Effect of some sterilization treatments and growth regulators on *Ruscus hypoglossum* L.

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ABSTRACT

This study has been undertaken at Tissue Culture Lab., El-Zoharya Bot. Garden, Ministry of Agric., Egypt, and Fac. of Agric. Cairo Univ., during the period 2001-2004 to study the effect of sterilization treatments on percentage of free of contamination explants and the effect of benzyladenine (BA), Kinetin (Kin) and Thidiazuron (TDZ) and different combinations of benzyladenine (BA) and Kinetin (Kin) on shooting behavior and chemical composition of Ruscus hypoglossum L.

The best treatment which can be recommended to obtain free of contamination explants was 50 or 60% chlorox with 0.4% mercuric chloride (M.C.).The addition of BA at 1.5 mg/1 in MS medium produced the highest number of shoots/explant. Increasing the number of subcultures significantly increased the number of shootlets, shootlet length, and number of leaves per explant. Using MS medium supplemented with 6.0 mg/l Kin gave the longest shoots, and the highest number of leaves. Using MS supplemented with TDZ at 4.5 mg/l produced the highest amounts of chlorophyll-A, and carotenoids, while TDZ at 6.0 mg/l produced the highest amount of chlorophyll-B. The highest content of indoles was produced by using MS medium supplemented with TDZ at 3.0 mg/l. Using 1.5 mg/L BA + 2.0 mg/l Kin produced the highest number of shootlets per explant, shoot length and number of leaves. Using MS medium free hormones (control) increased the amount of chlorophyll-A to the highest value, but the highest amount of chlorophyll-B and carotenoids was produced by using MS medium supplemented with 1.5 mg/l BA + 2.0 mg/l Kin. The amount of indoles was increased to the highest amount by using MS medium supplemented with 1.5 mg/l BA + 2.0 mg/l Kin. While total soluble phenols reached to the highest amount by using MS medium supplemented with 6.0 mg/l BA + 2.0 mg/l Kin.

Key words: *Ruscus hypoglossum, micropropagation, sterilization, growth regulators.*

INTRODUCTION

Foliage plants can be propagated by conventional methods such as seeds, cuttings, division, but the use of tissue culture technique in vegetative propagation of these plants can be considered an alternative method to obtain high quantities of plants and also the most extensive commercial use of tissue culture is in the rapid clonal propagation of plants.

Plants from the *Liliaceae* family are among the most widely grown as foliage plants, and include many species such as *Ruscus hypoglossum* L., *Aspidistra elatior* Blume and *Asparagus* spp. *Ruscus hypoglossum* L, a native to the region from Western Europe to Iran, is a compact evergreen shrub with a creeping rootstock that can reach 18 inches length. It is an interesting plant with leaf-like cladoles of 1½ to 3 inches with wide tapering at both ends (the true leaves are the smaller green appendages around the flowers). The success of tissue culture in propagation of ornamental plants is greatly influenced by the nature of culture medium used. The nutrient medium has two major functions, the first is to supply the basic nutritional ingredients for continued growth of isolated explants and subsequent propagules; the second function is to direct growth and development through hormonal control (George and Sherrington, 1984). 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) for propagation of *Vanilla walkeriae*, Pereira Pinto *et al*. (1996) for *Kielmeyera coriacea*, Torres and Mogollan (1997) for *Cattleya lueddemanniona*, Karhu, (1997) for *Lonicera caerulea* and Sakr *et al*. (1999) for *Yucca elephantipes.* The aim of this study was to investigate the effect of some sterilization treatments on percentage of free of contamination-explants and the effect of some growth regulators on shooting behaviour and chemical composition of *Ruscus hypoglossum.*

MATERIALS AND METHODS

This investigation was carried out in Plant Tissue Culture Laboratory in El-Zoharya Botanical Garden [Unit of Horticultural Services the Minsitry of Agric., Egypt and Faculty of Agriculture, Cairo University during the period from 2001 to 2004. Buds of rhizomes of *Ruscus hypoglossum* plant were used as a source of explants.

The buds were cut, put in polyethylene bags and transferred directly to the laboratory. The explants were washed in soap water using septol soap for 30 minuets and rinsed with running tap water for two hours. The length of the buds was 1.5 to 2 cm .Buds were surface sterilized. The MS (Murashige and Skoog, 1962) basal medium was prepared at full strength. Cytokinins, auxins and other culturing materials were added. After the preparation of the medium, the pH value was adjusted to 5.7±0.1 by using a few drops of either potassium hydroxide (KOH) or hydrochloric acid (HC1). Agar was used as a solidifying agent at 6 gm/L concentration. After mixing the different components, the culturing medium was heated to dissolve the agar and dispensed into Pyrex glass jars at the rate of 50 ml for each jar (350 ml) and plugged with polypropylene closure caps. The media were autoclaved at 121°C (1.2 kg/cm^2 for 20 minutes) and then cooled. Jars were kept in slanting position for a week.

