Bull. Fac. Agric., Cairo Univ.,58(2007):133-143.

ESTABlISHMENT OF AN *In vitro* MICROPROPAGATION PROTOCOL FOR *Trachleium caeruleum* L.

(Received: 4.9.2006)

By
T .A. M. Abou Dahab

Ornamental Horticulture Department, Faculty of Agriculture, Cairo University.

ABSTRACT

An *in vitro* propagation protocol has been developed from mature *Trachleium caeruleum* plants.

Experiment (1): Effect of disinfection treatments on free contamination explants was done. The results indicated that the highest free contaminated explants (100%) were recorded by using chlorox at 60% plus M.C. at 0.2%.

Experiment (2): Influence of BA, Kin, and TDZ at different rates on shooting behavior was studied. The data indicated that: MS medium plus Kin. at the rate of 4.0 mg/l produced the greatest shoot number/explant. Increasing the number of subcultures gradually significantly increased the shoot number/explant and the 3rd subculture produced the highest value. Shoot length/explant significantly was reduced by adding TDZ to the MS medium. MS medium plus Kin at the rate of 1.0 mg/l was the most effective treatment in producing significantly the longest shoot/explant. Culturing the explants on MS medium supplemented with Kin at 4.0 mg/l produced the highest number of leaves/explant.

Experiment (3): Influence of different media composition on shooting behavior. Full strength LS medium was the best used in producing significantly the greatest shoot number/explants. Increasing the number of subcultures significantly increased the shoot number/explant. The longest shoot was found when using full and half strength LS medium in the 3rd subculture. Full strength LS medium produced significantly the greatest number of leaves/explant.

Experiment (4): Influence of MS salt strength and activated charcoal on rooting behavior. The greatest number of roots/shootlet was recorded with ¼ MS salt strength and with activated charcoal. The longest roots were recorded with ¼ MS salt strength and with activated charcoal. The tallest plantlets were recorded when using ½ MS salt strength and activated charcoal. The highest number of leaves/shootlets was recorded by using ½ MS salt strength with activated charcoal.

Experiment (5): Effect of acclimatization media. During adaptation the tallest plants and the greatest number of leaves were observed when using peat moss, where the survival percentage was 100% in all growing media.

Key words: *acclimatization , activated charcoal, BA, Kin, micropropagation,TDZ, Trachleium caeruleum.*

1. INTRODUCTION

Trachleium caeruleum (Throatwort) family Campanulaceae is one of the most popular cut flower for using as a filling material for flower arrangement.

The plants are perennial herb half to one meter in height with ovate double , toothed, thin leaves and 8cm ,long flowers very numerous in clustered panicles with slender tubular corolla 2 cm long, violet- blue colour (Graf, 1992). The cut flowering branches are used for export and local market for flower arrangement.

Recently, *Trachleium caeruleum* plants been introduced to the Egyptian flower growers since few years, some Egyptian growers cultivated *Trachleium* for the local and for export to European market as cut flowers. They imported the seedlings from Holland.

The success of tissue culture on propagation of ornamental plants is greatly influenced by the nature of culture medium used. MS medium (Murashige and Skoog, 1962) is a common medium used in the plant tissue culture, so it has been used by many workers such as Agrawal *et al*., (1992), on orchid (*Vanilla walkeriae*); Pereira

Pinto *et al*., (1996) on *Kielmeyera coriacea*. Sakr *et al*., (1999) on *Yucca elephantipes*, Chen *et al*., (2000) on *Lilium speciosum*, Abd El Rhman (2002) on *Solidago* sp. and Haque *et al*., (2003) on garlic.

Up till now, there is no literature available on *Trachleium* propagation through tissue culture techniques in Egypt.

Therefore, the current experiments are achieved to study the micropropagation behavior of *Trachleium* explants *in vitro* as affected by some sterilization treatments, the effect of BA, Kin and TDZ and the effect of different media and strength on shooting behavior. However for rooting behavior the effects of MS salt strength and activated charcoal were studied, also the effect of some growing media on growth of plantlet during adaptation stage in order to establish a protocol for rapid propagation of *Trachleium caeruleum* plants.

2. MATERIALS AND METHODS

This investigation was carried out in plant tissue culture lab in El-Zohrya Botanical Garden, Ministry of Agriculture, Egypt, and the Fac. of Agric., Cairo Univ. during the years 2004 and 2005.

2.1. Plant material

Shoot tips (1-2 cm) from mature *Trachleium caeruleum* plants grown in the greenhouse in El-Zohrya Botanical Garden were used in this study.

2.1.1 Experiment (1): Effect of disinfection treatments on free contamination explants of *Trachleium caeruleum.*

The aim of this experiment was to study the effect of some sterilization treatments (chlorox solution at the rate of 30, 40, 50 and 60 % alone or with mercuric chloride ($HgC1_2$) at the rate of 0.0, 0.1, 0.2, and 0.4 %.

The explants were dipped in ethanol 70% for 30-seconds before sterilization treatments. One drop of Tween 20 (Polyoxyethlene sorbitan monolaurate) was used as a wetting agent per 100 ml of sterilizing solution for 20 minutes for each treatment. After sterilizing the explants, they were rinsed in sterilized distilled water (3 times) to remove all traces of the disinfectants. All steps of the sterilization method had been done under aseptic conditions inside the culture cabinet (Laminar airflow) using sterilized instruments.

