Effect of MS-salt strength, sucrose and IBA concentration and acclimatization media on *Ruscus hypoglossum* L. micropropagation

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ABSTRACT

This study was carried out 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 MS salts strength, sucrose, IBA and activated charcoal on shooting behavior, chemical composition of shootlets and rooting of Ruscus hypoglossum. The effect of some growing media during adaptation stage was also studied.Using MS medium at full salt strength produced the highest number of shoots. Increasing the number of subcultures significantly increased the number ofshootlets, shootlet length, and leaves per shootlet. Using MS medium at ½ and ¼ strength increased the shootlet contents of chlorophyll-A and carotenoids to the highest values. Culturing Ruscus explants on ¼ MS strength medium produced the greatest amount of indoles.Using 30 and 40 gm sucrose/l gave the highest number of shoots and leaves per shoot. While the highest shootlet length was produced by using 50 gm sucrose/l. Increasing the number of subcultures significantly increased the number of shoots, shootlet length, and number of leaves per shootlet. Using 10 and 30 gm sucrose /l produced the highest amount of chlorophyll-A.Using 10, 20, 30 and 40 gm sucrose /l produced the highest amount of total indoles and total soluble phenols.MS medium at ½ strength supplemented with IBA at 2.0 mg/l was the most effective treatment in increasing the number of roots / shootlet. The highest number of roots was recorded with 50 gm/l sucrose with activated charcoal. The longest root and shoot were recorded with 55 gm/l sucrose, while the highest number of leaves was recorded with 40 gm/l sucrose. Adding activated charcoal to sucrose concentrations was the best treatment to obtain the longest root and shootlet, while the highest number of leaves per shootlet was shown with treatments without activated charcoal.

During adaptaion the tallest plants and the greatest number of leaves were observed when using the peat moss as a growing medium, where the survival percentage was 100 %.

Keywords: *Ruscus hypoglossum, micropropagation, salt strength, sucrose, IBA, activated charcool, acclimatization.*

INTRODUCTION

Plants from the Liliaceae family are among those 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 of the region from Western Europe to Iran, is a compact evergreen shrub with a creeping rootstock that

reach 18 inches length. It is an interesting plant with leaf-like cladodes of 1½ to 3 inches length and wide tapering at both ends. *Ruscus hypoglossum* need partial shade to full shade.even though it tolerates full sun,the color is richer in shade. The plants grow under 70 % shade all year. A suitable soil mix consists of 1 part peat moss to 3 parts loam to 1 part sand or perlite. It is very drought tolerant, but should be watered twice a week for optimum growth. Plants are fertilized once during the growing season with a balanced fertilizer for folks with a warmer winter temperature (zone 8-10) they make outstanding groundcover under trees where nothing else will grow. *Ruscus hypoglossum* is propagated by division or by seed. MS medium (Murashige and Skoog, 1962) a ocommon medium used in plant tissue culture, has been used by many workers such as Agrawal *et al*. (1992) with orchid (*Vanilla walkeriae*), Pereira. Pinto *et al*. (1996) with *Kielmeyera coriacea*, Torres and Mogollan (1997) with orchid (*Cattleya lueddemanniona*), Karhu (1997) with *Lonicera caerulea* and Sakr *et al*. (1999a) with *Yucca elephantipes.*

Up till now, there is no literature available on *Ruscus hypoglossum* propagation through tissue culture techniques. Therefore, the objectives of this study was to investigate the following points:

1- Effect of sucrose concentration and MS salts strength on shoot formation.
2- Effect of MS strength, IBA, activated charcoal and sucrose concentration on root formation.
3- Effect of some growing media during adap-tation stage on the establishment of propagated plants.

MATERIALS AND METHODS

This investigation was carried out in Plant Tissue Culture Lab. in El-Zoharya Bot. Garden, Minsitry of Agric.-Egypt. and Fac. of Agric. Cairo Univ. during the period from 2001 to 2004.

Buds of plant rhizomes of *Ruscus hypoglossum* plant were used as sources of explants. Buds were cut, 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. The growth regulators and other culturing materials were added; the pH value was adjusted to 5.7±0.1; agar was used as a solidifying agent at 6 gm/L. After mixing 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, photoperiod of 16 hours light/8hours' dark and illumination intensity of 3000 lux at the top of culture vessels using fluorescent lamps.