The cultures were incubated under a temperature of 24±2°C day/night. Photo-period of 16 hours light/8hours darkness and illumination intensity of 3000 lux at the top of culture levels resulted from fluorescent lamps (120 cm long) and measured by lux meter.

Experiment (1): Effect of sterilization treatments

After preparation of the explants, the buds were sterilized by using a combination of sterilization treatments of chlorox solution at 30, 40 , 50, 60 and 70 % and mercuric chloride ($HgC1_2$) at 0.0, 0.1, 0.2, and 0.4 % i.e. 20 sterilization treatments.

The explants were dipped in ethanol 70% for 30 second before Clorox and mercuric chloride treatments. One drop of Tween 20 (Polyoxyethlene sorbitan monolaurate) was used as a wetting agent per 100 ml of sterilizing solution. After sterilizing the explants for 20 minutes, 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 of the culture cabinet (Laminar airflow) using sterilized instruments.

After that the explants were cultured on full strength and free of hormones Murashige and Skoog basal medium. Each jar contained one explant. All treatments incubated for one month. After this period the percentage of free of contamination explants was recorded.

Experiment (2): Effect of growth regulators on shoot formation and chemical composition

The explants were cultured on (MS) medium supplemented with different concentrations of Benzyladenine(BA), Kinetin (Kin) and Thidiazuron (TDZ) at 0.0, 1.5, 3.0, 4.5 and 6.0 mg/l Concentrations. Every treatment consisted of 4 replicates (each jar contained one explant).

This experiment consisted of 13 treatments. The data were recorded after 6, 12 and 18 weeks (Representing 3 subcultures) from date of culturing the explants.

Experiment (3): Effect of different combinations of benzyladenine (BA) and Kinetin (Kin) on shooting behavior and shoot chemical composition

The explants were cultured on (MS) medium supplemented with combinations of (BA at 1.5, 3.0, 4.5 and 6.0 mg/l concentrations and Kin at 1.0 2.0 mg/l concentration) i.e.8 treatment plus the control treatment (0 mg/lBA+0 mg/l Kin). Every treatment consisted of 4 replicates (each jar contained one explants). The data were recorded after 6, 12 and 18 weeks (representing 3 subcultures) from date of culturing the explants:

Chemical analysis

In all experiments the chemical analysis done on the shoot leaves includes determination of pigments (chlorophyll A and B), total indoles, total phenols and total soluble sugars. Determination of pigments was done according to Saric *et al.* (1967).Determination of total indoles was done according to Larsen *et al.* (1962) and modified by Salim *et al.* (1978) Determination of total soluble phenols was done using Foline-Ciocaltea reagent (A.O.A.C., 1985).

Statistical analysis

For all experiments, the obtained data were statilically analyzed using a completely randomized design and the least significant difference (LSD) test was used for comparison among means according to Steel and Torri (1980).

RESULTS AND DISCUSSION

Experiment 1: Effect of some sterilization treatments

The data in Table (1) indicated that increasing chlorox concentration increased percentage of free of contamination explants .The highest percentage of free of contamination explants (50%) was recorded by using 60 or 70% (v/v) chlorox which resulted in healthy explants.

On the other hand, the data indicated that increasing mercuric chloride (M.C.) concentrations increased percentage of free of contamination explants. The highest percentage of free of contamination

explants (68%) was recorded by using 0.4% (v/v) mercuric chloride (M.C.) which produced healthy explants.

The data on the interaction between chlorox and mercuric chloride concentrations, indicated that the highest value (100%) of free of contamination explants was obtained when using 50 or 60% chlorox with 0.4% mercuric chloride.

Our results on sterilization of explants are in line with those of Hosni *et al.* (2000) in *Limonium siunuatum* var. "Citron Mountain"and Hussein (2002) in *Aglaonema cecilia* cv. (Freeman) and *A. commutatum* cv. (Silver Queen).

Table (1): Mean percentage of free of contamination explants of Ruscus hypoglossum resulted from different sterilization treatments.

Mercuric chloride	Clorox					
	30%	40%	50%	60%	70%	Mean (A)
0.0 %	0.0	0.0	0.0	20.0	20.0	8.0
0.1 %	0.0	0.0	20.0	40.0.	40.0	20.0
0.2 %	0.0	0.0	60.0	40.0	60.0	32.0
0.4 %	20.0	40.0	100.0	100.0	80.0	68.0
Mean (B)	05.0	10.0	45.0	50.0	50.0	____
L.S.D.0.05 (A)		17.1				
L.S.D.0.05 (B)		19.2				
L.S.D.0.05 (AXB)		38.4				

Experiment 2: Effect of BA, Kin, TDZ and subcultures on shoot formation and chemical composition:

