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IN VITRO PROPAGATION OF *Gerbera jamesonii* H. BOLUS

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ABSTRACT

Shoot tips of *Gerbera jamesonii* H. Bolus were used as explants in this study. Aseptic cultures of Gerbera were established by placing the isolated shoot tips on Murashige and skoog medium supplemented with different cytokinins (BA, Kin or 2ip) at different concentrations. The highest number of shoots/explant and the heaviest fresh weight/culture were obtained when MS medium contained 2.0 mg/L BA. Leaves of the obtained aseptic cultures were examined as explants to produce shoots (direct organogenesis). Interestingly, while intact leaves showed positive response in shoot formations on the petioles, there were no shoot formations on cut leaves (without petioles). This study recommended using intact old leaves of gerbera as they showed higher response on shoot formations when compared with the response of young leaves. While, the highest number of shoots was obtained on the petiole when the intact old leaf was cultured on MS medium contained 3 mg/L BA, the highest percentage of old leaves, that produced shoots, was achieved with the medium contained 1, 2 or 3 mg/L BA. While at rooting stage, the highest response of root formations was achieved when the medium supplemented with 0.5 mg/L IBA, in the same time proved to be the most effective in increasing the root lengths. The plantlets were successfully acclimatized when transferred to pots contained soil mixer of peatmoss: sand : vermiculite in equal volumes (1:1:1). The survival percentage was 100% after one month from tansplanting.

Abbreviations: MS: Murashige and Skoog; 2,4-D : dichlorophenoxyacetic acid; BA: Benzyladenine ; NAA: naphthalene acetic acid; IBA: indole-3-butyric acid; Kin: Kinetin ; 2ip (2-isopenteyladenine .

INTRODUCTION

Gerbera jamesonii is one of the cut flowers getting commercial significance. Gerbera was multiplied exclusively through the sexual pathway until 1971. An efficient method of vegetative multiplication through plant division of the crown was established by (Leffring 1971), the multiplication rate varying between eight and ten per year. Kaminek et al (1987) recorded a threefold increase rate following cytokinin spray. However, plant multiplication by divisions is too slow to be commercially practicable. In recent years, most of the commercial varieties are multiplied through tissue culture technique. This method enables a million fold expansion per year of a desired plant. The objectives of this work were to establish an efficient in-vitro method of propagation of gerbera. That was conducted by studying:

1- The effect of growth regulators at different concentrations on the growth of shoot tip and multiplication.
2- The effect of the same growth regulators that used before at different concentrations on induction of adventitious shoots on isolated leaves derived from aseptic cultures that established earliar.
3- The effect of growth regulators on root formation.

MATERIALS AND METHODS

This study was carried out at Plant Biotechnology Department, Institute of Genetic Engineering and Biotechnology, Minufiya University, Sadat City, Egypt. During the years of 2003 and 2004. Explants of shoot tips, 1-2 cm long, were excised from gerbera plants, then, washed under running water with soap for 30 minutes. Surface sterilization of explants was performed by immersion in solution containing sodium hypochlorite at concentration of 2% and two drops of tween 20 for 20 minutes. That was followed by transferring the explant to solution of mercuric chloride $HgCl_2$ at 0.1 % for 3 min. Finally, the explants were rinsed three times in autoclaved distilled water to remove all traces of the disinfectant. The external parts of explants were removed then the remained portion of a length 0.5 cm possessing 2 - 4 leaf primordia were cultured on nutrient medium. Murashige and Skoog basal medium (1962) was used at full strength supplimented with different types and concentrations of growth regulators.

Following preparation the medium and prior to addition of agar, the pH was adjusted to 5.8. The medium was poured into culture Jars (325 ml) where each jar contained 50 ml of the medium. The jars where capped with polypropene closures and autoclaved at 121°C and 1.2 Kg/cm^2 air pressure for 20 min.