Experiment (1): Effect of MS salt strength on shooting

In this experiment, three concentrations of MS salts (Full, 1/2 and 1/4 strength) were examined. All media were supplemented with 1.5 mg BA/l and 2.0 mg Kin/l. Three subcultures were done after 6, 12 and 18 weeks from culturing explants. Each treatment consisted of 4 replicates (each jar contained one explant). The data were recorded at the end of each subculture. Data on number of

shootlets/explant, shootlet length and number of leaves/shoot were recorded.

Experiment (2): Effect of sucrose concentration on shooting

Five concentrations of sucrose (10, 20, 30, 40 and 50 g/l) were tested with full strength MS medium supplemented with 1.5 mg BA/l and 2.0 mg Kin/l .Each treatment consisted of 4 replicates during three subcultures (each subculture 6 weeks). At the end each of subculture, data on number of shootlets/explant, shootlet length and number of leaves/shoot were recorded.

Experiment (3): Effect of MS salt strength, IBA and activated charcoal on rooting

MS medium at full, 1/2 and 1/4 strength were prepared and supplemented with indole butyric acid (IBA) at 1.0, 2.0 and 3.0 mg/l in the presence of activated charcoal (A.C) at 1.0 gm/L or without it and used for culturing shootlets. So, the following 24 treatments were used:

1- Full MS salt strength + zero, 1.0, 2.0 or 3.0 mg IBA/l.
2- 1/2 MS salt strength + zero, 1.0, 2.0 or 3.0 mg IBA/l.
3- 1/4 MS salt strength + zero, 1.0, 2.0 or 3.0mg IBA/l.
4- Full MS salt strength + zero, 1.0, 2.0 or 3.0 gm /l activated charcoal.
5- 1/2 MS salt strength + zero, 1.0, 2.0 or 3.0 gm /l activated charcoal.
6- 1/4 MS salt strength + zero, 1.0, 2.0 or 3.0 gm /l activated charcoal.

Each treatment contained 5 replicates; the data were recorded after 60 days from culturing the explants on number of roots, root length, plant height and number of leaves/shoot.

Experiment (4): Effect of sucrose concentrations on rooting

In this experiment, four concentrations of sucrose (40, 45, 50 and 55 gm /l) were added to ½ strength MS medium supplemented with 2.0 mg IBA/l. Each treatment consisted 5 replicates. At the end of this experiment (after 30 day), data on number of root/shootlet, root length, number of leaves/shoot and plant height, were recorded on the cultured shootlets.

Experiment (5): Effect of some growing media on growth of plantlets during adaptation stage

This experiment was conducted in the greenhouse to evaluate the effect of some growing media on the survival percentage of *Ruscus hypoglossum* plantlets during the acclimatization stage. The plantlets (8-10 cm length with 4-5 leaves) produced *in vitro* were individually transplanted into 8 cm plastic pots filled with: peat moss , peat moss + sand (1:1, v/v) , peat moss+ vermiculite (1:1, v/v), peat moss+ perlite (1:1, v/v) or peat moss + vermiculite + perlite (1:1:1, v/v/v) . Each treatment consisted of 3 replicates. At the end of this experiment (after four weeks), the data were recorded,on survival percentage, length of plantlet and number of leaves per plantlet.

Chemical analysis

In all experiments, the following chemical analyses were done on the shoots produced *in vitro*:

Determination of pigments (chlorophyll A and B), was according to Saric *et al.* (1967), that of total indoles according to Larsen *et al*. (1962) and modified by Salim *et al.* (1978) and that of total soluble phenols according to Foline-Ciocaltea reagent (A.O.A.C., 1985). All pigments, indoles and phenols were determined in mg per 100 g shoot fresh weight (FW).

Statistical analysis

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

RESULTS AND DISCUSSION

Experiment 1: Effect of MS strength on shoot formation of *Ruscus hypoglossum*

Number of shoots

The data in Table (1) and Fig. (1) clearly showed that different MS salt strengths (full, half and quarter) had a significant effect on the growth and development of *Ruscua hypoglossum* cultured *in vitro*. The number of shootlets produced per explant was the greatest in the case of full salt strength (12.50) as compared with ½ salt strength (9.50) and ¼ salt strength (6.16).Increasing the number of subcultures significantly increased the number of shoots/explant.

Full strength MS medium at the 3rd subculture gave the greatest number of shoots/explant (18.0). This result was in agreement with Pereira-Pinto *et al*. (1996), who noticed that the use of total force and half strength of growing medium salts gave proportionally higher shoot ratios of *Kielmeyera coriacea*. Sakr *et al*. (1999b) stated that with *Magnolia grandiflora*, using MS medium at full-strength was more effective in increasing the number of shoots/explant than other medium strengths.

