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A PROTOCOL FOR MICROPROPAGATION OF *Chrysanthemum morifolium*, RAMAT. PLANTS.

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ABSTRACT

An in vitro propagation was carried out at Plant Tissue Culture Laboratory, Agricultural Development System Project (ADSP), Ministry of Agriculture, during the successive seasons of 2004/2005 and 2005/2006, to find out the favorable methodology for in vitro propagation of Chrysanthemum morifolium, Ramat. The results could be summarized in the following:

The largest shoot length and number of leaves /shoot tip or node explants occurred due MS medium supplemented with 0.1 mg /L TDZ, whereas shoot formation (%) was the best when shoot tips or nodes were subculture in WPM medium supplied with 0.1mg /L TDZ, but callus formation favoured the 1.0 mg/l TDZ on WPM. Application GA_3 at 1.0 mg /L to shoot tips, subculture on MS medium supplemented with 1.0 mg BA /L., resulted the best shoot length, number of leaves as well as shoot and callus formation. Applying 0.5 mg /L TDZ combined with 2.0 mg /L IAA to shoot tip explants gave the more number of leaves and shoot formation. While 2.0 mg /L IAA alone on shoot tips resulted the longest shoots. The heaviest callus formation occurred on node explants treated with 0.5 mg TDZ/l.

Shoot tips subculture on MS medium supplemented with 1.0 mg/l BA under light intensity of 1000 lux produced the larger number of leaves and shoot length.

Callus formation was the heaviest on node explants under 1000 lux light intensity. The largest number of leaves and shoot length occurred on shoot tips subculture on MS medium supplied with 1.0 mg /L BA under 24^0C. But,callus formation was the best on node explants under 17 ^{0}C. Adding sucrose to MS medium supplemented with 1.0 mg /L BA was the best compared to fructose and glucose as sources of carbon in producing shoot length, number of leaves and callus formation. The highest values of characters occurred due to 20 g /L sucrose combined with shoot tip explants. Shoot length and number of leaves/ shoot tip or node explants were the largest when treated with 1.0 mg /L BA, combined with 15 g. /L sucrose. Whereas number of shoots on shoot tips, or node explants was the highest at 1.0 mg /L BA with 10 g /L sucrose. The 1.0 mg /L IAA combined with 15 g /L sucrose led to the heaviest callus formation. While root length was the longest by 0.1mg /L BA combined with sucrose at 10 g /L. MS medium at 1/2 strength resulted the largest number of roots, root length and root formation, While full strength of MS failed to induce roots .Reducing MS strength caused concomitant decrease

in the growth; characters shoot length and number of leaves. MS salts medium at full strength gave the largest growth characters.

Key words: *In vitro*, micro-propagation, thidiazuron (TDZ), benzyl-adenine (BA), silver nitrate ($AgNO_3$), chrysanthemum morifolium, light intensity, ssucrose, type of medium-temperature.

INTRODUCTION

Chrysanthemum (*Chrysanthemum morifolium*, Ramat), plant is cultivated as an ornamental plant and as a source of pyrethrum. Several factors were found to affect the regeneration potential of plant tissue cultured *in vitro*. Of these factors, plant growth regulators. Flick *et al.* (1983) found that the cytokinin /auxin balance was more important for regeneration than the specific hormone utilized. Kushal and Arora (1994) observed the increasing BA level increased number of shoots of chrysanthemum in *in vitro*, but suppressed their growth. Haq *et al* (1998) mentioned that callus induction occurred only on MS medium supplemented with BA at 0.5-1.2 mg /L, but the best callus resulted by 1.2 mg. Kumari and Verghese (2003) stated that callus induction was highest in media supplemented with BAP at 2mg /L+ NAA at 0.2 mg /L , callus was elongated in media supplemented with GA3 at 5-10 mg /L and rooting was initiated on the same media after 10-12 days.

Explant type is another important factor affecting regeneration. Kaul *et al*. (1999) stated that stem explants were superior to leaf explants for *Chrysanthemum morfiolium* regeneration *in vitro*. El-Sayed (2005) mentioned that shoot tips explants increased survival percent and shootlet number /explants of *Sequoia sempervirens* compared to nodal cuttings, *in vitro.* Tombolato and Costa (1998) concluded that source of carbon (sucrose, glucose and fructose) was very important component *in vitro* culture media; because of the light energy deficiency and low CO2 concentration present *in vitro* conditions. De Faria *et al.* (2004) assured the importance of sucrose in *in vitro* culture media, and gained greater increments in plant height and high seedling multiplication of *Dendrobium nobile* Lindl. When added 60 g / L sucrose to culture media.