This work was designed as follow:

PART (1) SHOOT TIP CULTURE:

The aim of this experiment was to determine the optimal type and concentration of cytokines for shoot proliferation from shoot tip culture. Aseptic shoot tip explants (about 0.5 cm) were placed horizontally on MS medium containing 30 g/L sucrose and solidified with 6.0 g/L agar. For shoot regeneration, different concentrations of BA, Kin or 2ip were added at 0.0, 1.0, 2.0, 3.0 and 5.0 mg/L. The experimental design was a randomized complete block with five replications (the experiment was replicated two times). A replicate consisted of one jar containing three explants. After 6 weeks, number of shoots per explant and fresh weight (g.) were counted and analyzed by analysis of variance (ANOVA) according to Gomez and Gomes (1984).

Clusters of shoots were transferred to hormone free MS medium for plant elongation.

Part (2) LEAF CULTURE:

Leaves from aseptically grown plants (clusters of shoots from part one) were used as explants. Two types of leaves (old and young) were compared for *in vitro* shoot formations:

1- Basal leaves (old leaf)

The old leaves were a source of 2 types of explants:

A- intact leaf

B- leaf without petioles

2- Young leaves:

The young leaves were a source of 2 types of explants:

A- intact leaf

B- leaf without petioles.

In four different experiments these types of explants were placed on MS medium supplemented with BA, Kin or 2ip each at different concentrations (0.0, 1.0, 2.0, 3.0 and 5.0 mg/L).

The factorial experimental system in complete randomized block design with five replications was used. each replicate consisted of one Jar containing three explants. Frequency of shoot formation was evaluated. The number of adventitious shoots per explant was counted and analyzed by analysis of variance (ANOVA).

Part (3) ROOT FORMATION

For root induction, firstly, clusters of shoots grown on MS medium contained 2 mg/L BA at part one (multiplication experiment) were transferred to hormone-free MS medium for shoot elongation. Then, shoots were individually transferred to MS medium supplemented with three types of auxins IBA, NAA and 2,4-D each of which at different concentrations (0.0, 0.5, 1.0 and 1.5 mg/L). Rooting percentage as well as the number and length of roots per shoot were evaluated 6 weeks later.

Incubation conditions:

The cultures were incubated at 25 °C day and night. Light was provided by white fluorescent tubes giving light intensity of 2000 Lux at the explant levels for 16 hours per day.

PART (4) ACCLIMATIZATION

For acclimatization, rooted plantlets grown on MS medium contained 0.5 mg/L IBA were carefully washed in running tap water to remove the substrate., then transferred to plastic pots containing soil mixture of sand, peat moss and vermiculite 1:1:1 (v/v), and protected with plastic bags to maintain high relative humidity around the plants. The pots were maintained in a greenhouse. A solution of one half strength MS salts was added to the pots to enhance the development of plants. Data was recorded for percentage of survivals after 30 days from transplanting

RESULTS AND DISCUSSION

Effect of growth regulators on shoot proliferation from shoot tips:

Data on the main effect of growth regulators in Table (1) showed that BA was significantly effective in increasing the number of shoots when compared to the other used growth regulators (Kin and 2ip).

Results on the main effect of concentrations indicate that while all used concentrations showed high responses on number of shoots, without significant differences between each of them, while they significantly surpassed the hormone free medium (control).

Concerning the original data in the same table, data showed that the addition of growth regulators (BA, Kin or 2ip) each alone to the medium was significantly beneficial for shoot proliferation when compared with hormone-free medium (control). However, the highest value of shoot number (8.70) was significantly recorded with the medium contained 2.0 mg/L BA followed by 1.0 mg/L BA. It was observed that BA at 2.0 mg /L surpassed the control treatment, and both of other examined concentrations of BA and all examined concentrations of Kin and 2ip (Fig 1). While In that concern , Posada (1999) recorded that the best results for the shoot propagation were obtained with the treatments 1 and 2 mg/L BA respectively, and Mandal et al (2002) found that the medium supplemented with 2mg/l BA and 0.5 mg/L IAA ensured the optimum conditions for shoot proliferation. Shiquing *et al* (2002) stated that the optimum media for micropropagation of gerbera were MS contained 3 mg/L BA and 0.1 mg / L NAA for shoot induction and 3 mg / L BA and 0.2 mg / L NAA for shoot multiplications.