Number of shoots

Concerning the effect of BA, the results (Table2 and Fig.1) clearly indicated that, regardless the number of subcultures there were significant increases in the number of shoots with the use of BA at different concentrations as compared with control (MS alone), The addition of BA at 1.5 mg/1 in MS medium produced the highest number of shoots/explants (6.83), while the addition of BA at 6.0 mg/l produced the lowest number of shoots (3.66) and exhibited vetrifications. Concerning the effect of Kin, the results indicated that addition of Kin at 6.0 mg/l in MS medium produced the highest number of shoots/explant (5.66) as compared to control with no observation of any vetrification. The results cleary indicated that the addition of TDZ at 1.5 mg/l in MS medium produced the highest number of shoots/explant (5.58) as compared to control, and addition TDZ at 4.5 or 6.0 mg/l resulted in vetrification of the shoots. Increasing the number of subcultures significantly increased the number of shoots/explant. It was 3.55 in the 1st subculture and increased to 4.25 and 5.73 for the 2nd and 3rd subculture, respectively (Table 2).

Concerning the interaction of concentrations of BA, Kin and TDZ with the number of subcultures, the highest number of shootlets/explant (10) was obtained when adding 1.5 mg/l BA at the 3rd subculture as compared with the control

medium (free of hormones) in 1st subcultures (2.25 shootlets / explants).

From the above mentioned results, it can be concluded that supplementing 1.5 mg BA/l to MS medium was the best for shoot multiplication of *Ruscus hypoglossum*.

Shoot length

It is clear that shoot length was significantly affected by adding BA, Kin and TDZ to MS medium as compared with free of hormones (control) medium .The average shoot length ranged between 1.62 and 6.08 cm. The longest shoot (6.08 cm) was recorded for explants grown on MS medium supplemented with 4.5 mg Kin/l. 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 concentration. Culturing the explants on MS medium supplemented with 6.0 mg TDZ/1 resulted in the shortest shoots, (1.62 cm) as compared to the control (4.16cm).

A significant effect of number of subcultures on the length of shootlet was recorded .The shootlet length was more significantly higher at the 3rd subculture (3.80 cm) than at 2nd (3.17 cm) and 1st subculture (2.44 cm).

The data indicated that in the 1st subculture, growing the explants on MS medium supplemented with 4.5 mg Kin/l resulted in the longest shoots (4.12 cm), whereas, explants grown on MS medium supplemented with 4.5 and 6.0 mg TDZ/l gave the shortest shoots (1.50 cm). In the 2nd and 3rd subcultures, similar results were recorded (Table 2).

In conclusion, in this study the best shoot length was recorded when adding 4.5 mg/l Kin to the MS medium.

As for cytokinins, Kushal-Singh *et al.* (1994) showed also that in chrysanthemum cv. Riot, increasing levels of BAP in the medium suppressed shoot length. Mujib et al., (1995) reported also that kinetin increased height of shoots produced when shoot tips of carnation (cvs Royal Crimson and Candy Sim) were cultured on MS medium. Cuenca et al. (1999) found that maximum shoot elongation was achieved when culturing *Centaura paui* inflorescence nodel segments on medium without growth regulators and the addition of cytokinins significantly decreased their height.

Number of leaves

The highest number of leaves/shoot (12.83) was recorded by culturing the explants on MS medium supplemented with Kin at 6.0mg/L. whereas, the lowest number (2.41) was recorded for explants grown MS medium supplemented with 6.0 mg/l TDZ .There were significant increases in the number of leaves per explant as the number of subcultures increased. The average number of leaves was 4.86 in the 1st subculture and then increased to 6.59 and 8.21 in the 2nd and 3rd subculture, respectively.

In the 1st subculture, the data indicated that, explants grown on MS medium supplemented with TDZ at 6.0 mg/l gave the lowest number of leaves (2), whereas the highest number (9.5) resulted from growing on MS medium supplemented with Kin at 6.0 mg/L. The same trend was found in the 2nd and 3rd subcultures. Therefore, it can be concluded that the highest number of leaves/explant was obtained when Kin was included in the MS medium at 6.0 mg/l concentration.

The same trend was observed by Nofal *et al.* (1996) when they studied the effect of kin sprays at the levels of 25, 50 and 75 ppm on the vegetative growth of *Crinum*

longifolium and *Hemerocallus aurantiaca*. Their data showed that all treatments significantly increased the mean number of leaves per plant. Sakr *et al.* (1999) showed that the highest number of leaves was obtained for the *Yucca elephantipes* green cultivar when its explants were grown on medium supplemented with 5 mg kin + 0.125 mg NAA/litre and for the *Yucca elephantipes* variegated cultivar when its explants were grown on medium supplemented with 5 mg kin + 0.5 mg NAA/litre.

Table (2): Mean number of shoots/explant; shoot length and leaves/shoot of Ruscus hypoglossum as affected by different concentrations of BA, Kin and TDZ and subcultures In vitro.

