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STUDIES ON *Gerbera jamesonii Bolus* CV. AURANTIACA, UNDER MICROPROPAGATION CONDITION

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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 2007 and 2008 years. It was intended to find out the most suitable treatments for propagation of *Gerbera jamesonii* Bolus cv Aurantiaca by using the tissue culture technique. So, the study was done using explants from juvenile leaves and seeds of the plant. The results emphasized that using 30% Clorox for 15 min and 30% Clorox for 10-20 min, respectively for sterilization of explants gave the best result of (100%) survival and free of contamination of leaf and seeds explants. BA at 0.5 mg/l and 1.0 or 2.0 mg/l NAA were the best concentrations for callus formation from leaves. Furthermore, the use of BA on plantlet formation of seeds was not significant when BA was used at either 0.0 or 0.5 mg/l with the different concentrations of NAA and 1.0 mg/l BA plus either 2.0 or 4.0 mg/l NAA. Leaves explant was better than seeds for number of shoots when cultured on the multiplication medium. MS medium plus 1.0 mg/l Kin and 0.5 mg/l NAA was mor the most appropriate for shoot formation from leafs callus. MS medium plus 2.0 mg/l Kin was favoured for number of shoot from seeds. The shoots of *Gerbera jamesonii* Bolus cv Aurantiaca were successfully rooted when cultured in MS medium supplemented with 1.5 mg/l IBA. Plantlets after root development exhibited 100% survival in plastic pots filled with peat moss and sand at a ratio of 1:1, 2:1 and 3:1 (by volume).

Keywords: Micropropagation, *In vitro*, Tissue culture, gerbera, Callus, Shoot tips.

Abbreviations: MS = Murashige & Skoog medium, KIN= Kinetin NAA = Naphtaleneacetic acid, BA = BAP = 6-benzyladenine = 6-benzylaminopurine, IAA = Indolacetic acid, IBA = = Indolbutyric acid

INTRODUCTION

Gerbera was named in refer to Trang Gerber, a German naturalist who travelled in Russia. Gerbera is belonged to family Compositae as an ornamental plant (Bailey and Bailey, 1960).

Moreover, only juvenile leaf explants regenerated in the presence of BA and NAA. In order to enhance regeneration from mature Gerbera leaf explants, the effect of inoculation with *A. tumefaciens* strain 82139 was investigated. This strain induced shooty tumours, and this capacity was related to high levels of zeatin riboside as revealed by hormone quantification (Reynoird *et al*, 2000).

Organogenic cultures were established from immature flower heads of *Gerbera jamesonii* on modified MS medium supplemented with IAA and BA (Mandal, *et al*, 2002). An efficient *in vitro* method of propagation of gerbera (*G. jamesonii* cv. AV 101) was developed. Shoot tips were cultured on MS

medium containing 30 g/litre sucrose and 8 g/litre agar (Aswath and Choudhary, 2002a).

The optimum callus was developed on MS basal medium supplemented with 0.4 mg/l BAP, 4.0 mg/l NAA and 3% (w/v) sucrose (Aswath and Choudhary, 2002b). Regeneration of adventitious shoots from leaf and petiole pieces of *Gerbera. jamesonii* has been obtained on MS medium supplemented with different concentrations of auxins and cytokinins. About 75-77% of the calls from both types of the explants produced 12-15 shoots per callus with 3 mg/l BAP or BA. Auxins and kinetin, separately failed to produce shoots. The shoots regenerated on the callus induction medium (Kumar *et al*, 2004).

Leaf bits excised from gerbera (*Gerbera jamesonii* cv. Mummut) plants 25-30 or 45-50 days old were cultured in MS medium**.** The combination of NAA and BA was more effective than either NAA or BA for enhancing callus formation, and this effect increased with increasing concentrations of both regulators. Callus formation was greater (80%) and earlier (20 days) in 25- to 30-day-old plants cultured in the medium containing 2.0 mg NAA + 0.75 mg BA/litre (Prasanth and Sekar, 2004).

A protocol was developed for the *in vitro* culture of gerbera cultivars Kozak and Gold Disk. Shoot tip explants were cultured on MS medium supplemented with 0.5-3 mg/l BA (Thakur *et al*, 2004). An efficient protocol was developed for large-scale propagation of *Gerbera jamesonii* using young capitulum as explants. MS medium supplemented with BAP at 7 mg/l in combination with 0.1 mg/l IAA was the most effective on initiating shoots, when placed in continuous dark after 30 days in culture (Tui *et al*, 2005).