The explants were cultured on Murashige and Skoog basal medium at full strength and free hormones. Each jar contained one explant. All treatments were incubated for one month. After this period the free contaminated explants were calculated. In this experiment 16 treatments replicated 3 times in each replicate 10 jars were used.

2.1.2 Experiment (2): Influence of BA, Kin and TDZ on shooting behaviours of *Trachleium caeruleum*

To study the effect of Benzyladenine (BA), Kinetin (Kin) and Thidiazuron (TDZ) on shoot development of *Trachleium caeruleum* explants. The MS medium supplemented with BA, Kin and TDZ each at the rate of 0.0, 0.5, 1.0, 2.0 and 4.0 mg/l was used. Every treatment (13 treatments) consisted of 4 replicates,each replicate jars were used jar contained one explant.

After 4, 8 and 12 weeks (representing 3 subcultures) the following data were recorded: number of shoot/explant, shoot length(cm), and the number of leaves /explant.

2.1.3 Experiment (3): Influence of different media composition on shooting behaviors of *Trachleium caeruleum*

In this experiment, four media were examined, Murashige and Skoog (MS), woody plant medium (WPM), Gamborg medium (B5) Gamborg *et al*., (1968) and Linsmaier and skoog(1965) medium (LS) at full and half strength. After 4, 8 and 12 weeks (representing 3 subcultures) from the date of culturing, the explants,the following data were recorded: number of shoot/explant, shoot length (cm), and the number of leaves /explant. In this experiment 8 treatments replicated 3 times in each replicate 4 jars were done.The chemical components of the media used are presented in Table (1).

2.1.4 Experiment (4): Influnce of MS salt strength and activated charcoal on rooting behaviors of *Trachleium caeruleum*

This experiment was carried out to study the effect of MS salt strength at full strength, ¾ strength, ½ strength, and ¼ strength on root formation in the presence of activated charcoal (A.C) at 1.0 g/L or without it.

Eight treatments contains 5 replicates each replicate contain three explant were done. The following data were recorded after 30 days on number of roots/explant, root length (cm), plant height(cm), and number of leaves/shoot.

2.1.5 Experiment (5): Effect of some growing media on acclimatization of *Trachleium caeruleum.*

This experiment was conducted in the greenhouse to evaluate the effect of some growing media on the survival percentage, plant length and the number of leaves of *Trachleium caeruleum* plantlets during the acclimatization stage.

Table (1): Chemical components of the media used, (MS, WPM, B5, LS).

Chemical components	Recommended media, type and concentration mg l^{-1}.			
	MS	**WPM**	**B5**	**LS**
Macro elements : NH_4NO_3	1650	400	-	1650
KNO_3	1900	-	2500	1900
$CaCl_2$	440	96.0	113.23	332.02
$MgSO_4.7H_2O$	370	370	121.56	180.54
KH_2PO_4	170	170	-	170
$(NH_4)_2SO_4$	-	-	134	-
$NaH_2PO_4.H_2O$	-	-	-	-
$Ca(NO_3)_2.4H_2O$	-	556	-	-
K_2SO_4	-	990	-	-
Micro elements : H_3BO_3	6.2	6.2	3.00	6.20
$MnSO_4.H_2O$	16.9	22.3	10.00	16.90
$ZnSO_4.7H_2O$	8.6	8.6	2.00	8.60
KI	0.83	-	0.75	0.83
$Na_2MoO_4.2H_2O$	0.25	0.025	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025
$COCl_2.6H_2O$	0.025	-	0.025	0.025
$FeSO_4.7H_2O$	27.80	27.8	-	-
Na_2 EDTA ($2H_2O$)	37.30	37.3	36.70	36.70
Organic components: Myo-inositol	100.0	100.0	100.0	100.0
Nicotinic acid	0.50	0.5	1.00	-
Thiamine HCl	0.1	1.0	10.00	0.40
Pyridoxine HCl	0.5	0.5	1.00	-
Glycine	-	2.0	-	-

The plantlets (6-7 cm length with 10-12 leaves) produced *in vitro* were individually transplanted into 8 cm plastic pots filled with: peatmoss 100%, peat moss + sand (1:1 v/v), peat moss + vermiculite (1:1 v/v) or peat moss + perlite (1:1 v/v).

At the end of this experiment (after four weeks), the data were recorded on survival percentage, length of plantlet, and the number of leaves/plantlet.

In this experiment four treatments replicated three times and in each replicate 10 plantlets were used.

2.1.6 Experimental design and data analysis

Every experiment was arranged in a completely randomized design in factorial experiment. Data analysis of variance was carried out according to Steel and Torrie (1980), using Duncan's Multiple Rang Test $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1. Experiment 1: Effect of disinfection treatments on free contaminated explants

The data in Table (2) indicate that using chlorox alone at different concentrations increased the percentage of free contaminated explants, and this increase positively correlated by increasing the concentration from 30 to 60%. The highest free contaminated explants (0.60) were recorded by using 50 and 60% chlorox alone. In general the highest free contaminated explants (1.00) resulted from using chlorox at 60% plus M.C. at 0.2 or 0.4%. However, in the case of using chlorox at the rate of 50% plus M.C. at 0.4% the free contaminated explants also recorded the highest value (1.00). Therefore these two treatments were the most effective in producing 100% of free contaminated explants in *Trachleium caeruleum*.