The present study was achieved to evaluate effects of some types of media, cytokinins (TDZ and BA), auxins (1AA, 1BA), GA_3, light intensity, temperature and source of carbon (sugars), individually or combined on shoot tip or node explants from *Chrysanthemum morifolium*, Ramat, under *in vitro* culture conditions.

MATERIALS AND METHODS

This work was conducted during the two successive seasons of 2004/2005 and 2005/2006 at Plant Tissue Culture Laboratory, Agricultural Development System Project (ADSP), Ministry of Agriculture.

Shoot tips (2.0 cm. long) from *Chrysanthemum moriflorum,* Ramat. Plants grown in the nursery of Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, were obtained on 10th November, during both seasons. Shoot tips

were washed thoroughly under current water for about an hour. The disinfection of shoot tips was accomplished by stirring for 20 minutes in 1.0% sodium hypochlorite (NaOCL) solution with few drops of tween 20 (polyoxyethlene sorbitan monolaurate) as a welting agent. The disinfected tips were rinsed three times in sterile de-ionized water and blotted to dry on sterilized filter paper before culturing *in vitro*. Outside tissues, accompained stem portions and leaflets were cut and removed to leave shoot tips at 0.3 cm long. Five explants were cultured in jar (200 ml) containing 40ml of MS (Murashige and Skoog, 1962) medium supplemented with 1.0 mg/l BA (Benzyl-adenine), as described by Karim *et al* (2003), for six months. The free contaminated explants were monthly subcultured on similar media. Six months later, shoots were transferred on MS media free hormones for one month to the resulting shoots, which became the source for the following experiments, starting from 15th June 2005 or 2006.

The 1st experiment: Shoot tips, 0.3cm long, were cultured on 3 type of media: MS (Murashige and Skoog, 1962) medium, B5 (Gamborg *et al,* 1968) medium and WPM, Woody Plant Medium (Lloyd and McCown, 1980), supplemented with TDZ (thiodiazuron) at the five levels of 0.0, 0.1, 0.5, 1.0 and 5.0 mg /L. The same treatments were applied on node explants, 0.3cm. Combination treatments amounted to 15 (3media and 5 levels of TDZ) were done for each explant. Eight weeks later the data were recoded.

The 2nd experiment was carried out to study the effect of five levels of GA_3 (Gibberellic acid) at 0.5, 1.0, 2.0, 4.0 and 6.0mg/l on shoot tip or node explants (0.3cm long) subcultured on MS medium supplemented with 1.0mg/l BA. Ten treatments (5GA_3 levels and 2 explants) were applied in this experiment, which lasted for 6 weeks.

The 3rd experiment was undertaken to detect the influence of 2.0 mg /L IAA combined with two levels (0.5 and 5.0 mg /L) of TDZ or BA on 0.3 cm long shoot tip or node explants. This experiment included ten treatments (5 levels of growth substances and 2 types of explants). After six weeks the following data were obtained for the three aforementioned experiments: shoot length (cm), number of leaves, callus formation (g) and shoot formation (%) /shoot tip or node explants.

The 4th experiment: Shoot tips and nodes (0.3cm long) were cultured on MS medium supplied with 1.0 mg /L BA were used to detect the effect of various light intensities (1000, 2000, 3000 at 4000 lux) on shoot length (cm), number of leaves and callus formation (g), after six weeks. The experiment included 8 treatments (4 light intensities with two sources of explants).

The 5th experiment was consummated to test the influence of different degrees of temperature (10, 17, 24 and 32^{0}C) on growth characters of two sources of explants (shoot tip and node, each 0.3 cm long) cultured on MS medium supplied with 1.0mg/l BA. After six weeks, the same data obtained on the foregoing the 4th experiment were recorded.

The 6th experiment was carried out to detect the effect of 3 sugars as different sources of carbon (sucrose, fructose and glucose each at 10, 20, 30 and 40 g /L) on two types of explants (shoot tip and node,0.3cm long) sub-cultured on MS medium

supplemented with 1.0 mg /L BA. After six weeks, shoot length (cm), number of leaves and callus formation (g) were recorded. This experiment contained 24 treatments: 12 sugars levels by 2 explants (shoot tip and node), and was done fore one season during 15th June to 31st July 2005.