Multiplication stage:

Gerbera jamesonii cultivars multiplication on BA media is not recommended. Permanent high K level must also be avoided. It must be decreased gradually during a long-term culture. After the eighth or ninth subculture the shoots are to be transferred to the resting medium with 0.1 mg/l K and 1.0 mg/l IAA (Vardja and Vardja 2001). The highest rate of *Gerbera jamesonii* of shoot proliferation was recorded from MS medium supplemented with BAP at 1.5 mg/l (Aswath and Choudhary, 2001). The effect of Kin or BAP in half MS medium suppressed shoot proliferation, but the suppression was more pronounced with BAP. The optimum pH for the medium was 5.7-6.7 (Aswath and Choudhary, 2002a).

Proliferated mass of shoots were obtained from immature capitula of Gerbera cv. Atella on MS medium supplemented with BA. Increasing sucrose level up to 3% increased the multiplication rate and improved the vigour. Above 3% sucrose level, shoot multiplication and growth were declined (Modh *et al*, 2002). Shoot multiplication of *Gerbera jamesonii* was first initiated in the half-strength medium. In the full-strength medium, though shoot multiplication started relatively late, the number, length and weight of shoots were better. Results also show that increasing the IAA concentration resulted in increasing shoot number and decreased length and weight of shoots (Aswath *et al*, 2002).

Shoot multiplication was early on half strength MS medium, while the full strength MS medium produced a higher number of shoots with better

shoot length and shoot weight. GJ-1 and GJ-3 performed best under full-strength MS medium with IAA at 0.2 mg/l + BAP at 1 mg/l or kin at 5 mg/l. GJ-2 performed best in the treatment combination of IAA at 0.1 mg/l + BAP at 1 mg/l or 5 mg/l kin (Aswath *et al*, 2003). The response of shoots to varying concentrations of BAP in both media was studied and finally it was noted that optimum requirement of BAP in the medium was 1 mg/l for gerbera to get maximum number of shoots per explant. Out of these two media used, MS medium proved to be superior than the B5 medium (Chikhale *et al*, 2004).

MS medium supplemented with BAP at 7 mg/l in combination with 0.1 mg/l IAA was the most effective on multiplication of shoots (10 per explant). By repeated subculturing of the capitulum explant, a high frequency of shoot multiplication was established (Tui *et al*, 2005).

Rooting stage:

The regenerated shoots of *Gerbera jamesonii* were transferred to rooting media containing different IBA concentrations. The best results were obtained on MS medium supplemented with 1.75 mg IBA/l (Aswath and Choudhary, 2001). Root formation was 100% on half MS medium with 2,4-D at 0.5-2.0 mg/l or NAA at 0.5-1 mg/l (Aswath and Choudhary, 2002a). *Gerbera jamesonii* shoots were rooted on growth regulator-free medium (Mandal *et al*, 2002).

For inducing rooting on *in vitro* shoots, only IAA was effective and no root induction was observed with IBA or NAA treatments (Modh *et al*, 2002). After 4 weeks of rooting on a medium with NAA at 2 mg/l, gerbera shoots were rooted from this medium (Aswath *et al*, 2003). The regenerated shoots of Gerbera, multiplied with 1 mg/l BAP, were rooted on MS medium containing 1 mg/l BAP + 0.1 mg/l IAA (Kumar *et al*, 2004).

Rooting of *in vitro* shoots was achieved on MS medium with 0.5 mg/l IAA (Purnima and Kothari, 2004). The regenerated shoots were then transferred on a rooting MS medium supplemented with 0.2-1 mg IAA/l (Thakur *et al*, 2004). One-hundred percent rooting was achieved in MS medium supplemented with 1.5 mg/l IAA in combination with 0.5 mg/l IBA (Tui *et al*, 2005).

Acclimatization stage:

Plants grown in peat + coco dust had the highest dry weights of leaves and roots. There were non significant differences in the fresh and dry weights of leaves and roots due to the concentration of nutrient solution (Nowak and Gabryszewska, 2001). Plantlets after root development exhibited 100% survival in plastic pots filled with coco peat, red soil and sand at a ratio of 3:1:1(Aswath and Choudhary, 2002a). Regenerated plantlets were transferred to soil where they grew normally with a survival rate of 95% (Aswath and Choudhary, 2002b).

The micropropagated plants were removed from the medium, transferred to soil, acclimatized for 3 weeks and subsequently cultured in the greenhouse with a transplantation success rate of 95% (Aswath *et al*, 2003).

The aim of this studying was to investigate the best protocol for *in vitro* propagation of *Gerbera jamesonii* Bolus cv Aurantiaca for commercial production. This was made by study the following steps: The effect of sterilization treatments, effect of growth regulators IBA and NAA on explants,

effect of explants on establishment stage, the effect of Kin and NAA on multiplication stage, the effect of IBA and NAA on rooting behavior, effect of peatmoss and sand for acclimatization stage.

MATERIALS AND METHODS

2.1. Location and duration:

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 carried out during the period from 2007 to 2008. The objective of this study was to investigate the most suitable treatments for micropropagation of *Gerbera jamesonii* Bolus cv Aurantiaca.