These results may be due to the liability of plant tissue of *Trachleium* to excessive surface sterilization with $HgCl_2$ which has a lysis effect on microbial cells as stated by Russel and Chopra (1990), Arafa *et al*., (1999) and Hussien (2002). They reported that surface sterilization with $HgCl_2$ followed by chlorox resulted in the highest decontamination and survival percentage of *Dieffenbachia exotica* cv. 'Tropic' and *Aglonema* spp., respectively.

The obtained results are in agreement with the findings of Hosni *et al*., (2000) on *Limonum sinulatum* var 'Citron Mountain', and El-Sayed (2005) on some woody plants.

Table (2): Effect of disinif ection treatments on free contamination of *Trachleium caeruleum* **explants.**

Mercuric chloride (M.C.)	Chlorox percentage				
	30%	**40%**	**50%**	**60%**	**Mean (B)**
0.0%	0.200 D	0.400 CD	0.600 BC	0.600 BC	0.450 B
0.1%	0.200 D	0.400 CD	0.600 BC	0.600 BC	0.450 B
0.2%	0.600 BC	0.800 AB	0.900 AB	1.000 A	0.825 A
0.4%	0.700 A-C	0.800 AB	1.000 A	1.000 A	0.875 A
Mean (A)	0.425 C	0.600 B	0.775 A	0.800 A	——

Means with different letters in the same column are significantly different ($P < 0.05$) using Duncan's multiple range test.

3.2. Experiment 2: Influence of BA, Kin, and TDZ on shooting behavior of *Trachleium caeruleum*.

3.2.1 Number of shoots (Table 3 and Photo 1).

The results clearly indicated that the addition of Kin at the rate of 2.0 or 4.0 mg/l significantly increased the number of shoots/explant compared to the other treatments. These two concentrations produced significantly the highest shoot number /explants (28.33 and 28.27) respectively. Whereas, the addition of TDZ at the concentrations of 0.5, 1.0, 2.0, and 4.0 mg/l significantly decreased the number of shoots/explant. The lowest number of shoots/explant (2.60) resulted for MS free hormone (control).

The number of subcultures had a high significant effect on the number of shoots/explant. The greatest number of shoots/explant (26.58) resulted from the 3rd subculture, whereas the 1st subculture produced the lowest value (7.61).

The interaction effect of BA, Kin, and TDZ and the number of subcultures showed that in the 1st and 2nd subcultures the highest number of shoot/explants (13.40 and 28.80) were produced on MS medium plus BA at 4.0 mg/l respectively. However, in the 3rd subculture the greatest number of shoot/explants (51.60) was resulted for MS medium plus Kin at 4.0 mg/l.

In general, MS medium supplemented with Kin at the rate of 2.0 mg/l was the best treatment in producing significantly the greatest number of shoot/explant.

The above mentioned results are in agreement with the findings of El-Sawy and Bekheet (1999) on *Dieffenbachia picta* cv. 'Tropica' and El-Sawy *et al*., (2000) on *Dracaena margenata* cv. 'Tricolour'.

3.2.2 Shoot length

It is clear from the data that shoot length was significantly affected by adding Kin, BA and TDZ to the MS medium as compared with free hormones (control) medium. The average shoot length ranged between 1.83 and 4.90 cm. The longest shoot (4.90 cm) was recorded for explants grown on MS medium supplemented with 1.0 mg/l Kin. Using MS medium supplemented with BA or TDZ significantly reduced the length of shoots ,and the rate of reduction in shoot elongation was in proportion with the rate of increase in BA or TDZ concentrations. Culturing the explants on MS medium supplemented with 4.0 mg/l TDZ resulted in the shortest shoots, (1.83 cm) as compared to the MS free hormones (3.467 cm).

A significant effect of number of the subcultures on the length of shootlet was recorded. The shootlet length was significantly higher at the 3rd subculture (3.81 cm) than at 2nd (3.47 cm) and 1st subculture (2.53 cm).

The data indicated that in the 1st subculture, growing the explants on MS medium supplemented with 1.0 mg/l Kin resulted in the longest shoots (3.10 cm), whereas, the explants grown on MS medium supplemented with 4.0 mg/l TDZ gave the shortest shoots (2.00 cm). In the 2nd and 3rd subcultures, similar results were recorded.

3.2.3 Number of leaves

The highest number of leaves/explant was recorded by culturing the explants on MS medium supplemented with Kin at 1.0, 2.0 and 4.0 mg/l. and BA at 0.5 mg/l, whereas, the lowest number was recorded for explants grown MS medium free hormones (control) or MS supplemented TDZ at 2.0 and 4.0 mg/l. (18.47, 23.93 or 23.73) respectively.

There were significant increases in the number of leaves per explant as the number of subcultures increased. The average number of leaves (24.42, 87.45 and 152.2) in the three subcultures respectively.

The most effective treatment in this character was the MS medium supplemented with Kin at 4.0 mg/l.

Kin increased the number of shoots/explant over the control by 112%, the shoot length by 13% and the number of leaves by 66%.

In the case of TDZ the percentage of increase in the number of shoots/explant over the control reached 40%. Following the same trend it caused a reduction in shoot length by 63%. On the other hand BA treatments increased the number of shoot/explants over the control by 94%, and decreased the shoot length by 9% compared to the control and increased the number of leaves, the percentage of increase reached 60%.