Culturing media (concentration/L)	Shoots / explants				Shoot length (cm)				Leaves/shoot			
	No. of subcultures			Mean (A)	No. of subcultures			Mean (A)	No. of subcultures			Mean (A)
	1	2	3		1	2	3		1	2	3	
MS (control)	2.25	2.75	3.25	2.75	2.75	4.00	5.75	4.16	6.00	8.75	11.00	8.58
MS + 1.5 mg BA	4.50	6.00	10.00	6.83	2.25	2.62	2.75	2.54	5.50	6.75	9.50	7.25
MS + 3.0 mg BA	3.75	5.00	8.50	5.75	2.12	2.25	2.50	2.29	5.00	7.00	8.50	6.83
MS + 4.5 mg BA	3.00	4.25	6.50	4.58	2.00	2.12	2.25	2.12	4.25	6.50	7.75	6.16
MS + 6.0 mg BA	2.50	3.50	5.00	3.66	1.62	1.87	2.12	1.87	3.50	5.25	6.50	5.08
MS + 1.5 mg Kin	2.75	3.25	3.75	3.25	3.25	4.37	5.25	4.29	6.75	9.50	11.50	9.25
MS + 3.0 mg Kin	3.25	3.50	4.50	3.75	3.50	4.75	6.12	4.79	7.50	10.50	13.50	10.50
MS + 4.5 mg Kin	3.50	3.75	5.50	4.25	4.12	6.37	7.75	6.08	8.50	11.75	14.25	11.50
MS + 6.0 mg Kin	4.70	5.25	7.25	5.66	3.75	5.50	6.75	5.33	9.50	13.50	15.50	12.83
MS + 1.5 mg TDZ	5.25	5.50	6.00	5.58	1.75	2.12	2.37	2.08	3.00	3.25	4.00	3.41
MS + 3.0 mg TDZ	4.75	5.25	5.75	5.25	1.62	1.87	2.25	1.91	2.75	3.00	3.75	3.16
MS + 4.5 mg TDZ	3.50	4.00	4.50	4.00	1.50	1.75	1.87	1.71	2.25	2.50	3.25	2.66
MS + 6.0 mg TDZ	2.75	3.25	3.50	3.16	1.50	1.62	1.75	1.62	2.00	2.25	3.00	2.41
Mean (B)	3.55	4.25	5.73	——	2.44	3.17	3.80	——	4.86	6.59	8.21	——
L.S.D. 0.05 Culturing media (A) 0.503					0.237				0.479			
L.S.D. 0.05 No. of subcultures (B) 0.242					0.113				0.230			
L.S.D. 0.05 (AX B) 0.872					0.410				0.829			

Chemical composition

Chlorophyll-A

The data in Table (3) showed that the different concentrations of hormones (BA, Kin, and TDZ) caused a significant influence on chlorophyll-A content for *Ruscus hypoglossum* shootlets. Using MS supplemented with TDZ at 4.5 mg/l concentration produced the highest amount of chlorophyll-A (140.8 mg/100g FW), while the lowest amount of chlorophyll-A (13.09 mg/100g FW) was found by using MS medium supplemented with 3.0 mg/l BA.

These results revealed that using TDZ generally increased chlorophyll-A content of the shoots while using BA or Kin did not significantly change chlorophyll-A as compared to the control (free of hormones) medium.

BA

Kin

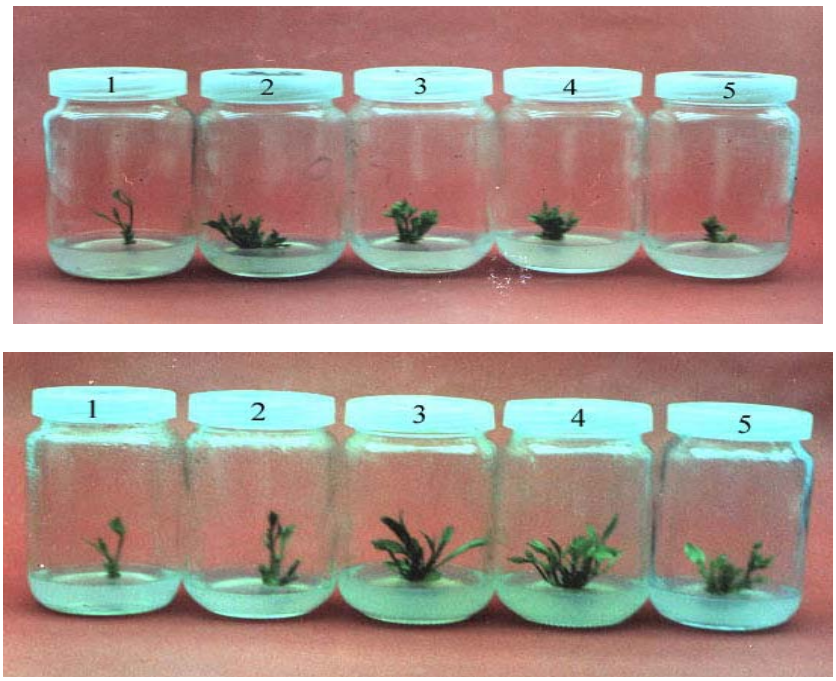

Fig. (1): Effect of different BA, Kin and TDZ concentrations on shooting behavior of Ruscus hypoglossum.

