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STUDIES ON CONSERVATION OF GLADIOLUS PLANTS AND CORMEL FORMATION BY USING TISSUE CULTURE TECHNIQUE

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ABSTRACT

The experimental trail was consummated in Plant Tissue Culture Laboratory at El-Zohria Botanical Garden, Horticulture Research Institute, Agriculture Research Center, during 2008 – 2010 years. It intended to find out the most suitable treatments for propagation of *Gladiolus primulinus* var Atom by using tissue culture technique. Buds from corms of *Gladiolus primulinus* were effectively surface sterilized with a mixture of Clorox (commercial bleach) and mercuric chloride (Hg_2Cl) at 40 % Clorox plus 2.0 g/L Hg_2Cl. Explants were transferred to a solidified MS medium containing 1.0 mg /l NAA for establishment stage. The second stage, shoots were transferred to MS medium containing of 2.0 mg/l BA which was used in the current study to improve number of shoots production. Sucrose and MS strength was used to try *in vitro* producing of higher number of cormels. Sourbitol or manitol and MS medium were more appropriate for germplasm from shoots conservation. The addition of sucrose at 90 g/l to 3/4 MS-strength medium enhanced number of cormels/shoot. The same and the best results of medium for rooting stage was 3/4 MS + 2 mg/l IBA.

Key words: Micropropagation, *In vitro*, Tissue culture, Gladiolus, Shoot tips, cormel and germplasm.

INTRODUCTION

Gladiolus (Family Iridaceae) is considered one of the most important flowering bulbs grown in Egypt. The flowers are very popular for local use and also demanded for exportation. The

production of flowers and corms faces yield decline yearly under local condition. So, the solution of this problem is very necessary in this concern. Accordingly, the work embodied in this paper was an attempt to solve this problem by using tissue culture technique in propagation of such plant.

Stolon tips and root explants were used for the *in vitro* propagation of gladiolus. Explants were washed thoroughly under running tap water, and surface sterilized with 0.1% HgCl2 for 10 min. Corms were rinsed in a distilled water. Shoot tips and axillary buds were excised and corms were cut into segments. Shoot tips, axillary buds and corm segments were surface sterilized with 80 % (v/v) sodium hypochlorite for 15 min. They were then rinsed three times in a sterile distilled water **(Mohamed-Yasseen, 2000)**.

MS medium plus NAA with or without kin were capable of both callus formation and root development in gladiolus. MS free-hormone was considered better for callus formation on corm segments. MS plus 0.5 mg/l BAP resulted in plant regeneration from callus. It was found that with the increase of sucrose concentrations, a noticeable gradual increase was recorded in all growth parameters studied including number of cormels/shoot. Sucrose at 120g/l was used for *in vitro* cormel formation of gladiolus which would give higher number of cormels **(Lasheen *et al*., 2001).**

Slices of cormel sprouts of *Gladiolus primulinus* cv. Golden Wave were cultured in MS with 4.0 mg/l NAA, 2.0 mg/l BAP or 1.0 mg/l 2,4-D to induce callus initiation. Highest number of shoots was regenerated in MS with 2.0 mg/l BAP from one-month-old callus which was initiated in the medium with NAA **(Pinaki and Shyamal, 2002)**. *Gladiolus grandiflorus* were established using intact cormels in MS medium. Shoot proliferation was maximum in MS medium fortified with 4 mg/l BA + 0.5 mg/l NAA. Low concentrations of BA (1 or 2 mg/l) were suitable for further shoot multiplication **(Priyakumari and Sheela, 2005)**. Cormel and meristem explants of Gladiolus showed maximum multiplication on MS medium containing 1mg/l BAP. Thus, cormel explants proved to be a better source for shoot multiplication as compared to meristem **(Faheem *et al*., 2007)**. An *In vitro* propagation method was developed for *Gladiolus anatolicus* (Boiss.) Stapf using corm sections. The highest rate of callus formation occured in MS medium containing 8.5 mg/l NAA.