Shoot length

A significant difference was detected between means of the three of medium strength in their effect on the length of shootlet (Table1). The longest shoot was found when using ½ strength (5.29 cm) followed by full-strength (4.50 cm), whereas the lowest value (3.66 cm) was produced for the ¼ -strength of MS salts.

The data revealed a significant increase in shoot length as subculture number increased; the longest shoot (5.20 cm) was found at the 3rd subculture. In the 1st subculture no significant difference was observed between full and half MS strength, but in the 2nd and 3rd subcultures, growing the explants on half strength MS medium significantly increased the length of shoots as compared with other tested MS salt concentrations.

Thus, it can be concluded that the best treatment for increasing the shoot length of *Ruscus hypoglossum* was half MS salt strength. In this regard, Mantell *et al*. (1998) and Boggetti *et al. (1999),* noted that MS medium containing half-strength macro-elements was the most effective for shoot elongation of cashew (*Anacardium occidentals*).

Number of leaves

The highest number of leaves/shoot (11.5) was recorded by culturing the explants on full strength MS medium, whereas, the lowest number of leaves (6.41) was recorded on ¼ strength MS medium. There were significant increases in the number of leaves per shoot as the number of subcultures increased. The data indicated that in the 1st subculture, growing explants on full strength MS medium, gave the highest number of leaves (10.0), whereas the smallest number of Leaves (5.75) resulted from ¼ strength MS medium. Similar results were recorded in the 2nd and 3rd subcultures. The highest number of leaves (13.0) was obtained by using full strength medium at 3rd subculture. Thus, it can be concluded that full strength MS medium was the best treatment. This result was in agreement with Sawsan (2002) on *Solidago altissima* var "Tara".

Table (1): Mean number of shoots/explant, shoot length and number of leaves/shoot of Ruscus hypoglossum explants as affected by salt strength of MS medium.

Strength of MS salts	Shoots (No.)/explant				Shoot length (cm)				Leaves(No.)/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	
Full strength	7.50	12.25	18.00	12.50	3.75	4.75	5.12	4.50	10.00	11.50	13.00	11.50
Half strength	5.75	9.25	13.5o	9.50	3.87	5.50	6.50	5.29	6.00	8.75	10.75	8.50
Quarter strength	4.25	5.75	8.50	6.16	3.37	3.62	4.00	3.66	5.75	6.50	7.00	6.41
Mean (B)	5.83	9.08	13.33	——	3.66	4.62	5.20	——	7.25	8.91	10.25	——
L.S.D. 0.05 Culturing media				(A) = 0.493				0.288				0.829
L.S.D. 0.05 Number of subcultures				(B) = 0.493				0.288				0.829
L.S.D. 0.05 (A x B)				= 0.854				0.499				1.437

Chemical composition

Chlorophyll-A, B and carotenoids

The data (Table 2) showed that the strength of MS salts had a significant influence on chlorophyll-A content of *Ruscus hypoglossum* shoots. Using MS salts at ½ and ¼ strength, produced the highest amounts of chlorophyll-A (127.2, and 126.7 mg/100g FW, respectively), while the lowest content (110.8 mg/100g FW) was found by using full strength MS. In this regard, Sawsan (2002) on *Solidago altissima* var. "Toto", reported that the highest amount of chlorophyll-A was obtained by using MS medium at ¾ strength, while, in var. "Tara", the highest amount of chlorophyll-A was recorded by using half-strength medium. It is quite clear that the MS salt strength had no significant effect on the chlorophyll-B content (Table2). The highest amount of carotenoids (42.65 and 42.11 mg/100g FW) were produced when using MS at ½ and ¼ strength, respectively, while the lowest content of carotenoids (26.13 mg/100g FW) was found by using full MS salt strength.

Thus, it can be concluded that the highest amount of chlorophyll-A, and carotenoids was recorded when ½ and ¼ strength MS media were used.

Fig. (1): Effect of salt strength of MS medium on shooting behavior of Ruscus hypoglossum:
1- Full MS salt strength. 2- ½ MS salt strength. 3- ¼ MS salt strength

Indoles and total soluble phenols

The data (Table 2) indicated that the indoles content in the *Ruscus hypoglossum* plantlets was significantly affected by MS salt strength. The highest content of indoles (6.293 mg/100g FW) was produced by using MS salts at ¼ strength, while the lowest ones (2.687, and 2.101 mg/100g FW) were observed by using MS salts at full and ½ strength, respectively. The data indicated that MS salt strengths had no significant effect on the content of phenols of *Ruscus hypoglossum*.