The 7th experiment was conducted to find out the influence of IAA and IBA auxins each at 0.1 and 1.0 mg /L levels combined with sucrose at the levels of 5.0, 10.0 and 15.0 g /L on 0.3 cm shoot tip or node explants, individually. The experiment consisted of 12 treatments (4 auxins levels by 3 levels of sucrose) for every source of explants. After six weeks, shoot length (cm), number of leaves, root length (cm), number of shoots, and callus formation (g) were assessed.

The 8th experiment was carried out on 15th June 2005, for one season only in order to evaluate the influence of MS salts at full, three quarter, half and quarter strengths on some root and growth characters. The experiment continued for four weeks when such parameters were assessed: root formation (%), number of roots, root and shoot length and number of leaves/ shoot tip.

All experiments except the 6th one were carried out during the two seasons. The layout of the eight experiments, except the 8th one were completely randomized factorial with three replications, whereas the eight experiment was complete randomized blocks, with three replications. The averages data were subjected to statistical analysis of variance procedure, in case of zero and percentage value, the original data were firstly arcsine-transformed prior to statistical analysis and the values of L.S.D. were obtained whenever the calculated "F" value were significant at 5% level (Snedecor and Cochran, 1984).

RESULTS AND DISCUSSION

The 1st experiment: Effect of three types of media and TDZ (Thidiazuron) levels on some growth characters and calls formation from shoot tips or node explants.

Data shown in Tables (1a & b and 2a & b) indicated that the results on shoot tip and node explants were nearly close. The 0.1mg /L TDZ (Thidiazuron) level resulted the longest and the more percentage of shoot formation, while number of leaves and callus formation were largest at 1.0 mg /L TDZ. Such increases were significant compared to most other treatments. MS medium significantly produced the largest length of shoots and number of leave (Figure1); whereas the WPM (Wood Plant Medium) was significantly the best medium for callus and shoot formation on shoot tips as well as on node explants these results are in agreement with those obtained by Ipekci *et al*, (2001) on *Paulowina elongata*; Nassar *et al*, (2001) on *Bixa orellana*, L and Gad,Mervat and Shehata(2003) on *Quercus robur*, L.

In the first season, the interaction between TDZ levels and type of media, showed that the 0.1 mg /L TDZ level supplemented to MS significantly resulted the longest shoots (3.27 cm on shoot tip and 7.04 cm on nodes) and the largest number of leaves (10.10 and 7.90 for shoot tip and node explants, respectively). While callus formation was significantly the heaviest due to 1.0 mg/l TDZ on WPM medium compared to most combination treatments, as it resulted in 2.94g from shoot tip and

2.80 from node explants. But the 0.1mg /L TDZ added to WPM was significantly the best treatment for shoot formation which gave 60.0 (7.75) and 54.0 (7.32) for shoot tip and node explants, successively. The second season's results showed same trend as in the first one. Poovaiah *et al* (2006 a &b) found that ,the highest mean number of shoots per internodes of *Mentha spicata* on medium MS containing TDZ .

Table 1a: Effect of different type of media and TDZ concentration on shoot length (cm) and number of leaves after 8 weeks from shoot tips of *Chrysanthemum morifolium*, during two seasons (2004/2005 and 2005/2006).

TDZ level (mg /L)	Shoot length (cm) /shoot tip				Number of leaves /shoot tip			
	Type of medium			Mean	Type of medium			Mean
	MS	B5	WPM	(A)	MS	B5	WPM	(A)
(2004/2005)								
0.0	2.71	1.47	1.78	**1.98**	7.35	4.30	3.50	**5.05**
0.1	3.27	1.77	2.09	**2.38**	10.10	5.15	4.50	**6.58**
0.5	2.23	2.61	1.69	**2.18**	7.95	6.85	4.05	**6.28**
1.0	2.95	2.13	1.67	**2.25**	9.40	5.80	4.75	**6.65**
2.0	3.02	1.81	2.01	**2.28**	9.15	5.25	4.70	**6.37**
Mean(B)	**2.84**	**1.96**	**1.85**		**8.79**	**5.47**	**4.30**	
L.S.D 0.05								
(A)	0.25				0.53			
(B)	0.20				0.41			
(AxB)	0.44				0.92			
(2005/2006)								
0.0	2.77	1.45	1.73	**1.98**	7.40	4.35	3.60	**5.12**
0.1	3.23	1.80	2.06	**2.36**	10.05	5.15	4.55	**6.58**
0.5	2.18	2.72	1.68	**2.19**	7.90	6.90	3.95	**6.25**
1.0	2.99	2.18	1.65	**2.27**	9.50	5.90	4.85	**6.75**
2.0	3.08	1.89	2.03	**2.33**	9.10	5.40	4.60	**6.37**
Mean(B)	**2.85**	**2.01**	**1.83**		**8.79**	**5.54**	**4.31**	
L.S.D 0.05								
(A)	0.21				0.48			
(B)	0.17				0.37			
(AxB)	0.37				0.83			