Table (1): Effect of different growth regulators at different concentrations on shoot proliferation from shoot tips of *Gerbera jamesonii* that were cultured in vitro for 6 weeks.

Concentrations (B)mg/l	Growth regulators (A)			Means of B
	BA	Kin	2ip	
Control	2.10	2.10	2.10	2.10
1.0	7.20	4.60	4.00	5.27
2.0	8.70	4.40	4.40	5.83
3.0	6.50	5.70	5.00	5.73
5.0	4.70	6.1	5.50	5.43
Means of A	5.84	4.58	4.20	
LSD at 5%	Growth regulators = 0.57 Concentration = 0.74 Interaction A x B = 1.28			

The highest rate of shoot proliferations was recorded from MS medium supplemented with BA at 1.5 mg/L (Aswath, 2001). On the contrary to our results, Hempel *et al.* (1985) found that, the best shoot multiplication was obtained with kin at 5 mg/L when compared with different concentrations of BA and 2ip.

Effect of growth regulators on fresh weight:

Data on the main effect of growth regulators in Table (2) (g) showed that BA significantly increased the fresh weight per explant compared to Kin and 2ip. Concerning the main effect of the used concentrations of growth regulators, results showed that heavest fresh weight was obtained with the concentration of 2.0 mg/L followed by 3.0 mg/L when both were compared with the other concentrations and control.

Table (2): Effect of different growth regulators at various concentrations on fresh weight, (g) when shoot tips of *Gerbera jamesonii* H. Bolus were cultured *in vitro*.

Concentration (B)mg/l	Growth regulators (A)			Means of B
	BA	Kin	2ip	
Control	0.329	0.329	0.329	0.329
1.0	0.825	0.425	0.536	0.595
2.0	2.101	0.436	0.612	1.050
3.0	1.848	0.575	0.610	1.011
5.0	1.508	0.791	0.690	0.996
Means of A	1.322	0.511	0.555	
LSD at 5%	Growth regulators 0.058 Concentration 0.075 Interaction A x B 0.130			

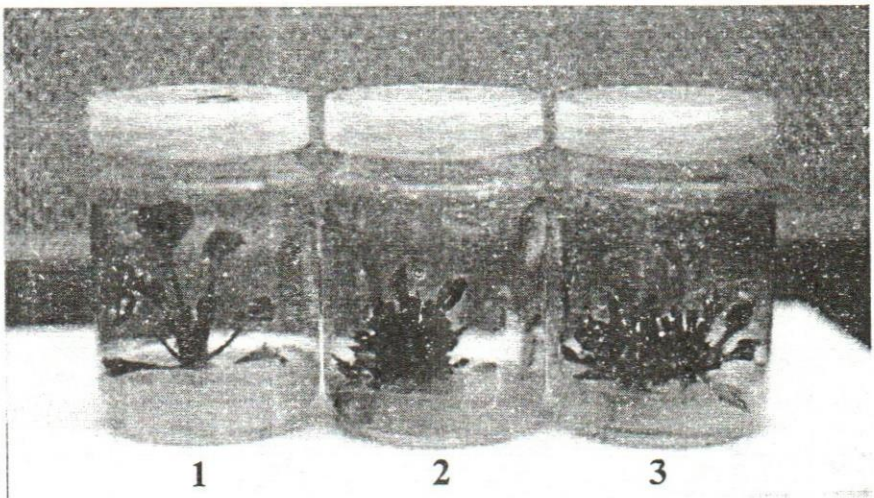

Fig. (1): 1- Shoots grown on MS free hormone medium (control)
2- Shoots grown on MS medium contained 2 mg/L Kin.
3- Shoots grown on MS medium contained 2 mg/L BA.