Table (2): Contents (mg/100g FW) of chlorophyll-A and B, carotenoids, indoles and phenols of Ruscus hypoglossum shoots as affected by salt strength of MS medium.

Strength of MS medim	Chlorophyll-A	Chlorophyll-B	Carotenoids	Indoles	Phenols
Full strength	110.8	10.18	26.13	2.687	0.3983
Half strength	127.2	13.01	42.65	2.101	0.3930
Quarter strength	126.7	12.69	42.11	6.293	0.5450
L.S.D.0.05	8.523	NS	14.73	1.071	NS

Experiment 2: Effect of sucrose concentration on shooting and chemical composition

Number of shoots

The data in Table (3) and Figure (2) showed that the MS medium supplemented with sucrose at 30 g/l or 40 g /l gave the highest number of shoots/explant (13.00 and 13.58, respectively). The lowest number of shoots (5.50) was recorded with the use of MS medium supplemented with sucrose at 10 g/l. A significant increase in number of shoots was found with increasing the number of subcultures. The data indicated that adding 30 or 40 g/l sucrose to medium gave the highest number of shoots, with no significant difference between them at the 3rd subculture.

Shoot length

The longest shoots (7.00 cm) were obtained when the explants were cultured on MS medium supplemented with sucrose at the highest level (50 g/l), and the shortest shoots (3.25 cm) were recorded when using the lowest concentration (10 g/l sucrose) (Table 3)..Increasing the number of subcultures caused a significant increase in shoot length. Decreasing the level of sucrose from 50 to 10 g/l significantly decreased the average of shoot length. The promoting effect of the high sucrose level on shoot length was observed by Domiano *et al*. (1985) on *Eucalyptus gunn,* and Kozak (2000) on *Gloriosa rothschildiana* cv. Red Dark.

Number of leaves

As shown in Table (3) the highest number of leaves (10.67) was obtained when the explants were grown on MS medium supplemented with sucrose at 40 g/l .Increasing the number of subcultures, significantly increased the number of leaves/explants.

The data indicated that increasing the sucrose concentration in the media from 10 to 30 g/l significantly increased the average number of leaves, but using sucrose at 50 gm/l significantly reduced it the number of leaves as compared with both 30 or 40 g/l sucrose concentration. Thus, it can be concluded that the greatest number of leaves could be obtained by using MS medium

supplemented with 30 gm/l sucrose. The same result was also observed by Pasqual *et al*. (1994) on *Nephrolepis exaltata* and Nower (1998) on *Syngonium podophyllum.*

Table (3): Mean number of shoots/explant, shoot length and number of leaves/ shoot of Ruscus hypoglossum as affected by different concentrations of sucrose.

Concentration of sucrose	shoots / explants				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	
10 g/l	4.25	5.75	6.50	5.50	2.25	2.87	3.25	2.79	4.25	5.50	6.75	5.50
20 g/l	6.25	7.75	11.25	8.41	2.87	3.87	4.50	3.75	6.25	7.50	8.75	7.50
30 g/l	7.50	12.75	18.75	13.00	3.75	4.75	5.12	4.50	7.50	8.50	12.25	9.41
40 g/l	7.75	14.50	18.50	13.58	4.25	5.62	5.87	5.25	9.50	10.75	11.75	10.67
50 g/l	5.50	10.00	11.50	9.00	4.87	6.25	7.00	6.04	7.00	8.50	10.00	8.50
Mean (B)	6.25	10.15	13.30	—	3.60	4.67	5.15	—	6.90	8.15	9.90	—
L.S.D. 0.05 for sucrose concentrations				(A) 0.558			0.257				1.416	
L.S.D. 0.05 for No. of subcultures				(B) 0.432			0.199				1.096	
L.S.D. 0.05				(AXB) 0.966			0.446				2.452	