1- Control (free of hormones). 2- 1.5 mg/l. 3.0 mg/l . 4- 4.5 mg/l. 5- 6.0 mg/l.

Chlorophyll-B

From the data (Table), it is quite clear that the various concentrations of hormones exhibited significant influence on the chlorophyll-B content for *Ruscus hypoglossum*, the highest amount of chlorophyll-B (56.12 mg/100g FW) was recorded by using MS medium supplemented with 6.0 mg/l TDZ, while the lowest value (4.590 mg/100g FW) was recorded by using MS medium supplemented with 6.0 mg/l Kin. The data also showed that there was no significant difference between BA concentrations (1.5, 3.0, 4.5, and 6.0 mg/l), Kin concentrations (1.5, 3.0, and 4.5 mg/l), and TDZ concentrations (3.0, and 4.5 mg/l).

Carotenoids

Evident influence of different concentrations of hormones (BA, Kin, and TDZ) on the shootlet content of carotenoids could be noticed from data illustrated in Table (3). Adding the TDZ at concentration (4.5 mg/L) increased the amount of carotenoids to the highest value (99.48 mg/100g FW), but using BA at (3.0 mg/L) decreased the amount of carotenoids to the lowest value (19.04 mg/100g FW).This means that TDZ concentrations, which increased the amount of carotenoids, were 4.5 and 6.0 mg/L as compared with other hormones and control.

In conclusion, the highest amount of chlorophyll-A, and carotenoids was recorded with MS medium supplemented with TDZ at 4.5 mg/l, while the highest amount of chlorophyll-B was recorded with MS medium supplemented with 6.0 mg/l TDZ.

In this respect, Tung (1997) on *Cucumis sativus* studied the effect of different concentrations of cytokinins BA, BA-ribosid (BAR), BA-3G, BA-7G, BA-9G, zeatin (Z), Z-riboside, dihydrozeatin and 2iP on the formation capacity of chlorophyll-A, chlorophyll-B and carotenoids. They declared that the production of such pigments was negatively related with the concentrations of dihydrozeatin, whereas it was positively related with the concentrations of the other cytokinins examined. Gao *et al.* (2000) concluded that modified white medium (MWM) containing 1.07 μM NAA and 18.40μM kinetin resulted in relatively high pigment formation in *Carthamus tinctorius*.

Indoles content

The data (Table 3) pointed out that the indoles content was significantly affected by various concentrations of hormones (BA, Kin, and TDZ). Data showed that the highest content of indoles (8.62 mg/100g FW) was produced by using TDZ at 3.0 mg/L concentration, while the lowest content (1.29 mg/100g FW) was observed by adding BA at 4.5 mg/L concentration. In this concern, Youssef (1994) on *Acacia salicina* recorded that using BA at high (5 mg/L) concentration remarkably augmented the endogenous level of total indoles.

Total soluble phenols

The data (Table 3) showed that there were significant differences among concentrations of BA, and those concentrations of Kin and TDZ. The highest amounts of total soluble phenols were recorded with all BA concentrations (1.5, 3.0, 4.5, and 6.0 mg/l) as compared with the corresponding concentrations of Kin and TDZ as well as with control.

Table (3): Chemical contents (mg/100 g FW) of Ruscus hypoglossum shoots as affected by different concentrations of BA,,Kin and TDZ.

Treatment	Chlorophyll-A	Chlorophyll-B	Carotenoids	Indoles	Phenols
MS (control)	31.55	26.78	58.92	3.92	0.35
MS + 1.5 mg BA	17.61	6.71	23.19	2.53	1.77
MS + 3.0 mg BA	13.09	5.23	19.04	1.56	1.81
MS + 4.5 mg BA	16.25	7.22	21.04	1.29	1.88
MS + 6.0 mg BA	21.46	8.93	25.49	1.82	1.82
MS + 1.5 mg Kin	55.00	14.93	50.12	3.60	1.45
MS + 3.0 mg Kin	16.04	25.48	49.77	4.41	0.63
MS + 4.5 mg Kin	35.53	8.39	46.93	5.53	0.85
MS + 6.0 mg Kin	29.13	4.59	41.63	5.17	0.63
MS + 1.5 mg TDZ	65.81	35.08	33.41	5.39	0.45
MS + 3.0 mg TDZ	35.76	6.05	42.78	8.62	0.52
MS + 4.5 mg TDZ	140.80	19.17	99.48	7.27	0.69
MS + 6.0 mg TDZ	56.12	56.12	70.99	6.17	0.77
L.S.D.0.05	45.49	30.00	29.95	2.25	0.66