The original data in the same table indicated that the using of growth regulators was effective in enhancing the fresh weight when compared with control. The heaviest fresh weight (2.101) differed significantly when was with the medium contained 2.0 mg/L BA compared to control and all concentrations of the used growth regulators.

Effect of type of leaf and growth regulators (at different levels) on the number of adventitious shoots from leaves of gerbera:

It is very interesting to mention that, explants of leaves without petioles did not show any response in shoot formation in all treatments of this study, but on the contrary shoot formation was only obtained with using explants of intact leaves. Accordingly, all our experiments were conducted using the intact leaves either old or young.

Data on the main effect of type of growth regulators on shoot proliferation from leaves (regardless the type of leaf) are presented in Table (3). Results showed that the highest value of shoots explant (1.318) was obtained with significant effect on the medium contained BA followed by Kin and 2ip respectively when compared with each other. Our results was in agreement with Jerzy and Lubomsk, (1991) Since, they observed that bud regeneration at the base of leaf petioles on a modified MS medium with BA or Kin at 24o after 6 weeks , BA being more efficient than Kin.

Table (3): Effect of type of leaves and growth regulators (at different levels) on the number of adventitious shoots that proliferated from leaves of *Gerbera. jamesonii* after 6 weeks *in vitro*.

		Concentrations mg/L (C)					Means of (A x B)	Means of (A)
Leaf types (A)	Cytokinen (B)	0.0	1.0	2.0	3.0	5.0		
Old leaves	BA	0.00	1.30	1.80	3.78	2.30	**1.83**	1.465
	Kin.	0.00	0.30	2.30	1.30	3.80	**1.54**	
	2ip	0.00	0.60	0.80	2.00	1.70	**1.02**	
Young leaves	BA	0.00	0.50	0.80	1.50	1.20	**0.80**	0.647
	Kin.	0.00	0.40	0.40	0.80	1.80	**0.68**	
	2ip	0.00	0.20	0.50	1.20	0.40	**0.46**	
Means of (C)		0.00	0.55	1.10	1.76	1.86	Means of (B)	
Means of (B x C)	BA	0.00	0.90	1.30	2.64	1.75	1.318	
	Kin.	0.00	0.35	1.35	1.05	2.80	1.110	
	2ip	0.00	0.40	0.65	1.60	1.05	0.740	
Means of (A x C)	Old leaves	0.00	0.73	1.63	2.36	2.60		
	Young leaves	0.00	0.36	0.56	1.16	1.13		
LSD at 5%		A = ** B = 0.15 C = 0.20 A x B = 0.22 A x C = 0.28 B x C = 0.35 A x B x C = 0.49						

However, when the effect of type of growth regulators was examined to induce shoots from leaves either old or young each alone, data in the same table (3) indicate that BA showed higher effect with both types of

leaves, That effect of BA was significant (1.836) with explants of old leaves. It was clear that BA was more effective on shoot proliferation when the explants were old leaves compared to either the effect BA on the young leaves or other growth regulators on both old and young leaves.

Regarding the main effect of type of leaves on shoot proliferation, data show that using the old leaves was effective significantly on shoot formation compared to the response of the young leaves. Higher value of shoots (1.465) was obtained with the old leaf. Data on the main effect of concentrations of growth regulators indicate that no shoots were proliferated on hormone–free medium (control). The gradual increase in the concentration resulted in gradual increase in the formation of adventitious shoots. The highest value of shoots was obtained with the medium contained 5 mg/L of growth regulator followed by the concentration 3mg/L without significant difference.