The highest number of shoots was obtained in MS medium containing 0.2 mg/l BA and 2 mg/l NAA. Corm formation in the base of shoots was observed in the medium with 0.1/l mg BA. Additionally, 5-6 cormel per shoot occured during subculturing **(Yelda and Beng, 2007)**.

The shoots produced roots in half strength of MS with 2.0 mg/l IBA and 6 % sucrose. Rooted shoots produced cormels at the base of shoots in the same nutrient medium. About 80 - 90 % cormels germinated when sown in the field and 20 % of them blossomed in the first year **(Pinaki and Shyamal, 2002)**. The treatment of 2 mg /l IBA produced earliest rooting and longest roots of *Gladiolus grandiflorus* **(Priyakumari and Sheela, 2005)**. Rooting of *in vitro* grown shoots was achieved on MS medium supplemented with 0.5mg/l NAA or 2mg/l IBA of Gladiolus plants **(Faheem *et al*., 2007)**.

The aim of this study was to investigate the best protocol for *in vitro* propagation of *Gladiolus primulinus* for commercial production, producing cormels and conservation plants. Accordingly, the following steps were studied: The effect of sterilization treatments, effect of NAA on establishment stage, the effect of BA on multiplication stage, the effect of IBA and MS strength on rooting behavior, effect of sucrose and MS strength on producing cormels and the effect of sourbitol or manitol on germplasm shoots conservation.

MATERIALS AND METHODS

This study was carried out in the Laboratory of Tissue Culture, Zohria Botanical Garden, Cairo, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture. The experiments were conducted during the period from 2008 to 2010. The objective of this study was to investigate the most suitable treatments for micropropagation of *Gladiolus*. The mother plants were imported from Holland. The parts used as explants were corm buds.

Number of measurements were taken on *Gladiolus primulinus* var. Atom i.e., number of survived explants, number of leaves, shoot length (cm), number of shoots, number of roots, root length (cm), number of cormels per shoot, cormel height (mm), cormel width (mm) and number of survived plants after six months.

Growth regulators of NAA were used during establishment stage, cytokinin, i.e. BA were used for multiplication stage, IBA and MS

strengths were used during rooting stage and sucrose, sorbitol or manitol was used during *in vitro* cormel formation stage and conservation of plants.

1-Experiment 1: Effect of some sterilization treatments on contamination of explants:

The aim of this experiment was to study the effect of some sterilization treatments, i.e. Clorox at 20, 30, 40 and 50 % with mercuric chloride (Hg_2Cl) solution at concentrations of 1, 2, 3 and 4 g/l on survival of *Gladiolus* explants *in vitro*.

Buds of *Gladiolus* were excised from the corms, initially 1-2 cm in length then were washed by a soapy water for 5 minutes followed by 1 hour under a running tap water. The explants were sterilized by immersion in mercuric chloride (Hg_2Cl) solution at the rate of 1, 2, 3 and 4 g/l containing 3-5 drops of Tween-20 for 5 minutes, followed by rinsing three times in a sterile distilled water, then immersed in a commercial Clorox solution at 20, 30, 40 and 50 % containing 3-5 drops of Tween-20 for 20 minutes. Finally, explants were washed 5 times with a sterile distilled water. Each sterilized explant was cultured separately under sterile conditions in 100 ml jar.

For surface sterilization of explants, 16 treatments were initiated, each treatment consisted of 10 jars.

2. Experiment 2: Effect of NAA and kin on explant establishment:

Murashige and Skoog (MS) medium **(Murashige and Skoog, 1962)** was used as culture medium.

For establishment stage, 4 treatments were studied, i.e. NAA at 0.0, 0.5, 1.0 or 1.5 mg/l.

3. Experiment 3: Effect of BA on multiplication stage:

For multiplication stage, 18 treatments were studied using BA at 0.0, 0.5, 1.0, 1.5, 2.0 or 2.5 mg/l and three subcultures.