Experiment 3: Effect of different combinations of BA and Kin concentrations and subcultures on shoot formation and chemical composition:

Number of shoots

The data in Table (4) showing the effect of concentrations of cytokinins revealed that the highest number of shoots/explant (12.92) was produced when explants were grown on MS medium containig 1.5 mg/l BA + 2.0 mg/l Kin as compared with MS medium free of hormone (2.75).Increasing the number of subcultures significantly increased the number of shoots formed on the explants. The number of shoots was 4.77 in the 1st subculture and increased to 6.94 and 9.36 for the 2nd and 3rd subcultures, respectively.

The interaction between hormone treatments and the number of subcultures was significant at 5% level. The highest number of shoots/explant (18.5) was obtained by adding 1.5 mg/l BA + 2.0 mg/l Kin in the 3rd subculture as compared with the control medium (without hormones).

From the above mentioned results, it can be concluded that MS supplemented with 1.5 mg/l BA + 2.0 mg/l Kin was the best treatment for shoot multiplication of *Ruscus hypoglossum.* In this trend, Bekheet (1999) cultured shoot tips of *Asparagus officinalis* cv. (U.C. 157) on basal (MS) medium and obtained multiple bud clusters when the MS-medium was supplemented with 1 mg/liter benzyladenine (BA). He also reported that adding kinetin to multiplication medium was more effective in increasing the number of initiated shoots than BA.

Shoot length

It is clear from Table (4) that shoot length was significantly affected by BA plus Kin treatments; the average shoot length ranged between 2.08 and 4.54 cm. The longest shoot (4.54 cm) was recorded for explants grown on MS medium supplemented with 1.5 mg/l BA + 2.0 mg/l Kin. Whereas, using MS medium supplemented with high concentrations of BA significantly reduced the length of shoots, and the rate of reduction in shoot elongation was in proportion with the increase in concentrations of BA, so culturing the explants on MS medium supplemented with 6.0 mg/l BA + 1.0 mg/l Kin resulted in the shortest shoots (2.08 cm) as compared to the control (4.16 cm).

A significant effect of number of subcultures on the length of shootlets was recorded (Table4). The shootlet was longer at the 3rd subculture (3.54 cm) than at 2nd subculture (3.06 cm) and 1st subculture (2.61 cm).

As for the interaction effect, the data indicated that at the 1st subculture, growing the explants on MS medium supplemented with 1.5 mg/l BA + 2.0 mg/l Kin resulted in the highest shoot length (3.75 cm), whereas, explants grown on MS medium supplemented with 6.0 mg/l BA + 1.0 mg/l Kin gave the shortest shoots (1.87 cm). In the 2nd subculture, a similar result was recorded. While in the 3rd subculture the longest shoots was recorded with MS medium free of hormones (control).

In conclusion, the longest shoots of *Ruscus hypoglossum* were generally recorded with 1.5 mg/l BA + 2.0 mg/l Kin treatment followed by control.

In this concern, Majumdar *et al.* (1998) found that *in vitro* differentiated shoots of *Albizia procera* were successfully produced on MS medium containing various BA levels. They reported that high frequency of shoot bud elongation was

observed on medium supplemented with BA at low concentrations.

Number of leaves

The effect of BA + Kin during 3 subcultures on number of leaves/explant of *Ruscus hypoglossum*, was studied (Table 4).It is clear that the highest number of leaves (11.67) was recorded by culturing the explants on MS medium supplemented with 1.5 mg /l BA + 2.0 mg/l Kin, whereas, the lowest number (5.58) was recorded for explants grown on MS medium supplemented with 6.0 mg /l BA + 1.0 mg/l Kin.

The results indicated that across all treatments there were significant increases in the number of leaves as the number of subcultures increased. The average number of leaves was 6.89 in the 1st subculture, and then increased to 8.25 and 9.77 in the 2nd and 3rd subcultures, respectively.

Table (4): Mean number of shoots/explants,shoot length (cm), leaves/shoot of Ruscus hypoglossum as affected bydifferent combinations of BA and Kin concentrations and subcultures in vitro.