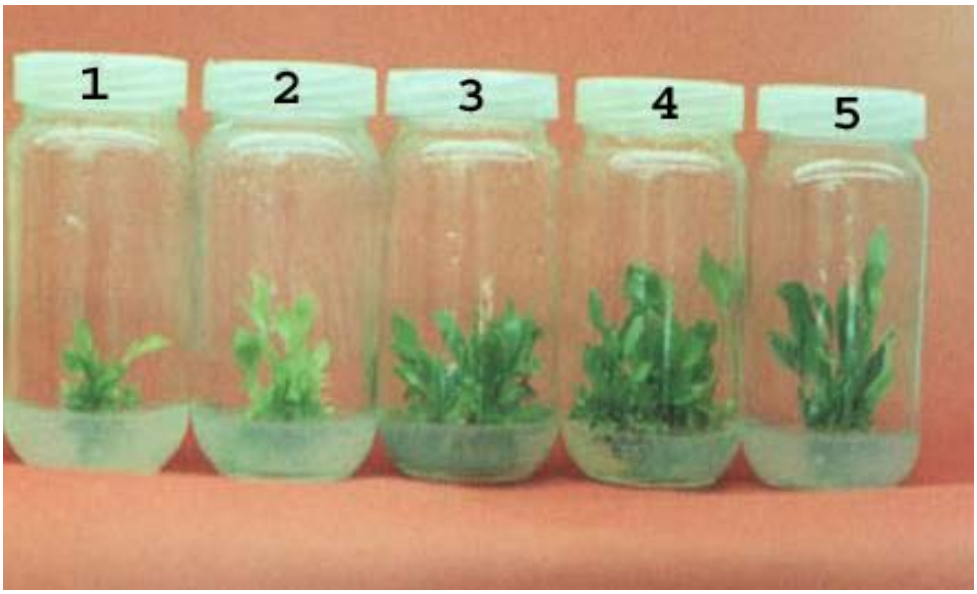

Fig. (2): Effect of sucrose concentration on shooting of Ruscus hypoglossum.
1- 10 g/l sucrose. 2- 20 g/l sucrose. 3- 30 g/l sucrose. 4- 40 g/l sucrose. 5- 50 g/l sucrose.

Chemical composition

Chlorophyll-A and B and carotenoids

The different concentrations of sucrose showed a significant influence on chlorophyll-A content (Table 4). Using sucrose at 30 g/l sucrose produced the highest amount of chlorophyll-A, while the lowest content of chlorophyll-A (133.8 mg/100g FW) was found by using sucrose at 20 g/l. The data also indicated that the various concentrations of sucrose had no significant effect on the chlorophyll-B content and carotenoids (Table 4).

Indoles and total soluble phenols

The indoles content was significantly affected by various concentrations of sucrose (Table 4). The highest content of indoles was produced by using 50 g/L sucrose, while the lowest content was observed by adding sucrose at 20 and 30 g/l concentrations. Sawsan (2002) on *Solidago altissima* var. "Toto" reported that the highest content of indoles was produced by using 50, 45, 30 and 40 g/l sucrose.

It is quite clear that the various concentrations of sucrose used in this study had no significant effect on total soluble phenols for *Ruscus hypoglossum* (Table 4).

Table (4): Contents (mg/100g) of chlorophyll A and B, carotenoids, indoles and phenols of Ruscus hypoglossum shoots as affected by different concentrations of sucrose.

Treatments	Chlorophyll-A	Chlorophyll-B	Carotenoids	Indoles	Phenols
10 gm/l	187.3	31.11	89.33	4.484	0.6420
20 gm/l	133.8	25.78	70.56	2.589	0.6023
30 gm/l	191.4	10.20	94.93	2.543	0.6770
40 gm/l	152.7	30.12	59.51	3.607	0.6077
50 gm/l	142.9	18.29	53.06	7.958	0.5420
L.S.D.0.05	51.68	NS	NS	1.487	NS

Experiment 3: Effect of MS-strength and IBA on root formation and shootlet growth

Number of roots

The data in Table (5) were recorded after 60 days from culturing shootlets of *Ruscus hypoglossum*. Results indicated that, the highest number of roots (6.16) was recorded when culturing on ½ strength MS medium supplemented with IBA at 2.0 mg/l in the absence or presence of activated charcoal (A.C.), while the lowest number of roots (0.50) was recorded when growing on full strength MS media with or without A.C. In conclusion, the best number of roots/shootlet (6.33) was recorded with ½ MS salt strength supplemented with 2.0 mg/l IBA and without activated charcoal, after 60 days.

Root length

The data showed that after 60 days, the highest root length (6.00 cm) was recorded when using MS medium at ½ salt strength supplemented with 3.0 mg/l IBA. The data indicated that the average root length of shootlets grown on MS media supplemented with A.C (3.63 cm) was significantly higher than those grown on MS media free of A.C. (2.90 cm) Growing the shootlets on MS at ½

salt strength supplemented with 3.0 mg/l IBA and A.C., gave the longest root (7.16 cm), while using MS medium at full salt strength without IBA, and without A.C. produced the shortest roots (0.16 cm).

