42.44 respectively. Kumar and Kanwar (2006) observed callus induction on leaf and petal explants on MS medium supplemented with 1, 1.5 and 2 mg/dm3 2,4-D. BA and kinetin failed to induce callus on leaf and petal explants in cut flower gerbera.

Table 4: Effect of Different Concentration of BA, NAA on Callus Induction

0Treatment	Callus initiation (Days)	Callus initiation (%)
T_1	-	-
T_2	-	-
T_3	24	33.33
T_4	25.5	42.44
T_5	-	-
T_6	-	-
T_7	-	-
CV%	4.28%	17%

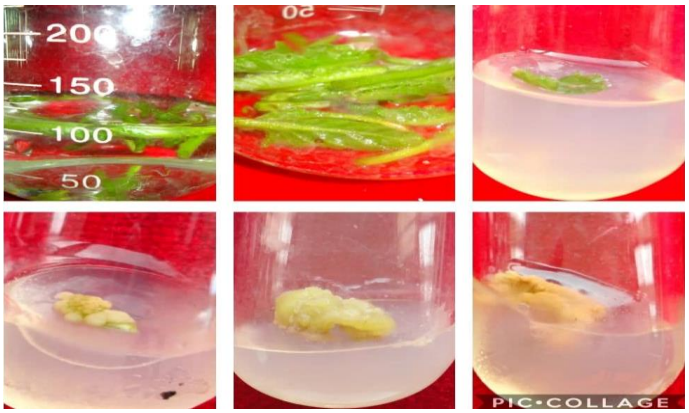


Fig. 1: Pictorial View of Callus Formation from Leaf Explants.

4. Conclusion

Different combinations of BA (4-6 ppm), BAP (0-2ppm), 2, 4-D (0-2.5 ppm), IAA (0-1.5ppm) and NAA (1-2 ppm) were used for in-vitro callus formation of gerbera from leaf explants. The highest (86%) callus initiation was started from BAP (1 ppm) + 2,4-D (1.5 ppm)

within 34.8 days compare to BAP (1 ppm) + 2,4-D (2.5 ppm) + IAA (1 ppm) within 50.8 days. Although lower percent callus initiation started from BA (6 ppm) + NAA (1 ppm) but it was much earlier (25.5 days).

5. Conflict of interest statement

Authors do not have any possible conflicts of interest.

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