4. Experiment 4: Effect of sucrose on *in vitro* cormel formation:

For cormel formation, 15 treatments were studied using sucrose at 10, 30, 50, 70 or 90 g/l and three MS-strength; (full, ½ MS or ¼ MS) were used.

5. Experiment 5: Effect of IBA and MS-medium strength on rooting growth:

In rooting stage, 15 treatments including combinations of 5 IBA levels (0.0, 0.5, 1.0, 1.5 or 2.0 mg/l) and three MS-strength; (full, ½ MS or ¼ MS) were used.

6. Experiment 6: Effect of sorbitol and manitol on conservation of plants:

For conservation of plants, 10 treatments were studied using sorbitol and manitol at 0.1, 0.3, 0.5, 0.7 or 0.9 mol/l and this was prolonged for six months.

7. Experimental design and statistical analysis:

A complete randomized design was employed in all of the experiments. Analysis of variance was used to show the least statistical differences between treatments using the L.S.D at 5% probability level (**Snedecor and Cochran, 1989**).

RESULTS AND DISCUSSION

1. Effect of different concentrations of sodium hypochlorite and mercuric chloride for surface sterilization on explant survival:

Results registered in Table (1) indicate that the effect of clorox (sodium hypochlorite) as commercial bleach was positive for surface sterilization of Gladiolus buds. Number of uncontaminated was explants increased with the increase of clorox concentration, when mercuric chloride was present.

For clorox effect, number of uncontaminated explants was increased in survival of explants with the increase of concentration from clorox. But, Clorox at 40% produced 5.8 explants when compared to 0.03 at control on explants. There were non significant differences between 40 and 50 % of Clorox.

Regarding, the use of mercuric chloride (Hg_2Cl) on surface sterilization of explants, it was augmented when clorox was supplemented to the solution of surface sterilization.

The interactions between clorox and mercuric chloride were significant with the highest value of disinfected explants (0.9) when the mixture of 40 % clorox and 4 g/l mercuric chloride was used.

Results obtained here are in harmony with those obtained elsewhere when HgCl2 were used on its own **(Mohamed-Yasseen, 2000).**

2. Effect of different concentrations of NAA on explants establishment:

For the establishment stage (Table, 2), it was found that the best medium treatment was that which contained 1.5 mg/l NAA in both growth parameters investigated. However, there were no significant

differences in between 1.0 mg/l and 1.5 mg/l NAA treatments for shoot length.

After four weeks in 1.5 mg/l NAA the mean shoot length was 1.23 cm and number of leaves was 5.25 which was significant when compared with control only.

For the interaction, 1.5 mg/l NAA at the four weeks gave the highest values of shoot length and number of leaves. However, the highest value of shoot length (1.5 cm) the number of leaves (8) was at 1.0 mg/L NAA after four weeks.

Results here are in agreement with the reports elsewhere with using MS medium and for establishment stage of Gladiolus **(Pinaki and Shyamal, 2002)**.

Table (1): Effect of different concentrations of Clorox and mercuric chloride on surface sterilization explants of *Gladiolus primulinus*

Clorox %	Hg_2Cl (g/l)				
	1	2	3	4	Mean (A)
20	0.00	0.00	0.00	0.10	0.03
30	0.00	0.20	0.50	0.50	0.30
40	0.30	0.50	0.60	0.90	0.58
50	0.60	0.60	0.70	0.70	0.65
Mean (B)	0.23	0.33	0.45	0.55	
LSD at 5% Clorox (A)		0.13			
Hg_2Cl (B)		0.13			
AxB		0.27			

Table (2): Effect of different concentrations of NAA on explants establishment of *Gladiolus primulinus.*