The 2nd experiment: Influence of different GA_3 levels and source of explants on some growth parameters after six weeks.

It appears from data in Table 3 that raising GA_3 levels from 1.0mg up to 6.0 mg/l caused significant progressive reduction in shoot length (cm), number of leaves, callus formation (g) and shoot formation (%). The 1.0mg GA_3 level significantly produced the highest values of all parameters, as compared to other levels. For source of explants, shoot tip surpassed than node explants in producing the best growth. The differences between the two sources of explants were significant for both shoot length

1b

2a

2b

Tab 3

and number of leaves, but did not reach the level of significant for callus or shoot formation. For the interaction treatments shoot tip explants treated with 1.0 mg /L GA_3 significantly produced the highest records of shoot length, number of leaves, callus and shoot formation which were 4.57cm, 7.60, 0.88gm and 66.67% ,respectively, in the first season and 4.37, 7.50, 0.92 and 73.33%, consecutively, in the second one.

GA_3 stimulated shoot elongation but not shoot bud proliferation of C*hrysanthemum morifolium*, Karim *et al* (2003). Kumari and Varghese (2003) on capitulum explants of C*hrysanthemum morifolium*, stated that calluses elongated in media supplemented with GA_3 at 5-10 mg /L, and rooting was initiated on the same media after 10-12 days.

The 3rd experiment: Influence of IAA added to some BA or TDZ levels and source of explants on some growth parameters after six weeks.

Data shown in Table 4 revealed that 2.0 mg/l IAA individually, significantly produced the longest shoots; but number of leaves and callus formation (g) were significantly improved by 2.0 mg /L IAA with 0.5 mg /L TDZ treatment during both seasons, whereas number of shoots were raised due to 5.0 mg /L BA added to 2.0 mg /L IAA treatment in the first season and 0.5 mg /L TDZ with IAA treatment in the second one.

Shoot tip explants was significantly better than node explants for shoot length (cm), number of leaves and shoots, but callus formation (g) was significantly heavier when explants was taken from node.

Concerning interaction treatments, the longest shoots (4.63 and 3.23 cm, in both seasons, successively) resulted by the 2mg/l IAA added to shoot tip explant. The largest numbers of leaves were 13.25 and 14.00 at the first and second seasons, consecutively, produced by shoot tip explant supplied with 2mg IAA /L plus 0.5 mg /L TDZ. But the heaviest callus formation (5.62 and 5.68g in both seasons, respectively) occurred due to IAA and 0.5 mg /L TDZ cultured from node explant. Number of shoots was more by node explant supplemented with 2.0 mg/l IAA and TDZ at 0.5 mg /L, in the first season and 5.0 mg /L in the second one.

Such results disagree with those of Amin *et al,(*1997)demonstrated that axillary and adventitious bud multiplication of chrysanthemum was possible from the nodal, shoot tip and petiole explants .Haq *et al* (1998) on chrysanthemum, stated that good callus induction occurred on shoot explants cultured on MS medium supplemented with 1.2 mg /L IBA. Kaul *et al* (1999) on chrysanthemum stem explant surpassed leaf explants. Karim *et al* (2003) reported that shoot multiplication of C*hrysanthemum morifolium* was achieved from the nodal and shoot tip explants of mature plant using MS medium with BA. El-Sayed (2005) noted that shoot tips of *Sequoia semperivens* increased shootlet number explant compared to nodal cuttings in *in vitro*. Poovaiah *et al*(2006a) showed that maximum number of shoots of *Mentha spicata* was observed when internodes were used as explant source .

Tab 4 present

The 4th experiment: Influence of different light intensities and source of explants on some growth parameters and callus formation (g), after six weeks.