The original data (interaction) reveal that the highest value of proliferated shoots/explant (3.780) was observed significantly with the medium contained 3.0 mg/L BA and explant of old leaf whene compared with any other interactions(Fig. 2). While, less sporadic values of proliferated shoots were obtained with the other treatments, on shoots were produced on hormone- free medium (control). Orlikowska *et al.* (1999) revealed that the addition of BA to the medium was very important to achieve sufficient shoot proliferations from leaves, as the lowest response was clear with the medium contained 0.2 µM thidiazuron and 0.3 µM IAA, but the medium contained 2.2 µM BA and 0.3 µM IAA showed higher response. Barbosa *et al.* (1994) found that shoot proliferation from young capitulums of gerbera was best on half strength MS medium with BA at 2.27 mg/L. In comparison to our results, low level of BA (0.75 mg/L) was used by Parthasaratny et al (1997) in combination with 1 mg/L IAA and 0.75 mg/L IBA to achieve adventitious shoot from leaves of gerbera. However, high concentration of BA (10 mg/L) was used in combination with 0.1 mg/L IAA for the induction of adventitious buds (Xiufang *et al.*, 2002). Although similar high concentration of BA (10 mg /L) was recommended by Modh *et al.* (2002) to be used for formation of adventitious shoots at the beginning of multiplication stage but at the following subcultures. The medium which contained 1.0 mg /L BA showed the highest shoot number.

Effect of type of leaves and growth regulators (at different levels) on the percentage of leaves that produced shoots:

In Table (4) data on the main effect of growth regulators (regardless the type of leaves) showed that BA significantly introduced the highest percentage of leaves (30.63) that produced shoots compared to kin and 2ip. In that contrary, there was no significant difference between the effect of kin and 2ip (15.96 and 14.87, respectively).

Fig.(2): Formation of shoots on the petiole of leaf cultured on MS medium contained 3 mg/L BA (direct organogenesis)..

Table (4): Effect of type of leaves and growth regulators (at different levels) on the percentage of leaves of *Gerbera. jamesonii* that produced adventitious shoots after 6 weeks *in vitro*.

		Concentrations mg/L (C)					Means of (A x B)	Means of (A)
Leaf types (A)	Cytokinen (B)	0.0	1.0	2.0	3.0	5.0		
Old leaves	BA	0.00	59.96	53.28	59.90	39.96	**42.62**	26.77
	Kin.	0.00	13.32	33.30	19.98	28.64	**19.05**	
	2ip	0.00	13.32	26.64	26.64	26.64	**18.65**	
Young leaves	BA	0.00	19.98	19.98	33.30	19.98	**18.65**	14.21
	Kin.	0.00	13.32	11.10	19.96	19.98	**12.87**	
	2ip	0.00	11.10	13.32	19.98	11.10	**11.10**	
Means of (C)		0.00	21.83	26.27	29.96	24.38	Means of (B)	
Means of (B x C)	BA	0.00	39.97	36.63	46.60	29.97	30.63	
	Kin.	0.00	13.32	22.20	19.97	24.31	15.96	
	2ip	0.00	12.21	19.98	23.31	18.87	14.87	
Means of (A x C)	Old leaves	0.00	28.87	37.74	35.51	31.75		
	Young leaves	0.00	14.80	14.80	24.41	17.02		
LSD at 5%		A = ** B = 4.20 C = 5.42 A x B = 2.93 A x C = 7.66 B x C = 9.38 A x B x C = NS						

Concerning the main effect of type of leaves (old and young) and growth regulators, from data in the same table (4) it was observed that BA showed the highest percentage of leaves that produced shoots, that effect of BA was significant with both old (42.62) and young (18.65) leaves compared to the effect of Kin and 2ip on both type of leaves. However, the highest percentage obtained with the medium contained BA and old leaf explant significantly was potent than either the medium had BA and young leaves or other growth regulators with old or young leaves. Data on the main effect of type of leaf explants indicate that explants of old leaves significantly showed higher percentage of responded leaves (26.77) compared to the young leaves (14.21).