NAA (mg/l)	shoot length (cm)					No. of leaves				
	No. of weeks				Mean (A)	No. of weeks				Mean (A)
	1	2	3	4		1	2	3	4	
0.0	0.0	0.0	0.5	0.5	0.25	0.0	0.0	1.0	2.0	0.75
0.5	0.0	0.0	0.5	1.0	0.37	0.0	0.0	2.0	4.0	1.50
1.0	0.0	0.5	1.0	1.5	0.75	0.0	1.0	4.0	8.0	3.00
1.5	0.0	0.5	1.0	1.5	0.75	0.0	2.0	5.0	7.0	3.75
Mean (B)	0.00	0.25	0.75	1.23		0.00	0.75	3.00	5.25	
LSD at 5% NAA (A)			0.47					0.42		
Weeks (B)			0.47					0.42		
AxB			0.93					0.83		

3. Effect of different concentrations of BA on multiplication stage:

Data recorded in Table (3) demonstrated that number of shoots was increased by increasing the concentration of BA when compared with the control. However, all concentrations of BA used resulted significantly shorter shoot length when compared to control. Following the same line, number of leaves was decreased as a result of BA concentrations.

Regarding subculturing effect, it was found that all studied parameters were positively increased with the subculture progress. The mean shoot length was fixed on 5.33 cm during the three subcultures of multiplication stage.

Raising the concentration of BA resulted in a significant increase in number of shoots (32 shoots at 2 mg/l BA after three subcultures). Meanwhile, the highest value of shoot length (11 cm) was formed at control level, whereas, the number of leaves (7) was recorded at control level.

Table (3): Effect of different concentrations of BA on multiplication stage of *Gladiolus primulinus*.

BA (mg/l)	Number of shoots				Shoot length (cm)				Number of leaves			
	Subculture				Subculture				Subculture			
	1	2	3	Mean (A)	1	2	3	Mean (A)	1	2	3	Mean (A)
0.0	2.0	3.0	4.0	3.00	7.0	9.0	11.0	9.00	3.0	5.0	7.0	5.00
0.5	3.0	7.0	18.0	9.33	7.0	8.0	8.0	7.67	3.0	4.0	5.0	4.00
1.0	7.0	15.0	21.0	14.33	6.0	6.0	5.0	5.67	4.0	5.0	5.0	4.67
1.5	5.0	12.0	24.0	13.67	5.0	4.0	4.0	4.33	4.0	4.0	3.0	3.67
2.0	7.0	18.0	32.0	19.00	4.0	3.0	3.0	3.33	3.0	3.0	2.0	2.67
2.5	6.0	11.0	18.0	10.67	3.0	2.0	1.0	2.00	3.0	2.0	2.0	2.33
Mean (B)	5.00	10.50	19.50		5.33	5.33	5.33		3.33	3.83	4.00	
LSD at 5% BA (A)			1.62				0.73				0.61	
Subculture (B)			1.15				0.52				0.43	
AxB			2.81				1.27				1.06	

3.5. Effect of different concentrations of sucrose and MS-strength on cormel formation:

Data demonstrated in Table (4) and plate (1) indicate that the highest number of cormels/shoot (0.73) was formed at 90 g/l sucrose. The same trend of results was also observed in cormel height and cormel width at 90 g/l sucrose (9.20 and 9.73 mm, respectively).

For MS-strength, maximum number of cormels/shoot, cormel height and cormel width were obtained at 3/4 MS-strength, in a respect order. It was found that the best medium was that which contained 3/4 MS-strength for every growth parameter investigated.

Also, the same concentrations of 90 g/l sucrose and 3/4 MS-strength showed the highest value of number of cormels/shoot, cormel height and cormel width (1.0, 12.0 and 12.6 mm, respectively).

Table (4): Effect of different concentrations of sucrose and MS-strength on cormel formation of *Gladiolus primulinus*.