In general Kin significantly increased shoot number/explant, shoot length and the number of leaves. However, BA and TDZ significantly increased the number of shoots/explant and number of leaves also, it significantly decreased the shoot length over the control.

TDZ is a synthetic cytokinin that has been used in micropropagation of woody species such as walnut, silver maple (*Acer saccharinum* L.) and white ash (*Fraxinus americana* L.) (Heutteman and Preece, 1993). It has been reported that it promotes shoot initiation but inhibits shoot

elongation at high concentrations (Donna and Preece 2003).

The above mentioned results indicated the same trend. Both BA and TDZ promote shoot initiation but inhibit shoot elongation.

3.3 Experiment 3: Influence of different media composition on shooting behavior of *Trachleium caeruleum*

3.3.1 Shoot number/explant (Table, 4)

The number of shoot/explants varied from 17.27 to 39.00. The largest number (39.00) resulted from using LS full strength medium. However, the lowest number (17.27) was recorded in the case of using WPM at half strength medium. LS and MS at full strength significantly promoted the shoot initiation compared to WPM and B5 media at full strength. One can observe also that half strength in all used medium inhibited the shoot induction compared to full strength . LS medium at full strength significantly increased shoot number / explants compared to MS at full strength and this increase reached32.7%.This means that LS at full strength was the most effective medium in promoting shoot initiation.

Increasing the number of subcultures significantly increased the shoot formation on the explants. The 3rd subculture significantly produced the greatest shoot number/explants (39.67).

The interaction effect showed that LS medium at full strength in the 3rd subculture recorded significantly the highest value (63.60) compared to the other treatments. The effect of different growing media on shoot number was studied by Douglas *et al*., (1986) on *Hydrangea macrophylla* explants. They found similar results as those observed in this study.

3.3.2 Shoot length

LS and MS media at half strength significantly produced the longest shoots (4.26 and 4.33 cm) respectively. However, the B5 medium gave the shortest shoots (3.30 cm). Full strength from MS, WPM or LS media gave the same results in shoot length. However, B5 at full strength produced significantly the shortest shoots. This means that LS or MS medium at full strength were the most effective medium in increasing the shoot length.

The 3rd subculture produced the longest shoots (4.91 cm). The data on the interaction indicated that LS or MS at half strength significantly increased shoots length. The present results are in agreement with the finding of Douglas *et al*., (1986) on *Hydrangea macrophylla* explants.

3.3.3 Leaf number/explant

The average number of leaves/explant varied from 60.5 to 219.6, the largest leaves number resulted from using LS medium at full strength, however, the lowest leaf number (60.5) was recorded from WPM medium at half strength. In general half strength from the different media reduced the number of leaves/explants. LS medium at full strength significantly increased the number of leaves over MS, WPM or B5 at full strength and this increase reached 27%, 181% or 36.6% respectively. This means that LS medium at full strength was the most effective treatment in producing the greatest number of leaves/explant.

The same trend was observed in the case of shoots number/explant. In the first subculture no difference in number of leaves/explant was observed. However, the number of leaves significantly increased in the 2nd and 3rd subcultures. The percentages of increase in leaf number/explant reached 12.3% and 108.9% respectively compared to the 1stsubculture.

3.4 Experiment (4): Influence of MS salt strength and activated charcoal on rooting formation and shootlet growth of *Trachleium caeruleum*.

3.4.1 Number of roots (Table, 5 and Photo, 2).

The average mean of root number/plantlet varied from 3.25 to 7.25. Using MS medium at ¼ strengh produced significantly the highest root number was (7.25), whereas, the lowest value (3.25) found in MS medium at full strength. The number of roots increased significantly by decreasing MS salt strength.

Concerning the use of activated charcoal the data indicated that activated charcoal significantly increased the number of roots/plantlet. The greatest number of roots (6.31) resulted from the use of activated charcoal. This treatment increased the number of roots compared to without A.C. This increment may be due to the application of activated charcoal to the media. Concerning the interaction, the data show that the highest number of root (10.50) was recorded when culturing on MS medium at ¼ salt strength in the presence of activated charcoal, while the lowest number of roots (2.75) was recorded when culturing on MS medium at full strength and without activated charcoal.

In conclusion, the best number of root/shoot was recorded with ¼ MS salt strength and with activated charcoal.

3.4.2 Root length

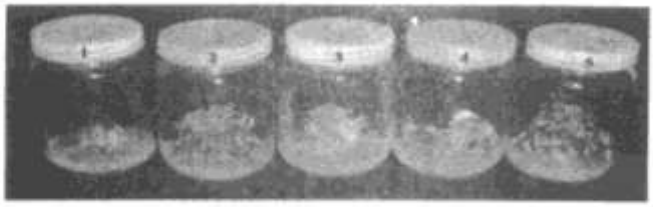
The data (Table, 5) showed that, the longest

Table (3): Influnce of BA, Kin, and TDZ concentrations and the number of subculture on shoot number/explant, shoot length (cm), and leaf number/explant of *Trachleium caeruleum* explants grown *in vitro*.