It is clear from data in Table 5 that increasing light intensity led to gradual reduction in the three parameter under study, however such decrease was not significant for both shoot length (cm) and number of leaves, while 1000 lux intensity significantly heaviest callus formation, in both seasons

Nower, (2002) found that light intensity at 2000 lux and photoperiod at 24 hr., significantly increased the fresh weight ,shoot length ,number of shoots of *Lilium* and *Gladiolus*. Shoot tip explant significantly surpassed the node one for improving shoot length and number of leaves, but callus formation showed the reverse. Shoot tip explant under 1000 lux light intensity significantly produced the longest shoots and number of leaves (1.48cm and 12.4, respectively, in the first season and 1.36cm and 11.2, consecutively, in the second one) compared to most other treatments. Whereas, the node explant subjected to 1000 lux intensity treatment resulted the heaviest callus formation (3.00 and 2.75g in both seasons, successively). Such results coincide with the findings of Zhi and Gao (2004) on *Chrysanthemum morifolium* ,found that the most suitable explant for tissue culture was the shoot tips with a diameter of 0.3 mm., and El-Sayed (2005) observed that shoot tips explant of *Sequoia sempervirnes* increased shootlet number compared to nodal explant *in vitro* conditions.

The 5th experiment: Influence of different temperature degrees and source of explants on some growth parameters and callus formation (g), after six weeks.

Subjecting chrysanthemum explants to 24°C significantly increased shoot length compared to most other degrees (Table 6). But, number of leaves and callus formation (g) were significantly increased by the 17°C as compared to most degrees. The differences between shoot length (cm) and number of leaves values as a result of sources of explants did not reach the level of significance. Node explant significantly improved callus formation (g) than shoot tip. The shoot tip explant subjected to 24°C significantly resulted the longest shoots (1.26 and 1.64cm, in the two seasons, respectively) compared to most treatments of combination. The larger number of leaves occurred significantly due to node explant under 17°C, in the first season (5.64 leaf) but resulted in the second on by the shoot tip explant under 24°C (5.60 leaf). Callus formation (g) was significantly the biggest: 1.84 (1.25) g in the first season and 1.46 (1.10) g in the second one, under node explant subjected to 17°C combined treatment in both seasons.

The 6th experiment: Influence of some kinds of sugars as a source of carbon at different levels on some growth parameters and callus formation.

It is evident from data in Table 7 that using sucrose as a source of carbon in MS medium supplemented with 1.0 mg /L BA surpassed other kinds of sugars, whereas glucose resulted the lowest results and failed to induce callus formation. The sucrose at 20 g /L, produced the highest record of shoot length (mm), number of leaves and callus formation (g) as compared to all other treatments Figure 1.Callus formation was significantly improved with shoot tip culture in comparison to node as the explants.

While the differences between shoot length and number of leaves due to source of explants did not reach the level of significance. Applying sucrose at 20 g /L level to shoot tip explant significantly resulted the longest shoots (4.07 cm) and the largest number of leaves (8.07) and the heaviest callus (2.97g) compared to all other treatments.

Table 5: Effect of different light intensities and source of explants on shoot length(cm), number of leaves and callus formation (g) after 6 weeks of *Chrysanthemum morifolium* durig two seasons (2004/2005 and 2005/2006).

Light intensity (lux)	Shoot length (cm)			Number of leaves			Callus formation (g)		
	Source of explants		Mean	Source of explants		Mean	Source of explants		Mean
	Shoot tip	Node		Shoot tip	Node		Shoot tip	Node	
				(2004/2005)					
1000	1.48	0.69	**1.08**	12.4	6.32	**9.36**	0.86	3.00	**1.93**
2000	1.24	0.88	**1.06**	12.0	7.68	**9.84**	0.71	2.25	**1.48**
3000	1.16	0.62	**0.89**	12.0	6.64	**9.32**	0.43	1.38	**0.91**
4000	0.92	0.71	**0.82**	10.8	6.96	**8.88**	0.21	0.44	**0.32**
Mean(B)	**1.20**	**0.73**		**11.80**	**6.90**		**0.55**	**1.77**	
L.S.D 0.05									
(A)	0.32			2.51			0.32		
(B)	0.22			1.78			0.23		
(AxB)	0.45			3.55			0.46		
				(2005/2006)					
1000	1.36	0.54	**0.95**	11.2	3.36	**7.28**	0.85	2.75	**1.80**
2000	0.88	0.84	**0.86**	9.00	7.84	**8.42**	0.56	2.24	**1.40**
3000	0.88	0.50	**0.69**	9.40	5.96	**7.68**	0.35	1.40	**0.87**
4000	0.50	0.67	**0.58**	5.40	7.44	**6.42**	0.21	0.42	**0.31**
Mean(B)	**0.91**	**0.64**		**8.75**	**6.15**		**0.49**	**1.70**	
L.S.D 0.05									
(A)	0.40			3.44			0.27		
(B)	0.29			2.43			0.19		
(AxB)	0.57			4.87			0.38		