MS-strength / Socrose (g/l)	Number of cormels/shoot				Cormel height (mm)				Cormel width (mm)			
	MS	3/4MS	1/2MS	Mean (A)	MS	3/4MS	1/2MS	Mean (A)	MS	3/4MS	1/2MS	Mean (A)
10	0.0	0.4	0.2	0.20	0.0	6.2	4.2	3.47	0.0	6.8	4.6	3.80
30	0.2	0.4	0.2	0.27	3.4	8.4	5.0	5.60	3.4	9.0	5.4	5.93
50	0.2	0.6	0.4	0.40	4.6	9.2	5.8	6.53	4.8	9.8	6.0	6.87
70	0.4	0.8	0.6	0.60	6.2	10.4	7.0	7.87	6.4	10.8	7.4	8.20
90	0.6	1.0	0.6	0.73	7.4	12.0	8.2	9.20	7.8	12.6	8.8	9.73
Mean (B)	0.28	0.64	0.40		4.32	9.24	6.04		4.48	9.80	6.44	
LSD at 5% Sucrose (A)			0.09				0.11				0.11	
MS-strength (B)			0.07				0.08				0.09	
AxB			0.15				0.18				0.19	

Plalte 1: Micropropagation of *Gladiolus primulinus* var. Atom and cormel formation.

6. Effect of different concentrations of IBA and MS-strength on *in vitro* rooting:

Results presented in Table (5) demonstrate that 2 mg/l IBA affected number of roots and root length (8 and 6 cm, respectively). IBA induced the formation of roots on Gladiolus shoots when compared with the remaining treatments.

The MS medium at three quarter MS strength was superior over other MS strengths for number of formed roots (6.96) and root length (5.60 cm).

The interaction between the different concentrations of IBA and MS strength treatments showed that the largest number of roots and root length was found when the shoots were cultured on three quarter MS-medium and 2 mg/l IBA (11.2 and 9.5 cm, respectively).

This later reported result seemed to be in harmony with the results obtained here with on Gladiolus, which on the whole seems at the end to favor IBA for the rooting stage of *Gladiolus* **(Priyakumari and Sheela, 2005 and Faheem *et al*., 2007)**.

Table (5): Effect of different concentrations of IBA and MS-strength on *in vitro* rooting of *Gladiolus primulinus*.

MS-strength / IBA(mg/l)	Number of roots				Root length (cm)			
	MS	**3/4MS**	**1/2MS**	Mean (A)	**MS**	**3/4MS**	**1/2MS**	Mean (A)
0.0	0.0	2.0	0.8	0.93	0.0	1.5	0.5	0.67
0.5	1.0	4.4	2.6	2.67	0.5	2.5	1.0	1.33
1.0	3.4	6.8	5.2	5.13	1.5	6.0	3.0	3.50
1.5	4.2	10.4	7.4	7.33	3.0	8.5	4.5	5.33
2.0	4.8	11.2	8.0	8.00	3.5	9.5	5.0	6.00
Mean (B)	2.68	6.96	4.80		1.70	5.60	2.80	
LSD at 5% IBA (A)			0.11				0.26	
MS-strength (B)			0.09				0.20	
AxB			0.19				0.45	

7. Effect of sorbitol and manitol on conservation of plants:

In plants the growing points that contain undifferentiated cells are called meristems. The meristematic cells can be tissue cultured to produce tissues, organs and whole plants. Tissue culture produced tissues, organs and plantlets, that constitute germplasm, can be

cryopreserved regenerated at a later date when needed using tissue culture protocols. This facility provides for the conservation of plant germplasm in germplasm banks, variously called *in vitro* banks or tissue banks or gene banks.

Data recorded in Table (6) demonstrate that number of survived shoots was increased by increasing the concentration of sorbitol or manitol when compared with other treatments. So in general, for number of survived shoots, sorbitol concentrations utilized were better than manitol ones.

Number of survived shoots was progressively increased from 0.57 at 0.1 mol sorbitol to 0.87 at 0.5 mol sorbitol. Meanwhile, there were significant differences between sorbitol and manitol when compared with all concentrations.

Table (6): Effect of different concentrations of sorbitol and manitol on conservation of plants.