2.2. Plant material:

The mother plants were grown naturally at the open field condition at Zohria Botanical Garden. The parts used as explants were juvenile leaves and seeds.

2.3. Culture room condition:

Cultures of Gerbera were placed in a growth chamber under 25 ± 2 ^{0}C and 16-h photoperiod provided white fluorescent light (Prasanth and Sekar, 2004). Callus was placed in continuous dark for 30 days in culture (Tui *et al*, 2005).

2.4. Experimental design and statistical analysis:

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

2.5. Experimental treatments:

Surface sterilization of explants:

The explants were excised from the mother plants and then washed by a soapy water for 5 min followed by one h under a running tap water. They were then sterilized by immersion in a Clorox (commercial bleach) solution at the rate of 20, 30 and 40 % plus 3-5 drops of Tween 20 for 10, 15 or 20 min. Finally, they were washed 5 times with a sterile distilled water. At the end of the experiments, the collected data included number of survived explants without contamination.

Establishment stage:

For establishment stage, 25 treatments were initiated from the use of two types of explants. Growth regulators (BA and NAA) were used for establishment stage, 5 treatments of BA levels (0.0, 0.5, 1.0, 2.0 or 4.0 mg/l) were combined with 5 treatments of NAA level (0.0, 0.5, 1.0, 2.0 or 4.0 mg/l). In each treatment nine explants in three replicates were cultured for one month. Finally both the shoot and callus formation were calculated.

Multiplication stage:

For multiplication stage, 75treatments were initiated from the use of Kin and NAA (i.e. 0.00, 0.25, 0.50, 1.00, 2.00 mg/l and 0.00, 0.50, 1.00, 2.00, 4.00 mg/l, respectively) during three subcultures.

After three subcultures, the rate of shoot proliferation was determined for each subculture by recording the following parameters:

- Number of shoots.
- Shoot length (cm).
- Number of leaves/shoots .

Rooting stage:

In rooting stage, 36 treatments were initiated from the use of IBA and NAA (0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l)

After 30 days on the rooting media the following data were recorded:

- Number of roots/plantlet
- Root length(cm).

Acclimatization stag:

Rooted plantlets were pricked out singly into 10 cm plastic pots filled with 1:1, 2:1 and 3:1 (v/v) peatmoss and sand, respectively. To maintain cultures at high humidity, pots were covered with clear transparent plastic sheets for three weeks. The plastic covers were then gradually removed to reduce humidity and to adapt plantlets to greenhouse conditions, after that survival capacity (%) was recorded.

RESULTS AND DISCUSSION

3.1. Effect of different concentrations of Clorox and periods on surface sterilization explants of *Gerbera jamesonii* Bolus cv. Aurantiaca :

Results recorded in Table (1) show that Clorox (a commercial bleach) at 30% for 15 min (i.e. not contaminated or died) gave the highest value of explants survival when compared to the other treatments of leaves. Notably, there were non significant differences between the most concentrations of Clorox at the different times for seeds. However, 20% Clorox at 10 min and 40% Clorox at 20 min gave 8.0 explants when compared to 9.0 at other treatments on seeds.

Table (1): Effect of different concentrations of Clorox and periods on surface sterilization explants of *Gerbera jamesonii* Bolus cv. Aurantiaca *explant .*

Time(min) / Concentration	Leaves				Seeds			
	10	15	20	Mean (B)	10	15	20	Mean (B)
Clorox %								
20	5.000	7.000	8.000	6.667	8.000	9.000	9.000	8.667
30	8.000	9.000	7.000	8.000	9.000	9.000	9.000	9.000
40	7.000	8.000	6.000	7.000	9.000	9.000	8.000	8.667
Mean (A)	6.667	8.000	7.000		8.667	9.000	8.667	
LSD at 5% time (A)		0.641				0.081		
Concent. (B)		0.141				0.081		
AxB		0.244				0.140		

On the other hand, data indicated that increasing the time of immersed explants decreased the survival percentage of explants at the high concentrations of Clorox (40%) while the best concentration (30%) increased the free contaminated explants (at 15 min) on leaves.

The interactions between Clorox and time were significant with the highest value of survived explants (9), when 30% Clorox for 15 min was used.

3.2. Effect of different concentrations of BA and NAA on explants establishment of *Gerbera jamesonii* Bolus cv. Aurantiaca:

Data in Table (2) demonstrated the effect of BA on callus formation of leaves, as it was decreased by increasing the concentration of BA. BA at 0.5 mg/l was superior than 1.0, 2.0 or 4.0 mg/l when compared to explants responded from leaves. Starting from 0.5 mg/l BA, No. shoots/seeds was decreased and showed significant differences when compared to 0.0 and 0.5 mg/l BA (0.8, 1.0 and 1.0, respectively).

There were non significant differences between the different concentrations of NAA at 0.0 mg/l BA were used on seeds. Callus formation/leaf explant, showed no effects of leaves on callus formation.