In conclusion, the best root length was recorded when culturing shootlets on half strength MS medium supplemented with 3.0 mg/l IBA and activated charcoal, after 60 days.

Plant height

After 60 days from culturing the shootlets, data (Table 5) showed that the tallest plants (10.83 cm) were recorded when using ½ salt strength MS medium supplemented with 3.0 mg/l IBA, while the shortest shootlet was recorded with full MS salt with 1.0 mg/l IBA. On the other hand, ¼ salt strength MS medium without IBA and full strength MS medium with 1.0 mg/l IBA produced the shortest plants (5.33 cm). The data also showed that the plants, grown on MS medium supplemented with A.C were (7.79 cm) significantly taller than those grown on MS media without A.C. (6.54 cm). The tallest plants (11.83 cm) were recorded when using MS medium at ½ salt strength supplemented with 3.0 mg/l IBA, and charcoal.

Number of leaves

Data in Table (5) indicated that after 60 days from culturing the shootlets on rooting media, the highest number of leaves/shoot (7.16) was recorded by using ½ strength MS medium supplemented with 3.0 mg/l IBA, while the lowest number (3.16) was produced by using ½ or ¼ strength MS medium without IBA. Moreover, adding activated charcoal to MS medium significantly increased the number of leaves/plant as compared with MS medium free of charcoal.

Experiment 4: Effect of sucrose concentration on rooting

Number of roots:

The data in Table (6) indicated that there was no significant difference in the number of roots between sucrose concentrations. Data also showed that there was no significant difference between the number of roots on media with or without A.C. The highest number of roots (4.20) was recorded on MS medium supplemented with 50 gm/l sucrose and activated charcoal, but the lowest number (1.6) was recorded with 40 g/l sucrose and activated charcoal.

Root length

It is quite clear from the data (Table 6) that the different sucrose concentrations exhibited significant influences on the root length. The longest root (2.75 cm) was recorded with 55 g/l sucrose, while the shortest one (1.75 cm) was recorded with 45 g/l sucrose treatment. Moreover, the highest root length (3.07 cm) was shown with activated charcoal, while the shortest (1.30 cm) was shown without activated charcoal. The longest root (4.50 cm) was recorded when using MS medium supplemented with 55 g/l sucrose and activated charcoal, while the shortest root (0.90 cm) was recorded when using MS medium with 50 g/l sucrose and without activated charcoal.

Table (5): Mean number of roots/shootlet , root lengh, plant height and number of leaves/plant of Ruscus hypoglossum shooltels grown in vitro as affected by salt strength of MS medium ,IBA concentration and activated charcoal (A.C.).

Treatment	Number of roots			Root length (cm)			Plant height (cm)			Number of leaves		
	With A.C	Without A.C	Mean (A)	With A.C	Without A.C	Mean (A)	With A.C	Without A.C	Mean (A)	With A.C	Without A.C	Mean (A)
Ms- Strength	0.66	0.33	0.50	0.83	0.16	0.50	6.83	5.83	6.33	4.66	3.66	4.16
MS + 1.0 mg/l IBA	1.33	1.66	1.50	1.83	1.33	1.58	5.83	4.83	5.33	4.66	2.66	3.66
MS + 2.0 mg/l IBA	2.66	3.00	2.83	2.83	2.16	2.50	6.83	5.66	6.25	5.66	3.66	4.66
MS + 3.0 mg/l IBA	2.33	2.66	2.50	3.50	3.50	3.50	7.83	5.83	6.83	6.66	3.66	5.16
½ MS- strength	0.66	0.66	0.66	1.66	0.33	1.00	6.33	4.83	5.58	3.66	2.66	3.16
½ MS + 1.0 mg/l IBA	3.00	3.33	3.16	4.16	3.33	3.75	7.83	7.83	7.83	7.66	4.66	6.16
½ MS + 2.0 mg/l IBA	6.00	6.33	6.16	6.50	4.16	5.33	9.83	8.66	9.25	7.66	5.66	6.66
½ MS + 3.0 mg/l IBA	4.33	4.66	4.50	7.16	4.83	6.00	11.83	9.83	10.83	8.66	5.66	7.16
¼ Ms- strength	1.00	1.33	1.16	1.16	0.33	0.75	5.83	4.83	5.33	3.66	2.66	3.16
¼ MS + 1.0 mg/l IBA	2.00	2.33	2.16	3.16	3.66	3.41	6.83	5.83	6.33	6.66	3.66	5.16
¼ MS + 2.0 mg/l IBA	4.33	4.66	4.50	5.00	5.16	5.08	7.83	6.66	7.25	7.66	4.33	6.00
¼ MS + 3.0 mg/l IBA	2.66	3.00	2.83	5.83	5.83	5.83	9.83	7.83	8.83	7.66	5.66	6.66
Mean (B)	2.58	2.83	——	3.63	2.90	——	7.79	6.54	——	6.25	4.05	——
L.S.D. 0.05 (A)		0.585			0.592			0.270			0.453	
L.S.D. 0.05 (B)		0.239			0.241			0.110			0.185	
L.S.D. 0.05 (A.B)		0.828			0.838			0.381			0.640	