In the same table (4) data on the main effect of concentrations of growth regulators revealed that no shoots were proliferated on hormone-free medium (control) Although, the highest response (29.96%) was obtained with the medium contained the concentration 3.0 mg/L followed by 2 mg/L without significant difference, but the results obtained with those concentrations significantly surpassed the results of the other concentrations 1.0 and 5.0 mg/L).

It was observed from the original data that, application of BA at 1.0, 2.0 and 3.0 mg/L resulted in high value of percentage of the responded leaves (59.96, 53.28 and 59.90%, respectively) without significant differences. These results showed to be true when the old leaves were used as explants. These treatments were effective in increasing the percentage of responded leaves compared to all other treatments either included old or young leaf explants. No proliferated shoots were observed with hormone – free medium (control) either with old or young leaf explants. In similar study, Reynoird *et al.* (1993) found that when leaf explant were cultured on MS medium supplemented with 10 μM BA and 2.5 μM NAA, up to 90% of excised developing leaves formed 3-5 shoots per explant. The explants were highly responsive (83.3%) in a medium containing 2 mg/L NAA and 1mg/L BA after 3 weeks of callus transfer to a medium (Aswath and Choudhary, 2002,a)

According to the previous results of leaf culture, for shoot formation from leaves, recommendation raised up to use Ms medium contained 3.0mg/L BA with explants of intact old leaves.

Effect of growth regulators at different concentrations on root formations.

In Table (5) data on the main effect of types of growth regulators showed that IBA was significantly more effective in root formation when compared with NAA and 2, 4-D. Data on the main effect of the different used concentrations of growth regulators showed that the highest value of root numbers was significantly obtained with the low concentration (0.5 mg/l) compared to the other concentrations (1 and 1.5 mg/L) and control. The gradual increase of the concentration resulted in gradual decrease in the values of root numbers.

Table(5):Effect of growth regulators at different concentrations on root formations on the shoot bases of *G.jamesonii* after 6 weeks *in vitro*.

Concentration (B) mg/l	Growth regulators (A)			Means of B
	IBA	NAA	2,4-D	
Control	0.10	0.10	0.10	0.10
0.5	5.80	4.50	4.50	4.933
1.0	3.35	3.40	3.30	3.350
1.5	2.62	2.10	2.20	2.307
Means of A	2.968	2.526	2.525	
LSD at 5%	Growth regulators 0.392 Concentration 0.452 Interaction A x B NS			

However, the addition of growth regulators to the medium at all concentrations was significantly effective in root formation when compared with the effect of hormone-free medium (control). The original data in table (5) indicated that the highest number of roots (5.8) was produced when the medium was supplemented with low concentration of 0.5 mg/L IBA (Fig.3) followed by the same concentration of NAA and 2, 4-D. Similar results were obtained by Radice and Maconi (1998) concerning rooting of gerbera shoots which were 70 – 100% with the medium contained 0.5 mg/L IBA, in contrary Modh *et al.* (2002) found that only IAA was effective and no root induction was observed with IBA or NAA. The gradual increase in the concentration of all used auxins showed a gradual negative effect on root formations. Hormone-free medium (control) showed the lowest effect on root formations. Some other studies were done on root formations of gerbera. such as Aswath and Choudhary (2001) who stated that the best root formation was obtained on MS medium supplemented with 1.75 mg/L IBA, while, Chun and Vouxiang (2001) found that 1/2 MS contained 0.3 mg/L IBA had the best rooting capacity.

In Table (6), data on the main effect of growth regulators on root lengths showed that IBA was significantly effective in comparison to NAA and 2,4-D. Data on the main effect of the used concentrations indicated that the low concentration (0.5 mg/L) was significantly effective when compared with the higher concentrations (1.0 and 1.5 mg/L) and control. However, all used concentrations significantly surpassed the control.

Table (6): Effect of growth regulators at various levels on root length of *G. jamesonii* after 6 weeks *in vitro*.