Culturing media (concentration/L)	Shoots / explant				Shoot length (cm)				Leaves /shoot			
	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)	2.25	2.75	3.25	2.75	2.75	4.00	5.75	4.16	6.00	8.75	11.00	8.58
MS + 1.5 mg /l BA + 1 mg /l Kin	5.50	7.50	11.25	8.08	2.62	2.87	3.25	2.91	8.50	9.25	13.00	10.25
MS + 3.0 mg /l BA + 1 mg /l Kin	4.75	7.00	9.50	7.08	2.37	2.37	2.62	2.45	6.00	7.00	8.50	7.16
MS + 4.5 mg /l BA + 1 mg /l Kin	4.00	6.25	7.25	5.83	2.25	2.25	2.5	2.33	5.25	6.25	7.00	6.16
MS + 6.0 mg /l BA + 1 mg /l Kin	3.75	5.00	6.00	4.91	1.87	2.00	2.37	2.08	4.75	5.50	6.50	5.58
MS + 1.5 mg /l BA + 2 mg /l Kin	7.50	12.75	18.50	12.92	3.75	4.75	5.12	4.54	10.50	11.50	13.00	11.67
MS + 3.0 mg /l BA + 2 mg /l Kin	6.50	9.25	13.50	9.75	2.87	3.37	3.75	3.33	8.25	9.75	10.75	9.58
MS + 4.5 mg /l BA + 2 mg /l Kin	5.50	7.25	9.75	7.50	2.75	3.12	3.50	3.12	7.00	8.75	9.75	8.50
MS + 6.0 mg /l BA + 2 mg /l Kin	3.25	4.75	5.25	4.41	2.25	2.87	3.00	2.70	5.75	7.50	8.50	7.25
Mean (B)	4.77	6.94	9.36	____	2.61	3.06	3.54	____	6.89	8.25	9.77	____

Concerning the interaction between the number of subcultures and the tested treatments of cytokinins, the data indicated that in the 1st subculture, explants grown on MS medium supplemented with 1.5 mg/l BA + 2.0 mg/l Kin gave the highest number of leaves (13.0), whereas the least number of leaves (4.75) resulted from MS medium supplemented with 6.0 mg /l BA + 1.0 mg/l Kin. Similar results were recorded in the 2nd and 3rd subcultures, where BA at 1.5 mg/l + kin at 2.0 mg/l produced the highest number of leaves/explant as compared with other treatments.

Thus, it can be concluded that the highest number of leaves of *Ruscus hypoglossum* was recorded when using MS medium supplemented with 1.5 mg/l BA +

2.0 mg/l kin.This result was in disagreement with Sawsan (2002) on *Solidago altissmia* var. "Tara".

Chemical composition

Chlorophyll-A

The data in Table (5) showed that the effect of different combinations of BA and Kin concentrations resulted a significant influence on chlorophyll-A content of *Ruscus hypoglossum* shootlets. Using MS medium free of hormones (control) produced the highest amount of chlorophyll-A (170.90 mg/100g FW), while the lowest content of chlorophyll-A (24.71 mg/100g FW) was found by using MS medium supplemented with 1.5 mg/l BA + 1.0 mg/l Kin.

Table (5): Chemical contents (mg/100g FW) of Ruscus hypoglossum shoots as affeceted by combinations of different BA and Kin concentrations.

Treatments	Chlorophyll-A	Chlorophyll-B	Carotenoids	Indoles	Phenols
MS (control)	170.90	7.78	79.73	2.83	0.77
MS + 1.5 mg /l BA + 1 mg /l Kin	24.71	11.86	26.22	0.67	1.70
MS + 3.0 mg /l BA + 1 mg /l Kin	40.58	8.94	37.63	0.89	1.31
MS + 4.5 mg /l BA + 1 mg /l Kin	64.08	29.61	58.21	0.85	1.65
MS + 6.0 mg /l BA + 1 mg /l Kin	32.25	8.97	30.82	1.06	1.74
MS + 1.5 mg /l BA + 2 mg /l Kin	116.00	39.44	99.84	5.47	1.58
MS + 3.0 mg /l BA + 2 mg /l Kin	47.90	20.82	43.68	0.94	1.39
MS + 4.5 mg /l BA + 2 mg /l Kin	47.45	23.83	44.56	0.96	1.46
MS + 6.0 mg /l BA + 2 mg/l Kin	30.35	15.47	30.61	0.58	2.18
L.S.D.0.05	38.57	12.40	30.31	3.42	0.76

Chlorophyll-B

It is quite clear that the various combinations of BA and Kin concentrations exhibited significant influence on the chlorophyll-B content of *Ruscus hypoglossum shoots (Table 5).* The highest amount of chlorophyll-B (39.44 mg/100g FW) was found by using MS medium supplemented with 1.5 mg/l BA + 2.0 mg/l Kin, while the lowest value (7.78 mg/100g FW) was recorded by using MS medium free of hormones (control). The data also showed that MS medium supplemented with BA concentrations (1.5, 3.0, 4.5, and 6.0 mg/l) plus 2.0 mg/l Kin gave higher amounts of chlorophyll-B than the same concentrations of BA plus 1.0 mg/l Kin.

Carotenoids

Evident influence of different combination of BA and Kin on the content of shootlet carotenoids was noticed from data illustrated in Table (5) for *Ruscus hypoglossum.* Adding 1.5 mg/l BA + 2.0 mg/l Kin increased the amount of carotenoids to the highest value (99.84 mg/100g FW), but using 1.5 mg/l BA + 1.0 mg/l Kin decreased the amount of carotenoids to the lowest value (26.22 mg/100g FW).