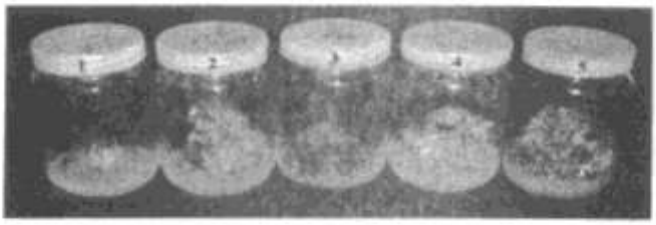
Culturing media (concentration/L)	Shoot number/explant				Shoot length (cm)				Leaf number/explant			
	No. of subculture			Mean (A)	No. of subculture			Mean (A)	No. of subculture			Mean (A)
	1	2	3		1	2	3		1	2	3	
MS (control)	2.20 N	2.40 N	3.2 MN0	2.60 C	2.30 I-N	3.40 D-H	4.70 BC	3.46 D	15.20 K	19.00 JK	21.20 JK	18.47 E
MS + 0.5 mg Kin	7.40 J-K	20.60 FG	36.20 C	21.40 CD	2.90 F-K	4.70 BC	5.90 A	4.50 AB	25.60 JK	102.2 FG	216.0 B	114.7 B
MS + 1.0 mg Kin	8.00 I-N	24.20 EF	43.80B	25.33 AB	3.10 E-J	5.50 AB	6.10 A	4.90 A	21.20 JK	138.0 C-E	292.0 A	150.4 A
MS + 2.0 mg Kin	8.200 I-N	20.20D-F	50.60 A	28.33 A	2.60 G-M	4.70 BC	5.90A	4.40 AB	26.46 JK	133.0 C-F	276.0 A	145.1 A
MS + 4.0 mg Kin	8.00 I-N	25.20D-F	51.60 A	28.27 A	2.40 I-N	4.30 CD	5.40 AB	4.03 BC	27.60 JK	128.8 D-F	296.0 A	150.8 A
MS + 0.5 mg BA	8.400 I-N	16.60 GH	43.60 B	22.87 B-D	2.80F-K	3.50 D-G	4.30 CD	3.53 CD	30.60 JK	112.2 EF	268.0 A	136.9 A
MS + 1.0 mg BA	8.00 I-N	25.60D-F	28.60 DE	20.73 CD	2.60 G-M	3.60 D-F	4.20 CD	3.46 D	25.00 JK	115.0 D-F	200.0 B	113.3 B
MS + 2.0 mg BA	12.60H-K	12.40 DE	31.00 CD	23.67 BC	2.50 H-N	3.20 E-I	3.90C-E	3.20 DE	31.00 JK	133.0 C-F	162.0 C	108.7 B
MS + 4.0 mg BA	13.40 H-J	28.80 DE	16.80 GH	19.67 D	2.50 H-N	3.10 E-J	1.70 M-O	2.433 FG	27.60 I-K	146.0 CD	74.00 GH	85.87 C
MS + 0.5 mg TDZ	6.60 K-N	17.20 GH	14.00 HI	12.36 E	2.400 I-N	3.11 E-J	2.70 F-L	2.73 EF	22.00 JK	40.60 I-K	66.00 HI	42.87 D
MS + 1.0 mg TDZ	6.80 K-N	12.00H-K	11.40 H-L	10.07 EF	2.50 H-N	2.30 I-N	1.80 L-O	2.20 GH	18.40 K	27.40 JK	50.00 H-J	31.93 DE
MS + 2.0 mg TDZ	5.00 L-N	9.40 I-M	8.60 I-N	7.66 F	2.30 I-N	1.60 NO	1.70 MO	1.86 H	20.00 JK	21.80 JK	30.00 JK	23.93 E
MS + 4.0 mg TDZ	4.40 MN	9.0 I-M	6.20 K-N	6.53 F	2.00K-O	2.20J-O	1.30 O	1.83 H	16.80 K	26.40 JK	28.00 JK	23.73 E
Mean (B)	7.61 C	18.82 B	26.58 A	___	2.53 C	3.47 B	3.81 A	___	24.42 C	87.45 B	152.2 A	___

Means with different letters in the same column are significantly different (P< 0.05) using Duncan's multiple range test.

BA



Kin



TDZ

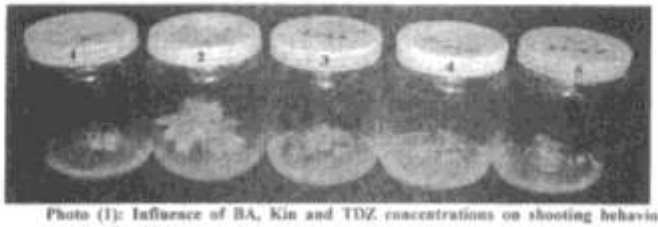


Photo (1): Influence of BA, Kin and TDZ concentrations on shooting behaviors of *Trachlelum caeruleum*.
1- Control (free hormones) 2- 0.5 mg/l. 3- 1.0 mg/l. 4- 2.0 mg/l. 5- 4.0 mg/l.

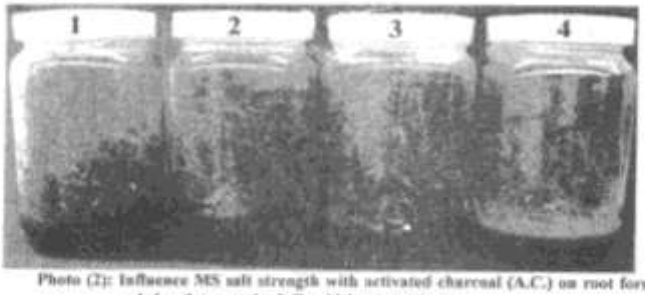


Photo (2): Influence MS salt strength with activated charcoal (A.C.) on root formation and shootlet growth of *Trachlelum caeruleum*.
1- Full MS (control). 2- 3/4 MS. 3- 1/2 MS. 4- 1/4 MS.