Concentration (B)	Growth regulators (A)			Means of B
	IBA	NAA	2,4-D	
Control	0.10	0.10	0.10	0.10
0.5	2.34	1.64	1.91	1.963
1.0	1.80	1.28	1.14	1.407
1.5	0.92	0.74	0.54	0.733
Means of A	1.29	0.94	0.922	
LSD at 5%	Growth regulators 0.127 Concentration 0.147 Interaction A x B 0.254			

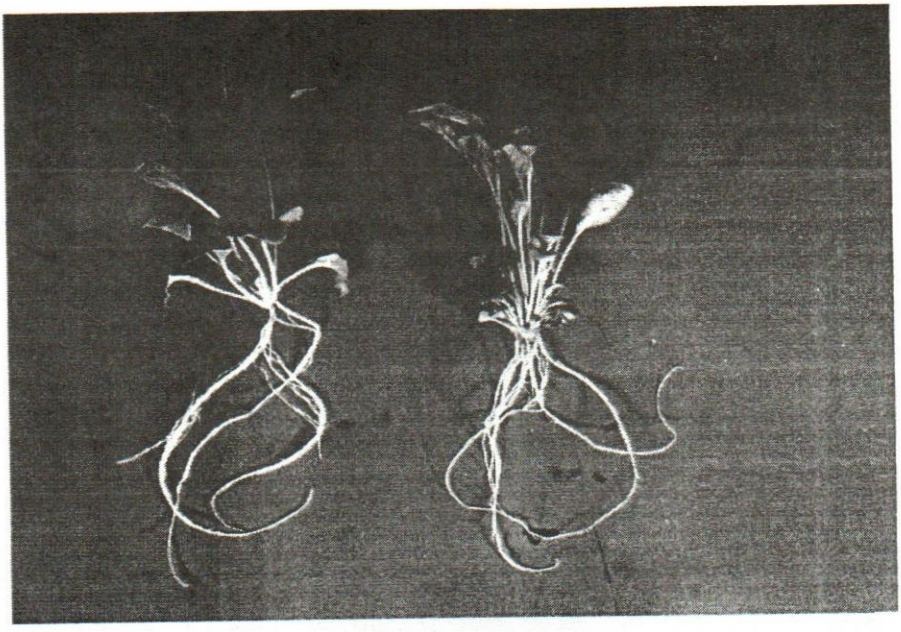

Fig. (3): Formation of roots on shoots of gerbera cultured on MS medium contained 0.5 mg/L IBA.

Concerning the original data, the highest value of root lengths was significantly observed when the medium contained 0.5 mg/L IBA followed by the same concentration of 2,4-D and NAA respectively . In that concern, Aswath and Choudhary (2002,a) revealed that the root length of gerbera was the best in half MS medium contained 0.5 mg/L 2,4-D and 0.5 mg/L NAA. The gradual increase in concentrations of growth regulators negatively affected the root lengths. However, the presence of any of the used growth regulators at any concentration was beneficial in increasing root lengths when compared with control.

Acclimatization:

At acclimatization, the survival percentage of plantlets was 100% when calculated in the green house after 30 days of transplanting to soil mixture contained sand :peatmoss :vermiculite in equal volumes V/V (Fig .4).

Our results were in agreement with Aswath and Chouhary (2002,a) They found that, when gerbera plantlets were removed from culture and planted in plastic pots filled with cocopeat ,red soil and sand at a ratio of 3:1:1., the survival rate was 100% and the young plants grew well. Regenerated plantlets were transferred to soil where they grew normally with a survival rate of 95% (Aswath and Choudhary ,2002,a)