Gao *et al.* (2000) concluded that modified white medium containing 1.07 μM NAA and 18.40μM kinetin resulted in relatively high pigment formation in *Carthamus tinctorius.* In the present study, it

could be concluded that the highest amount of chlorophyll-A was recorded with MS free of hormones (control), while the highest amount of chlorophyll-B and carotenoids was recorded when adding 1.5 mg/l BA + 2.0 mg/l Kin to the MS medium.

Indoles content

The data (Table 5) pointed out that the indoles content in *Ruscus hypoglossum* was significantly affected by adding various combinations of BA plus Kin to MS medium. Data showed that the highest content of indoles (5.47 mg/100g FW) was produced by using MS medium supplemented with 1.5 mg/l BA + 2.0 mg/l Kin, while the lowest content (0.58 mg/100g FW) was observed by adding BA at 6.0 mg/l + Kin at 2.0 mg/l.

The results revealed that, the highest amount of indoles was produced when using low level of BA concentrations plus high level of Kin.

Total soluble phenols

It was evident that the greatest content of total soluble phenols (2.18 mg/100g FW), was recorded when using MS medium supplemented with 6.0 mg/l BA + 2.0 mg/l Kin, and the lowest content (0.77 mg/100g FW) was recorded with using MS medium free of hormones (control). This means that, phenols content in *Ruscus hypoglossum* exhibited the highest level by using high level of BA concentrations.

In conclusion, the highest amount of indoles content was recorded with 1.5 mg/l BA + 2.0 mg/l Kin, while the highest amount of total soluble phenols was recorded with 6.0 mg/l BA + 2.0 mg/l K.

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الملخص العربي

تاثير بعض معاملات التعقيم و منظمات النمو على الاكثار الدقيق لنبات السفندر

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أجرى هذا البحث بمعمل زراعة الأنسجة بحديقة الزهرية (وحدة الخدمات البستانية – وزارة الزراعة) وكلية الزراعة جامعة القاهرة – جمهورية مصر العربية خلال الفترة ٢٠٠١--٢٠٠٤. و يهدف البحث إلى دراسة تأثير استخدام معاملات التعقيم المختلفة للحصول على أجزاء نباتية خالية من التلوث لنباتات السفندروتأثير التركيزات المختلفة من السيتوكينينات (BA و Kin و TDZ) والتوليفات المختلفة من البنزيل ادينين والكينتين معا على سلوك الإكثار الدقيق و المحتوى الكيماوي لنباتات السفندرالناتجة من خلال زراعة الأنسجة .أدى استخدام تركيز ٥٠ أو ٦٠ % كلوركس مع ٠,٤ % كلوريد زئبقيق للحصول على أعلى نسبة من الأجزاء النباتية الخالية من التلوث. ووجد أن استخدام BA بتركيز ١,٥ مجم/لتر نتج عنه أعلى معدل من التضاعف للعزلات النباتية أما استعمال Kin بتركيز ٦,٠ مجم/لتر أعطى أفضل طول و أكبر عدد من الأوراق للعزلات النباتية.كما أن زيادة عدد النقلات أعطى زيادة في كل من عدد الأفرع و طول النباتات و عدد الأوراق.وقد كان زيادة محتوى كلوروفيل-أ و الكاروتين إلى أعلى مقدار نتيجة لاستخدام TDZ بتركيز ٤,٥مجم/لتر أما أعلى كمية من كلوروفيل-ب كانت نتيجة استخدام TDZ بتركيز ٦,٠ مجم/لتروسجلت محتوى من الأندولات للمعاملة TDZ بتركيز ٣,٠ مجم/لتر بينما لم تظهر أى فروق معنوية بين المعاملات المختلفة مع الفينولات الكلية الذائبة.وعند استخدام توليفة ١,٥ مجم/لتر BA مع ٢,٠ مجم/لتر Kin نتج عنها أعلى معدل لتضاعف العزلات النباتية و أطول أفرع و أكبر عدد أوراق لكل نبات.كما أن زيادة عدد النقلات أعطى زيادة في كل من عدد الأفرع و طول النباتات و عدد الأوراق.وكانت زيادة كمية كلوروفيل-أ إلى أعلى كمية نتيجة لاستخدام بيئة MS خالية من الهرمونات أما أعلى كمية من كلوروفيل-ب و الكاروتين كانت نتيجة استخدام ١,٥ مجم/لتر BA + ٢,٠ مجم/لتر Kin.ونتج اعلي محتوى من الأندولات باستخدام ١,٥ مجم/لتر BA + ٢,٠ مجم/لتر Kin بينما أعلى محتوى من الفينولات الكلية الذائبة سجلت مع المعاملة ٦,٠ مجم/لتر BA + ٢,٠ مجم/لتر Kin.