The interaction between BA and NAA showed that the best concentrations were 0.5 mg/l BA and 1.0 or 2.0 mg/l NAA that gave the highest callus formation of leaves. In contrast, there were non significant differences between NAA concentrations at either 0.0 or 0.5 mg/l BA and 1.0 mg/l BA plus 2.0 or 4.0 mg/l NAA for shoot formation on seeds.

These results are in line with others obtained with different species like *Gerbera jamesonii* (Aswath & Choudhary, 2002a and Kumar *et al*, 2004). On the other hands, MS medium proved to be superior than B5 medium (Chikhale *et al*, 2004).

Table (2): Effect of different concentrations of BA and NAA on explants establishment of *Gerbera jamesonii* Bolus cv. Aurantiaca

NAA (mg/l) / BA (mg/l)	Leaves						Seeds					
	0.0	**0.5**	**1.0**	**2.0**	**4.0**	**Mean**	**0.0**	**0.5**	**1.0**	**2.0**	**4.0**	**Mean**
0.00	0.00	0.00	0.00	0.00	0.00	**0.00**	1.00	1.00	1.00	1.00	1.00	**1.00**
0.50	0.00	0.33	1.00	1.00	0.67	**0.60**	1.00	1.00	1.00	1.00	1.00	**1.00**
1.00	0.00	0.33	0.33	0.67	0.67	**0.40**	0.67	0.67	0.67	1.00	1.00	**0.80**
1.50	0.00	0.00	0.33	0.33	0.67	**0.27**	0.67	0.67	0.67	0.33	0.33	**0.53**
2.00	0.00	0.00	0.00	0.33	0.33	**0.13**	0.67	0.67	0.33	0.33	0.00	**0.40**
Mean	**0.00**	**0.13**	**0.33**	**0.47**	**0.47**		**0.80**	**0.80**	**0.73**	**0.73**	**0.67**	
LSD at 5% NAA (A)			**0.255**						**0.273**			
BA (B)			**0.255**						**0.273**			
AxB			**0.571**						**0.610**			

3.3. Effect of different concentrations of Kin on multiplication stage of *Gerbera jamesonii* Bolus cv. Aurantiaca :

Data in Table (3) indicated that increasing of Kin concentrations increased number of shoot formation and shoot length especially at 1.0 mg/l Kin.

Table (3): Effect of different concentrations of Kin and NAA on shoot formation/callus of *Gerbera jamesonii* Bolus cv. Aurantiaca .

Ki (mg/l)	NAA (mg/l)	No. of shoots					Shoot length(cm)				
		Sub. 1	Sub. 2	Sub. 3	Mean A	Mean B	Sub. 1	Sub. 2	Sub. 3	Mean (A)	Mean (B)
0.00	0.0	2.33	3.33	5.67	**4.49**	**3.78**	1.00	1.83	2.33	**2.24**	**1.72**
	0.5	2.33	3.67	6.00		**4.00**	1.00	2.00	2.67		**1.89**
	1.0	2.67	4.33	6.67		**4.56**	1.17	2.33	3.33		**2.28**
	2.0	2.67	5.00	6.67		**4.78**	1.17	3.00	3.67		**2.61**
	4.0	3.00	5.67	7.33		**5.33**	1.33	3.17	3.67		**2.72**
0.25	0.0	2.33	3.33	6.33	**5.18**	**4.00**	1.00	2.33	3.00	**2.74**	**2.11**
	0.5	3.00	4.33	7.33		**4.89**	1.33	2.67	3.33		**2.44**
	1.0	3.00	5.33	7.67		**5.33**	1.33	3.33	3.67		**2.78**
	2.0	3.33	5.67	8.33		**5.78**	1.50	3.67	4.33		**3.17**
	4.0	3.33	6.00	8.33		**5.89**	1.67	3.67	4.33		**3.22**
0.50	0.0	2.33	4.33	7.33	**6.42**	**4.66**	1.67	2.33	3.67	**3.73**	**2.56**
	0.5	3.33	5.67	9.33		**6.11**	1.33	4.00	4.33		**3.22**
	1.0	3.67	6.33	10.33		**6.78**	1.67	4.33	5.33		**3.78**
	2.0	3.67	6.67	11.33		**7.22**	1.83	5.67	6.33		**4.61**
	4.0	3.33	7.33	11.33		**7.33**	1.83	4.67	7.00		**4.50**
1.00	0.0	3.00	5.33	9.00	**7.78**	**5.78**	1.33	3.33	4.00	**4.93**	**2.89**
	0.5	4.67	8.67	14.67		**9.34**	2.67	6.67	9.67		**6.34**
	1.0	4.00	7.67	13.00		**8.22**	2.00	5.67	8.67		**5.45**
	2.0	3.67	7.33	12.33		**7.78**	1.83	5.67	7.67		**5.06**
	4.0	3.67	7.33	12.33		**7.78**	1.83	5.67	7.33		**4.94**
2.00	0.0	2.33	5.67	9.33	**7.29**	**5.78**	1.33	3.67	4.33	**4.63**	**3.11**
	0.5	3.67	7.33	13.67		**8.22**	1.83	6.00	8.33		**5.39**
	1.0	3.33	7.00	13.33		**7.89**	1.83	5.67	8.00		**5.17**
	2.0	3.33	7.00	12.67		**7.67**	1.67	5.33	7.33		**4.78**
	4.0	2.67	6.33	11.67		**6.89**	1.50	5.33	7.33		**4.72**
Mean C)		**3.15**	**5.87**	**9.68**			**1.55**	**4.08**	**5.35**		