Tombolato and Costa (1998) stated that the source of carbon (sucrose, glucose or fructose) was very important component in *in vitro* culture media, because of the light energy deficiency and low CO_2 concentration in *in vitro* conditions. Ishil *et al* (1998) on *Phalaenopsis* observed that the presence of sucrose in the culture medium cause protocorm formation but its absence caused callus proliferation. While, De Faria *et al* (2004) noted that sucrose at 60 g/l caused the highest increases in plant height and seedling multiplication of *Dendrobium nobile* in *in vitro.* The medium type and various carbon sources were markedly influenced *in vitro* propagation of *Eclipta alba* Baskaran and Jayabalan(2005).

Tab 6

Table (7): Effect of different sources of carbon levels (sugars) on shoot length (cm), number of leaves, and callus formation (g) after 6 weeks from different source of explants of *Chrysanthemum morifolium*.

Sugar levels (g /L)		Shoot length (cm)			Number of leaves			Callus formation (g)		
		Source of explants		Mean (A)	Source of explants		Mean (A)	Source of explants		Mean (A)
		Shoot tip	Node		Shoot tip	Node		Shoot tip	Node	
Sucrose	**10**	2.20	2.07	**2.13**	5.20	4.73	**4.97**	1.12	0.94	**1.03**
	20	4.07	3.97	**4.02**	8.07	7.83	**7.95**	2.97	2.77	**2.88**
	30	3.10	2.97	**3.03**	7.17	6.73	**6.95**	2.45	2.27	**2.36**
	40	2.70	2.57	**2.63**	6.60	6.30	**6.45**	1.67	1.74	**1.71**
Fructose	**10**	1.67	1.53	**1.60**	3.70	3.50	**3.60**	0.28	0.19	**0.24**
	20	2.30	2.17	**2.23**	4.30	4.30	**4.30**	0.52	0.49	**0.50**
	30	2.70	2.57	**2.63**	4.50	4.70	**4.60**	0.97	0.72	**0.84**
	40	2.07	1.93	**2.00**	4.17	3.93	**4.05**	0.27	0.29	**0.28**
Glucose	**10**	0.67	0.57	**0.62**	2.50	2.60	**2.55**	0.00	0.00	**0.00**
	20	1.30	1.20	**1.25**	3.37	3.17	**3.27**	0.00	0.00	**0.00**
	30	2.00	1.87	**1.93**	4.03	3.93	**3.98**	0.00	0.00	**0.00**
	40	1.53	1.47	**1.50**	3.50	3.50	**3.50**	0.00	0.00	**0.00**
Mean(B)		**2.19**	**2.07**			**4.76**	**4.60**		**0.85**	**0.79**
L.S.D 0.05										
(A)		0.29				0.35			0.09	
(B)		N.S.				N.S.			0.04	
(AxB)		0.41				0.14			0.13	

The 7th experiment: Influence of IAA and IBA auxins and sucrose levels supplemented to shoot tip or node explants on some growth and root parameters.

Data exhibited in Tables (8 and 9) revealed that the results of shoot tip attained a parallel trend to those of node explants and the findings at both seasons showed a close trend.

In the first season, growth parameters expressed as shoots number and length as well as number of leaves were significantly increased due to 1.0 mg IBA /L supplementation; while root length significantly favour the lower level of IBA (0.1mg /L), the 0.1 mg /L IAA gave the lowest values. IAA at 1.0 mg /L supplement significantly resulted the highest callus formation, whereas IBA at any level gave the lowest formation. Increasing sucrose concentration in media significantly gradually raised shoot and root length, number of leaves and callus formation values. But shoot length was significantly the longest under 10 g /L sucrose compared to higher or lower concentrations. Concerning treatments of the interaction between auxins and sucrose levels, it appeared that 1.0mg/L IBA and 15g/L sucrose significantly resulted the longest shoots (5.03 cm on shoot tip and 4.63 cm on node explants) and the largest number of leaves (8.07 and 7.70, upon shoot tip and node explants, respectively). The largest number of shoots (3.0 on shoot tip and node explants) was given by 1.0 IBA