Table (6): Mean number of roots/shootlet and root length of Ruscus hypoglossum as affected by Sucrose concentration and charcoal in the MS medium.

Concentration of sucrose (g/l)	Number of roots		Mean (A)	Root length		Mean (A)
	1 g/l activated charcoal	No activated charcoal		1 g/l activated charcoal	No activated charcoal	
40 g/l	1.60	3.40	2.50	2.20	1.80	2.00
45 g/l	2.80	2.80	2.80	2.00	1.50	1.75
50 g/l	4.20	2.20	3.20	3.60	0.90	2.25
55 g/l	3.80	2.40	3.10	4.50	1.00	2.75
Mean (B)	3.10	2.70	——	3.07	1.30	——
L.S.D (A)		0.940			0.569	
L.S.D (B)		0.665			0.402	
L.S.D (A x B)		1.330			0.804	

Plant height

The different sucrose concentrations exhibited a significant influence on the plant height (Table 7). The tallest plants (5.95 cm) were recorded with 55 g/l sucrose, while the shortest one was observed with 40 g/l sucrose.

The data indicated that the highest mean plant height (6.47 cm) was shown with activated charcoal, while the shortest height (3.60 cm) was shown without charcoal. The highest plant length (8.36 cm) was recorded when using MS medium supplemented with 55 g/l sucrose and activated charcoal, and the shortest one (3.20 cm) was recorded when MS medium was supplemented with 45 g/l sucrose without activated charcoal.

Number of leaves

Data in Table (7) indicated that the highest mean number of leaves (15.80) was recorded when the medium contained 40 g/l sucrose, while the lowest number (10.60) was produced with 55 g/l sucrose. The highest number of leaves per plant was shown with treatments without activated charcoal, while the lowest number of leaves was shown with treatments containing activated charcoal. The highest number of leaves per plant (19.00) was recorded with 40 g/l sucrose without activated charcoal, and the lowest number of leaves (7.60) was recorded with 55 g/l sucrose plus activated charcoal.

Table (7): Mean plant height and number of leaves of Ruscus hypoglossum as affected by sucrose concentration and charcoal in the MS medium.

Concentrations of sucrose (g/l)	Plant height (cm)		Mean (A)	Number of leaves		Mean (A)
	1 g/l activated charcoal	No activated charcoal		1 g/l activated charcoal	No activated charcoal	
40 gm/l	4.40	4.20	4.30	12.60	19.00	15.80
45 gm/l	5.90	3.20	4.55	9.60	17.00	13.30
50 gm/l	7.30	3.40	5.35	9.40	17.60	13.50
55 gm/l	8.30	3.60	5.95	7.60	13.60	10.60
Mean (B)	6.47	3.60	———	9.80	16.80	———
L.S.D (A)		0.431			1.102	
L.S.D (B)		0.305			0.779	
L.S.D (A.B)		0.610			1.559	

Experiment 5: Effect of acclimatization media

Survival percentage

The different growing media used in this study had no significant effect on the survival percentage (Table 8). This means that all growing media during rooting stage produced healthy plants during acclimatization and showed high survival percentage.

Plant height

It is evident from the obtained data (Table 8) that the different growing media had a significant effect on plant height after hardening-off. The tallest plants (10.40 cm) were observed with peat moss growing medium, while the lowest value (9.20 cm) came from plants cultured in peat moss + vermiculite growing medium.

Number of leaves per plant

The various treatments had a significant affect on the number of leaves per plant. The greatest number of leaves (5.20) was produced by using peat moss as a growing medium.Thus, peat moss was the best medium for giving the highest means of plant height and number of leaves/plant.