Concentration (mol)		No. of months							
		1	2	3	4	5	6	Mean A	Mean B
Sorbitol	٠,١	0.8	0.8	0.6	0.6	0.4	0.2		0.57
	0.3	1.0	0.8	0.8	0.6	0.4	0.2		0.63
	0.5	1.0	1.0	1.0	0.8	0.8	0.6	0.68	0.87
	0.7	1.0	1.0	0.8	0.8	0.6	0.4		0.77
	0.9	0.8	0.8	0.6	0.6	0.4	0.2		0.57
Manitol	٠,١	0.8	0.8	0.6	0.6	0.4	0.2		0.57
	0.3	0.8	0.8	0.8	0.6	0.6	0.2		0.63
	0.5	1.0	0.8	0.8	0.8	0.6	0.4	0.65	0.73
	0.7	1.0	0.8	0.8	0.6	0.6	0.4		0.70
	0.9	0.8	0.8	0.8	0.6	0.4	0.2		0.60
Mean C		0.90	0.84	0.76	0.66	0.52	0.30		

LSD at 5%		
	(A)	0.0694
	(B)	0.1097
	AB	0.1551
	(C)	0.1202
	AC	0.1699
	BC	0.2687
	ABC	0.3800

As for time, on the six month of culture on media shoots death was increased in all treatments. Decreasing number of survived shoots was positively correlated with prevailing in the medium.

There was a steady decrease in number of survived shoots with prolonging steadying in the medium. After the six month, the greatest number of survived shoots (0.6) was obtained when compared to other treatments.

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دراسات على حفظ نباتات الجلاديولس وتكوين الكريمات باستخدام تكنيك زراعة الأنسجة

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أجريت الدراسة خلال الفترة من سنة ٢٠٠٨ - ٢٠١٠ فى معمل زراعة الأنسجة بحديقة الزهرية التابعة لمعهد بحوث البساتين-مركز البحوث الزراعية-وزارة الزراعة-جمهورية مصر العربية.

وكان هدف الدراسة هو تحديد أنسب المعاملات لإكثار نبات الجلاديولس باستخدام تكنيك زراعة الأنسجة.

ويمكن تلخيص أهم النتائج التى تم التوصل إليها فى الأتى:

أمكن إكثار نبات الجلاديولس بواسطة براعم الكورمات كمنفصلات نباتية حيث تم تعقيمها بواسطة كلوركس بتركيز ٢٠و ٣٠و ٤٠ و٥٠ ٪ لمدة ٢٠ دقيقة وكذلك كلوريد الزئبقيق بتركيز ١، ٢، ٣، ٤ جم/لتر لمدة ٣ دقائق. بالإضافة إلى التفاعلات بينهما.

وقد تبين أن أفضل تركيز من الكلوركس للحصول على أعلى نسبة بقاء للنباتات وأقل نسبة تلوث هو ٤٠ ٪ كلوركس بالإضافة إلى ٤ جم/لتر كلوريد الزئبقيق.

وفى مرحلة التأسيس أستخدمت بيئة موراشيجى وسكوج والمضافة إليها ١,٠ مجم/لتر نفثالين حمض الخليك.

أما فى مرحلة التضاعف فقد أستخدمت بيئة موراشيجى وسكوج المضاف إليها ٢ مجم/لترمن البنزيل أدينين وذلك خلال ٣ نقلات متتالية من حيث عدد الخلف.

وفى مرحلة تكوين الكوريمات فقد أستخدمت بيئة موراشيجى وسكوج بتركيز ٤/٣ المضاف إليها ٩٠ جم/لتر سكروز حيث أدى ذلك إلى زيادة عدد الكوريمات وطولها وقطرها.

أما فى مرحلة التجذير فقد أستخدمت بيئة موراشيجى وسكوج بتركيز ٤/٣ قوة والمضاف إليهما إندول حمض البيوتريك بتركيز ٢ مجم/لتر.

وفى مرحلة حفظ وتخزين نبات الجلاديولس وجد أن إستخدم تركيز ٠,٥ مول من السوربيتول او المانيتول كان الأفضل من حيث نسبة البقاء بعد ستة أشهر (٦٠ ٪).