Fig 1

Tab 8a

Tab 8b

Tab 9a

Tab 9b

mg /L combined with 10g/l sucrose treatment. But root length significantly preferred by the 0.1mg /L IBA with 15g /L sucrose, which resulted 8.80 and 8.37 cm on shoot tip and node explants, consecutively. Callus formation was the heaviest (1.85g) on shoot tip and 1.37 g on node explant) due to IAA at 1.0 mg /L level combined with 15 g /L sucrose.

The results of the second season were similar to those obtained in the first one. Datta *et al* (2001) on *Chryanthemum morifalium*, mentioned that shoots in *in vitro* rooted in 1/2 MS + 1.5g/l sucrose + 0.2mg/l NAA. Sarker and Shaheen(2001) showed that, roots developed (100%) on MS medium containing 0.2 mg/l IBA of *Chryanthemum morifalium*.

The 8th experiment: Influence of MS salts strength on some growth and root parameters, from shoots after four weeks.

Data in Table 10 indicated that MS medium at full strength significantly highest records of the studied growth characters expressed in shoot length and number of leaves, but failed to produce roots. Decreasing MS strength led to progressive reduction in growth characters. The highest root formation, number of roots and root length significantly resulted by MS at half strength in both seasons. These results coincide with the findings of many investigators that reducing the strength of MS medium induced rooting of explants. Kushal and Arora (1994), Datta *et al* (2001) and Zhi and Gao (2004) on chrysanthemum, stated that rooting characters were the best on half MS strength. While shoot growth of chrysanthemum, *in vitro* was better 100-150% on MS (Oliviera *et al,* 1996).

Table 10: Effect of different MS salts strengths on root formation (%), number of roots, root length (cm), shoot length (cm) and leaves number after 4 weeks from shoot tips of *Chrysanthemum morifolium* during two seasons (2004/2005 and 2005/2006).

MS salts strength	Root formation (%)		Number of roots		Root length (cm)		Shoot length (cm)		Number of leaves	
	Seasons		Seasons		Seasons		Seasons		Seasons	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Full	0.00 (0.16)	0.00 (0.16)	0.00	0.00	0.00	0.00	5.67	6.00	8.73	8.83
Three quarter	32.00 (5.65)	33.33 (5.78)	1.60	1.73	3.77	3.97	4.10	4.10	6.17	6.17
Half	46.67 (6.83)	49.33 (7.02)	2.33	2.47	8.80	8.50	2.50	2.53	5.50	5.60
Quarter	26.67 (5.16)	26.67 (5.16)	1.33	1.35	4.67	4.40	1.63	1.73	4.70	4.80
L.S.D 0.05	0.43	0.32	0.25	0.18	0.94	0.76	0.51	0.51	0.64	0.55

CONCLUSION

From the previous results it would be concluded that:

- Supplemented 0.1mg /L TDZ to MS medium, increased shoot length and number of shoot /shoot tip or node explant. Callus and shoot formation were the best with WPM medium supplemented with 1.0 and 0.1mg /L TDZ, respectively.
- The highest shoot length, number of leaves, callus and shoot formation occurred by 1.0 GA_3 added shoot tip explant.
- Shoot tip explants supplemented with 0.5 mg /L TDZ caused the largest increase in number of leaves and shoots number. While the longest shoot resulted by 2.0 IAA added to shoot tip explant. But callus formation favoured node explant supplied with 0.5 mg /L TDZ.
- Shoot length and number of leaves were the largest on shoot tip explants illuminated with 1000 lux or put under 24°C. But callus formation was the best due to node explant subjected to 1000 lux light intensity or 17°C.
- Shoot tip explant supplied with 20g/l sucrose gave the largest shoot length, number of leaves and callus formation.
- 1.0 mg /L IBA combined with 15g/l sucrose resulted the largest shoot length, number of leaves and callus formation, but number of shoots favoured 1.0 mg /L IBA combined with 10g/l sucrose. The longest roots were produced by 0.1mg /L IBA with 10 g /L sucrose.
  Similar results occurred from shoot tip or node explants.
- Rooting characters (formation, length and number) were the largest on half strength of MS (Figure 1). Whereas full MS strength gave the longest shoots and the more number of leaves, but were suppressed as MS strength was reduced.