LSD at 5% Ki,(mg/l) (A)	0.226	0.195
NAA,(mg./l) (B)	0.226	0.195
AB	0.504	0.435
Sub culture (C)	0.175	0.151
AC	0.391	0.337
BC	0.391	0.337
ABC	0.873	0.753

Concerning shoot number, exhibited in Table (4) indicate that there were continuous additive increases in number of shoots due to the increase in Kin concentrations (Fig: 1). Following the same line, as in shoot length and number of leaves, they were increased as a result of Kin concentrations.

However, MS medium was reported elsewhere not to be suitable for gerbera multiplication. Permanent high K level must also be avoided (Vardja and Vardja 2001)

On the other hand, BA was more suitable for shoot proliferation of gerbera multiplication (Aswath and Choudhary, 2002b). By repeated subculturing of the capitulum explant, a high frequency of shoot multiplication was established (Tui *et al*, 2005).

Fig (1): *Gerbera jamesonii* Bolus cv. Aurantiaca in multiplication stage.

3.4. Effect of different concentrations of NAA on multiplication stage of *Gerbera jamesonii* Bolus cv. Aurantiaca :

Data presented in Table (3) showed that NAA was effective an manipulating the number of shoot formed and shoot length.

Data in Table (4) show also that NAA caused an increase in number of Gerbera shoots. This was true between the different concentrations of NAA used and also when compared with the control. Similarly, shoot length and number of leaves were significantly increased due to increases in NAA concentrations.

3.5. Effect of Kin and NAA during multiplication stage of *Gerbera jamesonii* Bolus cv. Aurantiaca :

Data of the interaction between Kin and NAA concentrations exhibited in Table (3) showed clearly that the highest number of shoot from callus (9.34) was formed at 1.0 mg/l Kin plus 0.5 mg/l NAA. Also, the same concentrations of NAA and BA showed the highest value of shoot length.

Results presented in Table (4) showed that the highest number of shoots/shoot (8.56) was formed from 2.0 mg/l Kin, while the highest value of shoot length (8.06 cm) was formed at 1.0 mg/l Kin plus 2.0 mg/l NAA. Whereas, the number of leaves was 7.89 at 1.0 mg/l Kin plus 0.5 mg/l NAA.

Data in Tables (3, 4) showed that during subculturing, the third subculture produced more shoots when compared to shoots produced from the other subcultures.

6. Effect of IBA and NAA during rooting stage of *Gerbera jamesonii* Bolus cv. *Aurantiaca* :

Data in Table (5) demonstrate that IBA clearly affected the rooting stage of Gerbera. IBA was superior than NAA in the number of roots and root length.

For IBA level, it was found that 1.5mg/l IBA gave the highest number of roots and root length (6.89 and 9.50 cm, respectively) and there were significant differences between it and the other concentrations. Similarly for NAA level, it was noted that 2.5mg/l NAA gave the highest number of roots, while NAA at 2.0 mg/l gave higher shoot length when compared to other concentrations.

The interaction between IBA and NAA were significant for increasing number of roots and root length, which were demonstrated clearly in almost all the different treatments. IBA at 1.5 mg/l plus 0.0 mg/l NAA was the best for inducing greater number of roots and root length when compared to the other combinations.

For inducing rooting *in vitro* shoots, only IAA was effective and no root induction was observed with IBA or NAA treatments (Modh *et al*, 2002).

Table (5): Effect of IBA and NAA during rooting stage of *Gerbera jamesonii* Bolus cv. *Aurantiaca* .