Table (8): Mean percentage of survival plant, length and number of leaves/plant of Ruscus hypoglossum as affected by different growing media during acclimatization stage.

Growing medium	Survival %	Plant height cm	Number of leaves/plantlet
Peat moss	100	10.40	5.20
Peat moss + sand (1:1)	100	9.80	4.40
Peat moss + perlite (1:1)	100	9.60	4.80
Peat moss + vermiculite (1:1)	100	9.20	4.40
Peat moss + perlite + vermiculite (1:1:1)	100	9.45	4.60
L.S.D.0.05	NS	0.521	0.476

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الملخص العربي

تاثير تركيزات املاح بيئة (MS) وتركيزات السكروز وحمض الاندول بيوتريك وبيئات الأقلمة على نبات السفندر

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**مركز البحوث الزراعية – معهد بحوث البساتين- وزارة الزراعة-جمهورية مصر العربية

أجرى هذا البحث بمعمل زراعة الأنسجة بحديقة الزهرية (وحدة الخدمات البستانية – وزارة الزراعة) و كلية الزراعة جامعة القاهرة - خلال الفترة من ٢٠٠١- ٢٠٠٤و يهدف البحث إلى دراسة تأثير تركيزات بيئة MS وتركيز السكروز على استيلاد الفروع و المحتوى الكيماوى لنبات السفندر من خلال زراعة الأنسجة وتاثير تركيزات الIBA والفحم النشط والسكروز على تجدير الفروع الناتجة. وتأثير البيئات المختلفة (البيتموس و البيتموس : الرمل ١:١ و البيتموس :فرميكوليت ١:١ و البيتموس : البرليت ١:١ و البيتموس : الفرميكوليت : البرليت ١:١:١) على سلوك نباتات السفندر المجدرة معمليا عند اجراء عملية الاقلمة لها بالصوبة وقد اظهرت النتائج ان استخدام التركيز الكامل من الأملاح الأساسية لل MS نتج عنه أعلى عدد من الفروع . بينما استخدام نصف تركيز من الأملاح الأساسية من MS أعطى أفضل استطالة فى حين أن أكبر عدد من الأوراق على كل فرع قد سجل مع ربع تركيز للأملاح الأساسية من ال MS.كما وجد ان استخدام نصف و ربع تركيز من الأملاح الأساسية من ال MS أعطى أعلى محتوى من كلوروفيل-أ و الكاروتين فى اوراق الفروع .ولوحظ أعلى محتوى من الأندولات بالفرع عند استخدام ربع تركيز من أملاح MS.كذلك وجد ان استخدام ٣٠ و ٤٠ جم/لتر من السكروز يعطى أعلى معدل تضاعف للافرع و أعلى عدد أوراق لكل فرع . بينما أطول الأفرع تم الحصول عليها باستخدام ٥٠ جم/لتر سكروز.وان استخدام تركيز ١٠و ٣٠ جم/لتر من السكروز نتج عنه أعلى محتوى من الكلوروفيل-أ.وان استخدام تركيزات ١٠و ٢٠و ٣٠و ٤٠ جم /لترمن السكروز أدت إلى زيادة كل من محتوى الأندولات و الفينولات الكلية الذائبة فى الفرع .وان استخدام نصف تركيز من الأملاح الأساسية لل MS مضاف اليه ٢,٠ مجم/لتر IBA نتج عنه أعلى عدد من الجذور على كل فرع .واستخدام نصف تركيز من الأملاح الأساسية لل MS مضاف اليه ٣,٠ مجم/لتر IBA نتج عنه أعلى طول للجذور واعلى عدد للأوراق.كذلك وجد أعلى عدد من الجذور عند استعمال ٥٠ جم /لتر سكروز فى وجود الفحم النشط بينما أكبر طول للجذور و أطول نباتات و أكثر عدد من الأوراق وجد عند استعمال ٤٠ جم/لتر سكروز.وان إضافة الفحم النشط إلى التركيزات المختلفة من السكروز أعطى أكبر عدد من الجذور و أطول جذور وكذلك أطول نباتات بينما أكبر عدد من الأوراق فسجل مع المعاملات الخالية من الفحم النشط. واظهرت جميع بيئات الأقلمة قدرة عالية جدا" على بقاء النباتات حية بينما اظهرت استخدام بيئة البيتموس اكبر استطالة للنباتات واكبر عدد من الأوراق لكل نبات.