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بروتوكول الأكثار الدقيق لنباتات الأراولة

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أجرى هذا البحث في معمل زراعة الأنسجة، مشروع تنمية النظم الزراعية،بوزارة الزراعة، خلال الموسمين المتتاليين ٢٠٠٤/٢٠٠٥ و٢٠٠٥/٢٠٠٦ لبحث الطريقة المناسبة لإكثارنبات الأراولة في المعمل. ويمكن إيجاز النتائج في الآتي :

- الأفرع الاطول وعدد الأوراق الأكثر نتجت بزراعة القمم النامية للأفرع وعقد السلاميات على بيئة مورشيجى وسكوج المضاف ١.٠ملليجرام ثيوديازول يوريا /لتر، بينما كانت النسبة المئوية لتكوين الأفرع أفضل عند زراعة قمم الأفرع أو عقد السلاميات في بيئة WPM مضاف إليها ١.٠ ملليجرام ثيوديازول يوريا ،و أفضل كمية كالوس(جم) تكونت على بيئة WPM المضاف إليها ١.٠ ملليجرام ثيوديازول يوريا .
- قمم الأفرع المنزرعةعلى بيئة مورشيجى وسكوج والمضاف إليها الجبريللين بتركيز ١.٠ ملليجرام/لتر و١.٠ ملليجرام اندول حامض البيوتريك/لتر، أنتجت أحسن طول فرع، عدد الأوراق وكذلك النسبةالمئوية لتكوين الأفرع وكمية الكالوس بالجرام.
- إضافة ٠.٥ ملليجرام ثيوديازول يوريا مرتبطة مع ٢.٠ ملليجرام اندول حامض الخليك/لتر في بيئة زراعة قمم الأفرع أعطى أكثر عدد للأوراق ونسبة مئوية لتكوين الأفرع، بينما ٢.٠ ملليجرام اندول حامض الخليك/لتر وحدة فى البيئة أنتجت أطول أفرع. وأثقل كالوس تكون بزراعة عقدالسلاميات على البيئةالمحتوية على ٠.٥ ملليجرام ثيوديازول يوريا /لتر.
- قمم الأفرع المعاد زراعتها على بيئة مورشيجى وسكوج والمضاف إليها ١.٠ملليجرام اندول حامض البيوتريك لكل لتر تحت شدة إضاءة ١٠٠٠ لوكس أنتجت أكبر عدد للأوراق وطول للأفرع. تكوين الكالوس كان أثقل على قمم الأفرع تحت شدة إضاءة ١٠٠٠ لوكس.
- حدث أكبر عدد أوراق وطول للفرع على قمم الأفرع المعاد زراعتها على بيئة مورشيجى وسكوج والمضاف إليها ١.٠ملليجرام إندول حامض البيوتريك /لتر تحت ٢٤درجةْمئوية لكن تكوين الكالوس كان أحسن على أجزاء من عقد السلاميات تحت١٧ْدرجة مئوية.
- إضافة سكروز إلى بيئة مورشيجى وسكوج المضاف إليها ١.٠ ملليجرام بنزيل ادنين /لتر كان الأفضل مقارناً بالفركتوز والجلوكوزكمصدر للكربون على طول الفرع، عدد الأوراق وتكوين الكالوس ٠إضافة٢٠جرام سكروز/ لترفى نيئة زراعة قمم الأفرع هو أفضل تركيز لانتاج أعلى قيم للقياسات السابقة٠
- -- الأفرع الاطول وعدد الأوراق الأكثر نتجت بزراعة القمم النامية للأفرع وعقد السلاميات في بيئة تحتوى على ١.٠٠ملليجرام /لتر إندول حامض البيوتريك و١٥جم سكروز / لتر.
- أنتجت بيئة مورشيجى وسكوج المنصفة الأملاح أكبر عدد جذور و طول للجذروكذلك اعلى نسبة مئوية للتجذير بينمابيئة مورشيجى وسكوج كاملة الأملاح فشلت في إنتاج جذور. بيئة مورشيجى وسكوج المنصفة الأملاح يصاحبها نقص فى صفات النمو طول الفرع وعدد الأوراق مقارنة ببيئة مورشيجى وسكوج كاملة الأملاح التى أعطت أعلى صفات نمو.