Table (4): Influence of different media composition and the number of subcultures on shooting behaviors of *Trachleium caeruleum*.

Culturing media	Shoot number/explant				Shoot length (cm)				Leaves number/explant			
	No. of subculture			Mean (A)	No. of subculture			Mean (A)	No. of subculture			Mean (A)
	1	2	3		1	2	3		1	2	3	
MS Full	**14.80 KL**	**31.00 E-G**	**43.80 BC**	**29.87 B**	**2.40 G**	**4.00 C**	**5.20 A**	**3.86 B**	**24.40 I**	**124.0 EF**	**270.0 C**	**139.5 CD**
MS Half	**10.60 LM**	**17.80 JK**	**29.80 EH**	**19.40 D**	**2.80 E-G**	**4.60 B**	**5.60 A**	**4.33 A**	**20.80 I**	**128.0 D-F**	**232.0 C**	**126.9 D**
WPM Full	**13.20 K-M**	**26.00 GH**	**36.00 DE**	**25.07 C**	**2.50 FG**	**4.40 BC**	**4.60 B**	**3.83 B**	**22.80 I**	**100.0E-G**	**174.0 D**	**98.93 E**
WPM Half	**7.20 M**	**19.20 I-K**	**25.40 G-I**	**17.27 D**	**2.60 FG**	**3.20 DE**	**4.60 B**	**3.46 C**	**19.60 I**	**72.00 GH**	**90.00 FG**	**60.53 F**
B5 Full	**7.00 M**	**27.60F-H**	**39.00 CD**	**24.53 C**	**2.40 G**	**3.40 D**	**4.40 BC**	**3.40 C**	**22.20 HI**	**118.0E-G**	**336.0 B**	**159.7 BC**
B5 Half	**7.80 M**	**19.20 I-K**	**33.00 D-F**	**20.00 D**	**2.80 E-G**	**3.00 D-F**	**4.10 BC**	**3.30 C**	**21.20 I**	**84.00 FG**	**266.0 C**	**123.7 DE**
LS Full	**15.40 KL**	**38.00 CD**	**63.60 A**	**39.00 A**	**2.30 G**	**4.20 BC**	**5.20 A**	**3.90 B**	**22.80 I**	**174.0 D**	**462.0 A**	**219.6 A**
LS Half	**10.00 LM**	**24.00 H-J**	**46.80 B**	**26.93 BC**	**2.60 FG**	**4.60 B**	**5.60A**	**4.26 A**	**22.40 I**	**138.0 DE**	**352.0 B**	**170.8 B**
Mean (B)	**10.75 C**	**25.35 B**	**39.67 A**	**___**	**2.55 C**	**3.92 B**	**4.91 A**	**____**	**22.40 I**	**117.3 B**	**272.8 A**	**____**

Means with different letters in the same column are significantly different (P< 0.05) using Duncan's multiple range test.

Table (5): Influence of MS salt strength and activated charcoal on rooting formation and shootlet growth of *Trachleium caeruleum*.

Culturing media	Root number/plantlets		Mean (A)	Root length (cm)		Mean (A)	Plantlet height (cm)		Mean (A)	No. of leaves plantlets		Mean (A)
	With Activated charcoal	Without Activated charcoal		With Activated charcoal	Without Activated charcoal		With Activated charcoal	Without Activated charcoal		With Activated charcoal	Without Activated charcoal	
Full MS	**3.75 C-E**	**2.75 E**	**3.25 C**	**3.75 D**	**2.12 E**	**2.93 B**	**7.625 A**	**5.875 C**	**6.75AB**	**15.00 A**	**10.50 BC**	**12.75A**
3/4 MS	**3.75 C-E**	**3.25 DE**	**3.50 C**	**5.00 CD**	**2.25 E**	**3.62 B**	**7.37 AB**	**5.37 C**	**6.37 B**	**16.50 A**	**10.50 BC**	**13.50A**
1/2 MS	**7.25 B**	**4.75 C**	**6.00 B**	**7.75 B**	**5.50 C**	**6.62 A**	**8.37 A**	**6.37 BC**	**7.37 A**	**15.00 A**	**12.25 B**	**13.63A**
1/4 MS	**10.50 A**	**4.00 CD**	**7.25 A**	**9.50 A**	**4.50 CD**	**7.00 A**	**7.75 A**	**6.25 C**	**7.00AB**	**9.25 C**	**10.00 C**	**9.62 B**
Mean (B)	**6.31 A**	**3.68 B**	**____**	**6.50 A**	**3.59 B**	**____**	**7.78 A**	**5.96 B**	**____**	**13.25A**	**11.50 B**	**____**

Means with different letters in the same column are significantly different (P< 0.05) using Duncan's multiple range test.

root (6.62 and 7.00 cm) was recorded when using MS medium at ½ and ¼ salt strength, respectively.

Concerning of activated charcoal the longest root (6.50 cm) was recorded with its presence in the media.The data recorded on the interaction revealed that, the longest root (9.50cm) was recorded when culturing on MS medium at ¼ salt strength in the presence of activated charcoal,while the shortest root length (2.12 cm) was recorded when culturing on MS medium at full strength and without activated charcoal (control).