NAA(mg/l) / IBA (mg/l)	Number of roots							Root length(cm)						
	0.00	**0.50**	**1.00**	**1.50**	**2.00**	**2.50**	**Mean**	**0.00**	**0.50**	**1.00**	**1.50**	**2.00**	**2.50**	**Mean**
0.00	4.33	5.33	6.00	6.33	6.33	6.67	**5.83**	7.33	7.33	8.00	8.33	9.33	8.33	**8.11**
0.50	5.33	6.00	6.67	6.67	6.67	6.33	**6.28**	8.33	8.67	9.33	9.33	8.67	8.67	**8.83**
1.00	7.33	7.00	6.33	7.00	6.67	6.33	**6.78**	8.67	9.00	8.33	8.00	7.67	7.67	**8.22**
1.50	8.67	7.67	6.33	6.33	6.33	6.00	**6.89**	10.67	9.67	9.67	9.33	9.00	8.67	**9.50**
2.00	7.67	6.67	6.00	5.33	6.67	7.67	**6.67**	9.67	9.33	9.33	8.67	8.33	8.33	**8.94**
2.50	6.67	6.33	6.00	5.33	4.67	4.33	**5.56**	9.00	8.67	8.33	8.33	7.67	7.67	**8.28**
Mean	**6.67**	**6.50**	**6.22**	**6.17**	**6.22**	**6.22**		**8.94**	**8.78**	**8.83**	**8.67**	**8.44**	**8.22**	
LSD at 5% NAA (A)			**0.394**							**0.378**				
IBA (B)			**0.394**							**0.378**				
AxB			**0.966**							**0.927**				

3.7. Effect of peatmoss and sand during Acclimatization stag of *Gerbera jamesonii* Bolus cv. Aurantiaca:

The plantlets of gerbera successfully lifted when they were transferred to culture in a mixture of peatmoss and sand (1:1; 2:1 and 3:1, respectively by udume) in the greenhouse. The survival of plantlet after one month was calculated and giving 100 % percentage.

REFERENCES

Aswath, C. and Choudhary, M.L. (2001): Effect of cytokinins on proliferation of multiple shoots in gerbera (*Gerbera jamesonii*). Indian Journal of Horticulture, 58(4): 383-386.

Aswath, C. and Choudhary, M.L. (2002a): Mass propagation of gerbera (*Gerbera jamesonii*) through shoot culture. Indian Journal of Horticulture, 59(1): 95-99.

Aswath, C. and Choudhary, M.L. (2002b): Rapid plant regeneration from *Gerbera jamesonii* Bolus callus cultures. Acta Botanica Croatica, 61(2): 125-134.

Aswath, C.; Deepaja, S.M. and Noorjahan, J.B. (2002): Morphogenetic response of *in vitro* gerbera shoots to IAA and BAP. Floriculture research trend in India Proceedings of the National Symposium on Indian floriculture in the New Millennium, 310-312.

Aswath, C.; Deepa, S.M. and Choudhary, M.L. (2003): Commercial multiplication of gerbera (*Gerbera jamesonii* Bolus) through *in vitro* shoot tip culture. Journal of Ornamental Horticulture New Series, 6(4): 303-309.

Bailey, L.H. and Bailey, E.Z. (1960): Hortus third A Concise Dictionary of Plants Cultivated in the United States and Canada, 3rd Ed, Macmillan, New York, 1333pp.

Chikhale, N.J.; Bokey, A.H and Rai, M.K. (2004): Micropropagation studies an gerbera (*Gerbera jamesonii*). Recent Trends in Biotechnology, 171-176.

Kumar, S.; Kanwar, J.K. and Sharma, D.R. (2004): *In vitro* regeneration of *Gerbera jamesonii* Bolus from leaf and petiole explants. Journal of Plant Biochemistry and Biotechnology, 13(1): 73-75.

Mandal, A.K.A.; Saxena, M. and Datta, S.K. (2002): Acclimatization of gerbera at Lucknow after *in vitro* multiplication. Indian Journal of Genetics and Plant Breeding, 62(4): 375-376.

Modh, F.K.; Dhaduk, B.K. and Shah, R.R. (2002): Factors affecting micropropagation of gerbera from capitulum explants. Journal of Ornamental Horticulture New Series, 5(1): 4-6.

Nowak, J. and Gabryszewska, E. (2002): Mineral nutrient requirements and effects of CO2 enrichment on Gerbera microcuttings. Journal of Horticultural Science and Biotechnology, 76(6): 670-673.

Prasanth, M. and Sekar, K. (2004): Studies on the age of explant on callus induction in gerbera cv. 'Mammut'. Scientific-Horticulture, 9: 207-211.

Purnima, T. and Kothari, S.L. (2004): Rapid *in vitro* regeneration of *Gerbera jamesonii* from different explants. Indian Journal of Biotechnology, 3(4): 584-588.

Reynoird, J. P.; Dewitte, W.; Prinsen, E.; Onckelen, H.v.; Noin, M.and Chriqui, D. (2000): Shooty tumours induced on *Gerbera hybrida* leaf explants by *A. tumefaciens* strain 82139 are characterized by high endogenous cytokinin levels . Acta Hort., 508: 261-263.

Snedecor, G.W. and Cochran, W.G. (1989): Statistical Methods (8th Ed.). Iow State Univ. Press, Ames, Iowa, U.S.A., 217- 236.