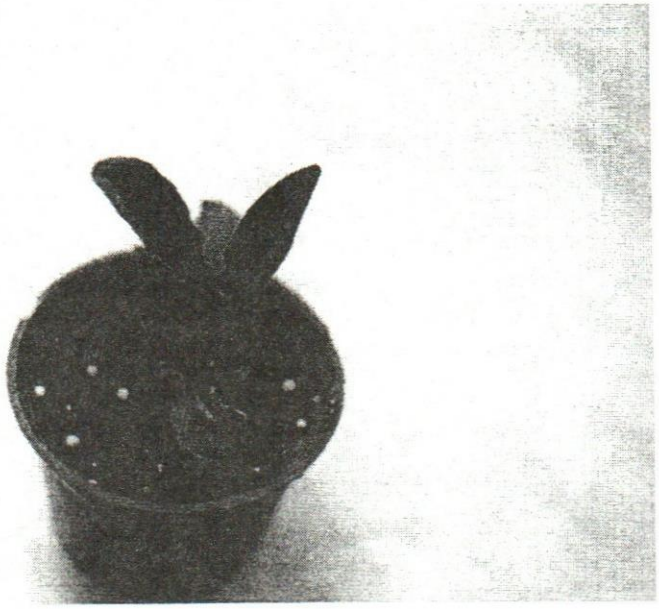

Fig (4): Three months old plantlets of gerbera were grown in soil mixture contained peatmos, sand and vermiculite 1:1:1 (v/v)

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إكثار الجربيرا معمليا

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خلال الجزء الاول لهذا العمل استخدمت القمم الناميه بطول (٥و سم) كاجزاء نباتيه لبدء اكثار الجربيرا معمليا، حيث تم زراعه تلك الاجزاء على بيئه موراشيج وسكوج المضاف اليها انواع مختلفه من منظمات النمو كل على حده بتركيزات مختلفه.

وقد اظهرت النتائج ان بيئه موراشيج وسكوج المحتويه على ٢ ملليجرام بنزيل ادنين ادت الى انتاج اكبر عدد من الافرع وكذلك اعلى وزن طازج من القمم الناميه المزروعه .

وخلال الجزء الثانى من هذا العمل تم زراعه اوراق مفصوله من نباتات ناتجه معمليا من الجزء الاول من الدراسه (معقمه) وذلك لدراسه تكوين افرع عليها ، وقد كان واضحا ان زراعه الورقه كامله (النصل والعنق) اظهرت استجابه عاليه لتكوين افرع عرضيه على عنق الورقه، الا انه عند زراعه الورق بدون عنق (نصل فقط) ادى ذلك لعدم تكوين ا فرع على الجزء المزروع، ،كما اوضحت الدراسه ايضا ان استجابه الاوراق الكامله المزروعه لتكوين الافرع العرضيه كانت اعلى عندما كانت تلك الاوراق اكبر عمرا وذلك بمقارنه بالاوراق الكامله الاصغر عمرا. وقد اظهرت النتائج ان بيئه موراشيج وسكوج المحتويه على ٣ ملليجرام / لتر بنزايل ادنين اعطت اعلى عدد من الافرع المتكونه على الاوراق الكامله الاكبر عمرا. وكذلك فان اضافه البنزيل ادنين الى البيئه المستخدمه بتركيزات١، ٢، او٣ ملليجرام/لتر ادى الى الحصول على اعلى نسبه مئويه للاوراق التى اعطت افرع على اعناقها.

وفى مرحله التجذير، ظهر ان استخدام بيئه موراشيج وسكوج المحتويه على ٥. ملليجرام / لتر اندول بيوتريك اسيد ادى الى الحصول على افضل استجابه سواء من حيث عدد الجذور التى تكونت على قواعد الافرع او طول تلك الجذور.

وقد تم اقلمه النباتات الناتجه بنجاح عند نقلها للصوبه وزراعتها فى خليط من التربه عباره عن ١ بيتموس: ١ رمل: ١ فرموكيوليت (بالحجم) وكانت نسبه النباتات الحيه بعد شهر من نقل النباتات الى الصوبه هى ١٠٠%.