In conclusion, the best root length was recorded with ½ and ¼ MS salt strength and with activated charcoal.

3.4.3 Plant height

The data indicated that, the tallest plantlets (7.37 cm) was recorded when using 1/2 MS salt strength medium.

MS medium with activated charcoal gave (7.78 cm) which was significantly taller than those grown on MS media without activated charcoal. The tallest plants (8.37 cm) were recorded when using MS medium at ½ salt strength and activated charcoal.

3.4.4 Number of leaves/plantlet

The data indicated that the highest number of leaves/plantlet (13.63, 13.50 and 12.75) were recorded by using MS medium at ½, ¾, and full salt strength respectively, while the lowest number (9.62) was produced by using 1/4 MS salt strength medium.

Moreover, adding activated charcoal to MS medium significantly increased the number of leaves/plantlet as compared with MS medium free of charcoal. The same trend was observed on the root number and length and plantlet height.

3.5 Experiment (5): Effect of cclimatization media of *Trachleium caeruleium*

3.5.1 Survival percentage: (Table, 6)

The different growing media used in this study had no significant effect on the survival percentage (Table, 6). This means that all growing media during rooting stage produced healthy plants during acclimatization and showed high survival percentage.

3.5.2 Plant height

It is evident from the obtained data (Table, 6) that the different growing media had a significant effect on plant height after harding off. The data indicated that, the tallest plant (14.67 cm) was recorded when using peatmoss, and the shortest plants (9.60 cm) were recorded with peatmoss + perlite (1:1 v/v).

3.5.3 Number of leaves/plant

The obtained data (Table, 6) showed that the different growing media had a significant effect on the number of leaves/plantlet after harding off. The data indicated that, the greatest number of leaves/plant (20.33) was recorded when using peatmoss, and the lowest number of leaves/plant (14.00) was recorded with peat moss + perlite (1:1 v/v).

Table (6): Mean percentage of survival, height and number of leaves/plantlet of *Trachleium caeruleum* as affected by different growing media during acclimatization stage.

Treatments	Survival %	Plant height (cm)	Number of leaves/plant
Peat moss	100 A	14.67 A	20.33 A
Peat moss + sand (1:1)	100 A	12.67 B	17.33 B
Peat moss + vermiculite (1:1)	100 A	10.67 C	15.67 C
Peat moss + perlite (1:1)	100 A	9.60 D	14.00 D

Means with different letters in the same column are significantly

The above mentioned results are in agreement with the findings of Abou Dahab *et al*., (2004 and 2005). Thus peat moss was the best medium for producing the highest means of plant height and the greatest number of leaves/plant.

4.REFERENCES

Abd El-Rahman S. S. (2002). Physiological Studies on the Microprpagation of *Solidago* sp. by *in vitro* Culture. Ph D. Thesis, Fac. of Agric. Cairo Univ., Egypt.

Abou Dahab A. M., Habib A. M. A., Hosni Y.A. and Gabr A. M. M. (2004). Studies on propagation of *Aspidistra elatior* Blume by tissue culture. Annals of Agric. Sci. Moshtohor, Vol 42 (1): 299-319.

Abou Dahab A.M., Habib A. M. A., Hosni Y. A. and Gabr A. M. M. (2005). Effect of some sterilization treatments and growth regulators on *Ruscus hypoglossum* L. Arab Journal of Biotechnology. Vol. 8, No. (1) Jan. 127-140.

Agrawal D. C., Morwal G. C. and Mascarenhas A. F. (1992). *In vitro* propagation and slow growth storage of shoot cultures of *Vanilla walkeriae* Wight – An endangered orchid. Lindlegana, 7 (2): 95-99.

Arafa A. M. S., Ibrahim M. K. H. and Ibrahim I. A. (1999). Role of benzyladenine and activated charcoal in optimizing the culture media of *in vitro* culture *Diffenbachia exotica*

cv. 'Tropica-Snow. Bull. Fac. Sci. Assuit Univ. 28 (2-D): 187-198.

Chen C., Cahng-Tesrm C., Yu-Ching T. and Wei-Chin C. (2000). A tissue culture protocol for propagation of a rare plant *Lilium speciosum*, Thunb. var. 'Gloriosoides Baker'. Bot. Bull. Acad. Sin., 41: 139-142.

Donna T. Ledbether and Preece I. E. (2003). Thidizuron stimulates adventitious shoot production from *Hydrangea quercifolia*. Leaf explants. Dep. Of plant, soil and General Agriculture, Souther, Illinois Univ. Carbondale, 1L 62901-4415, USA.

Douglas A. Bauley, Gary R. Seckinger and Allen Hammer P. (1986). *In vitro* propagation of Florists Hydrangea. Hort. Science. Vol 21 (3): 525-526.

EL-Sawy A. and Bekheet S. A. (1999). Propagation of *Dieffenbachia* through tissue culture. Egyption Journal of Botany. 39: (1), 97-107.

EL-Sawy A., Bekheet S. A. and Hosni Y. A. (2000). A protocol for micropropagation of *Dracaena marginata*. Egyption Journal of Horticulture, 27: (1), 29-40.

El-Sayed H. M. F. (2005). *In vitro* Clonal Propagation and Reservation of Genetic Resources of Some Woody Plants. Ph.D. Thesis, Fac. of Agric., Cairo University,Egypt.