Thakur, P.S.; Ghorade, R.B. and Rathod, T.H. (2004): Micropropagation studies in gerbera. Annals of Plant Physiology, 18(2): 133-135.

Tui, R.; Prasenjit, S. and Roy, S.C. (2005): *In vitro* plant regeneration from young capitulum explants of *Gerbera jamesonii*. Plant Cell Biotechnology and Molecular Biology, 6(1/2): 35-40.

Vardja, R. and Vardja, T. (2001): The effect of cytokinin type and concentration and the number of subcultures on the multiplication rate of some decorative plants. Proceedings of the Estonian Academy of Sciences Biology and Ecology, 50(1): 22-32.

دراسات على نبات الجيربيرا تحت ظروف الإكثار المعملى

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أجريت هذه الدراسة فى خلال الفترة من سنة ٢٠٠٧ إلى ٢٠٠٨ فى معمل زراعة الأنسجة بحديقة الزهرية التابعة لمعهد بحوث البساتين-مركز البحوث الزراعية-وزارة الزراعة-جمهورية مصر العربية.

و يهدف هذا البحث إلى معرفة أنسب المعاملات لإكثار نبات الجيربيرا عن طريق زراعة الأنسجة وذلك لوضع بروتوكول للإكثار الدقيق لهذا النبات.

ويمكن تلخيص أهم النتائج التى أمكن التوصل إليها فى الأتى:

أمكن إكثار نبات الجيربيرا بواسطة الأوراق الحديثة والبذور كمنفصلات نباتية تم تعقيمها بواسطة الكلوركس بتركيز ٢٠، ٣٠، ٤٠ ٪ لمدة ١٠ ، ١٥، ٢٠ دقيقة بالإضافة إلى التوليفات المختلفة .فتبين أن أفضل تركيز من الكلوركس للحصول على أعلى نسبة بقاء للنباتات وأقل نسبة تلوث بالنسبة لتعقيم الأوراق هو ٣٠ % لمدة ١٥ دقيقة

أما بالنسبة للبذور فقد أعطت كل المعاملات نتيجة جيدة ما عدا ١٠ % الكلوركس لمدة ١٠ دقائق حيث أدت لتلوث بعض النفصلات النباتية وكذلك تركيز ٤٠ % من الكلوركس لمدة ٢٠ دقيقة حيث أدى لموت بعض المنفصلات النباتية نتيجة إرتفاع تركيز الكلوركس وطول مدة التعقيم أيضا.

وفى مرحلة التأسيس أستخدمت بيئة موراشيج وسكوج المضاف إليها البنزيل أدينين بتركيز صفر، ٠,٥، ١,٠، ٢,٠، ٤,٠ مجم/لتر بالإضافة إلى نفثالين حمض الخليك بنفس التركيزات بالإضافة إلى التوليفات بينهما. فكان أفضل تركيز للأوراق هو ٠,٥ مجم/لتر بنزيل أدينين و ١,٠ مجم/لتر نفثالين حمض الخليك لتكوين الكالس , وكان أفضل تركيز للبذور هو صفر أو ٠,٥ مجم/لتر بنزيل أدينين مع كل تركيزات نفثالين حمض الخليك لتكوين البادرات.

وفى مرحلة التضاعف أستخدمت بيئة موراشيج وسكوج المضاف إليها الكينتين بتركيز صفر ،٠,٢٥، ٠,٥٠، ١,٠، ٢,٠ مجم/لتر بالإضافة إلى نفثالين حمض الخليك بتركيز صفر، ٠,٥، ١,٠، ٢,٠، ٤,٠ مجم/لتر, فكان أفضل تركيز للأوراق هو ١,٠ مجم/لتر كينتين و ٠,٥ مجم/لتر نفثالين حمض الخليك , وكان أفضل تركيز للبذور هو ٢,٠ مجم/لتر كينتين.

وفـى مرحلـة التجـذير أسـتخدمت بيئـة موراشـيج وسـكوج المضـاف إليهمـا إنـدول حمـض البيوتريك و نفثـالين حمض الخليك بتركيز صفر، ٠,٥، ١,٠، ١,٥، ٢,٠، ٢,٥ مجم/لتر.فتبين أن أفضل معاملة هى بيئة موراشيجى وسكوج المضاف إليها 1.5مجم/لتر إنول حمض البيوتريك.

وفى مرحلة الأقلمة أستخدم بيتموس ورمل بنسبة ١:١ ,٢:١ , ٣:١ (بـالحجم) تحت أنفـاق بلاستيكية داخل صوب بلاستيكية فكانت نسبة نجاحها ١٠٠% فى كل المعاملات.

Table (4): Effect of different concentrations of Kin and NAA on shoot formation/shoot of *Gerbera jamesonii* Bolus cv. Aurantiaca .