Gamborg O. L., Miller R. A. and Ojima K. (1968). Nutrient requirement of suspensions cultures of soybean root cells. Exp. Cell Res., 50, 151.

Graf A.B.(1992).Hortica Color Cyclopedia of Garden Flora. Roehrs Company Publishers USA. 1216.

Haque M.A., Nath U. K., Ahmed Q. N. and Alam S. (2003). Effect of 2,4-D and BAP pn *in vitro* regeneration of garlic. Online Journal of Biological Sciences, 2 (12): 771-774.

Heutteman C. A. and Preece J.E. (1993). Thidazuron a potent cytokinin for woody plant tissue culture. Plant cell Tiss. Org. Cult. 33, 105-119.

Hosni A. M., Hosni Y. A and Ibrahim M. A. (2000). *In vitro* micropropagation of *Limonium sinnuatum* "Citron Mountain", A hybrid statice newly introduced in Egypt. Annals Agriculture Science, Ain Shams Univ., Cairo, 45 (1): 327-339.

Hussein M. M. M. (2002). *In vitro* propagation of three species of *Aglonema* plants. Bull. Fac. Agric., Cairo Univ., 53: 465-488.

Linsmaier E. M. and Skoog F. (1965). Organic growth factor requirements of tobacco tissue cultures. Physiol. Plantarum, 18, 100-127.

Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473–497.

Pereira- Pinto J. E. B., Fonseca-Arello E, Pereira-Pinto C. A. B. and Pereira-Barbosa M. H. (1996). *In vitro* regeneration and growth response of *Kielmeyera coriaces* shoot when affected by salts and sucrose concentrations. Ciencia Rural, 26 (1): 57-61.

Russel A. D. and Chopra L. (1990). Understanding Antibacterial Action and Resistance. Elis Horwood, New York.

Sakr S. S., El-Khateeb M. A. and Abd El-Kareium A. H. (1999). *In vitro* production of *Yucca elephantips*, Bull of Fac. of Agric. Cairo Univ., 50 (2): 265-282.

Steel R. G. D. and Torrie J. H. (1980). Principles of Statistics. A Biometrical Approach. Second Ed., Mc Graw-Hill Kogakusha, L. T. D.

وضع بروتوكول للإكثار الدقيق لنباتات التراكليم عن طريق زراعة الأنسجة

طارق أبودهب محمد

قسم الزينة – كلية الزراعة – جامعة القاهرة – الجيزة – مصر

ملخص

أجرى هذا البحث في معمل زراعة الأنسجة بحديقة الزهرية و كلية الزراعة جامعة القاهرة خلال الأعوام 2004و2005 بهدف عمل بروتوكول للإكثار الدقيق لنباتات التراكليم عن طريق زراعة الأنسجة .

و أظهرت النتائج ما يلى:

التجربة الأولى: تأثير بعض معاملات التعقيم على إنتاج عزلات نباتية خالية من التلوث. حيث وجد أن استعمال الكلوروكس بتركيز 60% مضاف أليه كلوريد الزئبقيك .M.C بنسبة 0.2% أعطت أفضل النتائج.

التجربة الثانية: تأثير أنواع مختلفة من السيتوكينينات (BA, Kin, TDZ) بتركيزات مختلفة على سلوك الإكثار الدقيق و تكوين الأفرع. و أظهرت النتائج أن استعمال بيئة MS مضاف إليها الكينتين بتركيز 4 مجم/لتر أعطى اكبر عدد من الأفرع و الأوراق للعزلة النباتية. أدى زيادة عدد النقلات إلى زيادة معنوية في جميع الصفات وكانت النقلة الثالثة أفضل النقلات معنويا, كما أن عدد الأفرع انخفض معنويا نتيجة إضافة مادة TDZ للبيئة, بينما أدت إضافة الكينتين بتركيز 1 مجم/لتر إلى البيئة إلى الحصول على أفضل النتائج بالنسبة لطول الأفرع الناتجة.

التجربة الثالثة: تأثير البيئات المختلفة حيث أظهرت النتائج أن استخدام التركيز الكامل لبيئة LS أعطت أفضل النتائج معنويا بالنسبة لعدد الأفرع و عدد الأوراق, و أن زيادة عدد النقلات أعطى زيادة معنوية في عدد الأفرع الناتجة. أما بالنسبة لطول الأفرع فان أطولها نتج عند استعمال التركيز الكامل و نصف الكامل لبيئة LS في النقلة الثالثة.

التجربة الرابعة: تأثير التركيزات المختلفة من بيئة MS و الفحم النشط على تجذير الفروع الناتجة حيث أظهرت النتائج أن استخدام ¼ تركيز من بيئة MS مع الفحم النشط أعطى زيادة معنوية في عدد الجذور و طول الجذور كما أن أطول الأفرع و أكبر عدد من الأوراق نتج عند استخدام ½ تركيز من بيئة MS مع الفحم النشط.

التجربة الخامسة: تأثير بيئات الأقلمة: أظهرت النتائج أن استخدام البيت موس أعطى أفضل النتائج بالنسبة لطول النبتة و كذلك عدد الأوراق, كما أن جميع بيئات الأقلمة المختلفة أظهرت قدرة عالية جدا على بقاء النباتات بحالة جيـــدة و بنسبة 100%.