Ki (mg/ l)	NAA (mg/l)	No. of shoots					Shoot length (cm)					No. of leaves				
		Sub. 1	Sub. 2	Sub. 3	Mean (A)	Mean (B)	Sub. 1	Sub. 2	Sub. 3	Mean (A)	Mean (B)	Sub. 1	Sub. 2	Sub. 3	Mean (A)	Mean (B)
0.00	0.0	1.33	1.33	1.67	**1.18**	**1.44**	1.17	4.33	6.33	**4.69**	**3.94**	2.33	3.33	4.33	**4.15**	**3.33**
	0.5	1.00	1.33	1.67		**1.33**	1.33	4.67	6.33		**4.11**	2.33	4.00	4.67		**3.67**
	1.0	1.00	1.00	1.33		**1.11**	1.67	5.67	6.67		**4.67**	2.67	4.33	5.33		**4.11**
	2.0	1.00	1.00	1.00		**1.00**	1.83	6.33	7.33		**5.16**	3.00	5.67	5.33		**4.67**
	4.0	1.00	1.00	1.00		**1.00**	2.33	6.67	7.67		**5.56**	3.33	6.33	5.33		**5.00**
0.25	0.0	1.67	3.33	4.67	**3.24**	**3.22**	1.17	4.00	7.33	**5.07**	**4.17**	2.67	4.33	5.33	**4.80**	**4.11**
	0.5	1.67	3.67	4.67		**3.34**	1.67	4.33	7.67		**4.56**	2.67	4.33	5.67		**4.22**
	1.0	1.67	4.33	4.33		**3.44**	2.17	5.00	8.00		**5.06**	3.67	5.00	6.00		**4.89**
	2.0	1.33	4.33	4.33		**3.33**	2.50	5.67	8.67		**5.61**	3.67	5.33	6.67		**5.22**
	4.0	1.33	3.67	3.67		**2.89**	2.83	6.33	8.67		**5.94**	3.67	5.33	7.67		**5.56**
0.50	0.0	2.33	4.33	5.67	**4.09**	**4.11**	1.83	5.67	8.00	**6.08**	**5.17**	3.33	5.33	6.00	**5.51**	**4.89**
	0.5	2.67	4.67	5.67		**4.34**	2.17	6.33	8.67		**5.72**	3.67	5.67	6.33		**5.22**
	1.0	2.67	4.67	5.33		**4.22**	2.67	7.33	8.67		**6.22**	3.67	5.67	6.67		**5.34**
	2.0	2.67	4.33	5.00		**4.00**	2.83	7.33	9.33		**6.50**	4.33	6.33	7.33		**6.00**
	4.0	2.33	4.33	4.67		**3.78**	3.33	7.67	9.33		**6.78**	4.33	6.33	7.67		**6.11**
1.00	0.0	4.33	8.00	12.33	**7.58**	**8.22**	2.83	7.67	9.67	**7.51**	**6.72**	5.67	7.33	10.00	**7.18**	**7.67**
	0.5	4.33	7.67	11.67		**7.89**	3.33	8.33	10.33		**7.33**	6.00	7.33	10.33		**7.89**
	1.0	4.00	7.00	11.67		**7.56**	3.67	8.67	10.67		**7.67**	5.33	6.67	9.33		**7.11**
	2.0	3.67	7.00	10.67		**7.11**	4.17	8.67	11.33		**8.06**	5.33	6.67	8.67		**6.89**
	4.0	3.67	7.00	10.67		**7.11**	4.00	8.33	11.00		**7.78**	5.00	6.33	7.67		**6.33**
2.00	0.0	4.67	8.33	12.67	**7.91**	**8.56**	3.67	8.67	10.67	**7.40**	**7.67**	6.00	6.33	9.67	**6.91**	**7.33**
	0.5	4.67	8.33	12.00		**8.33**	3.67	8.67	11.00		**7.78**	6.33	6.67	10.00		**7.67**
	1.0	4.33	7.33	12.00		**7.89**	3.17	9.33	10.67		**7.72**	5.67	6.33	9.00		**7.00**
	2.0	4.33	7.33	11.33		**7.66**	3.00	8.67	9.67		**7.11**	5.67	5.33	8.33		**6.44**
	4.0	4.00	6.67	10.67		**7.11**	2.83	7.67	9.67		**6.72**	6.00	5.33	7.00		**6.11**
Mean C		**2.71**	**4.88**	**6.81**			**2.63**	**6.88**	**8.93**			**4.25**	**5.67**	**7.21**		
LSD at 5% Ki, (mg/l) (A)						0.226					0.209					0.254
NAA , (mg./l) (B)						0.226					0.209					0.254
AB						0.505					0.467					0.569
Sub culture (C)						0.175					0.162					0.197
AC						0.391					0.362					0.441
BC						0.391					0.362					0.441
ABC						0.875					0.808					0.985