Effect of some natural culture media on *in vitro* shootlet proliferation of *Ruscus hypoglossum* L. and *Aspidistra elatior* Blume

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ABSTRACT

The effect of some natural media on in vitro shootlets proliferation behaviour was tested for Ruscus hypoglossum and Aspidistra elatior. With respect of Ruscus hypoglossum, the maximum number of shootlets produced per explant was recorded after three times of subculture on half strength MS-medium, but the tallest shootlet length and the greatest number of leaves per shootlet were found when using full strength MS-medium. The highest amounts of chlorophyll-A and carotenoids were observed in shootlet tissues grown on MS- medium of half and quarter strength, respectively. Chlorophyll-B was produced in higher values when using 50 and 100 g/l of wheat medium. Using 50 and 100 g/l of either barley or chickpea medium resulted in the highest amount of indoles and phenols in the shootlet tissues. With respect of Aspidistra elatior, the greatest numbers of shootlets per explant were obtained in case of using full strength MS-medium, while the tallest shootlets were found when using 100 g/l of either chickpea or barley medium. Culturing on full strength MS-medium gave the highest values of chlorophyll-A and carotenoids, but culturing on 50 g/l faba bean medium induced the formation of chlorophyll-B and phenols in maximum amounts. Culturing on 150 g/l chickpea medium resulted in the highest values of total indoles. In conclusion, it is useful to use the same natural culture media for improving in vitro shootlet, proliferation behaviour of Ruscus hypoglossium and Aspidistra elatior.

Keywords: *Natural media, in vitro proliferation, Ruscus hypoglossum, Aspidistra elatior.*

INTRODUCTION

The success of tissue culture in shootlet proliferation of the ornamental plants is greatly influenced by the culture media used. The nutrient medium has two major functions; the first is to supply the basic nutritional ingredient 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 plant tissue culture for shootlet proliferation, so it has been used by many workers such as Agrawal *et al.*, (1992) in *Vanilla walkeriae*, Pereira-Pinto *et al.*, (1996) in *Kielmeyera coriacea*, Torres and Mogollon (1997) in *Cattleya lueddemanniona*, Karhu (1997) in *Lonicera caerulea* and Sakr *et al.*, (1999) in *Yucca elephantipes*.

To reduce the high expenses of the tissue culture technique, the MS medium could be substituted by some natural, cheap and easily

available products. In the recent years, the searches for the natural products of plants have been undertaken by many investigators. Janthranee-Kittipuriwong (1991) reported that the largest protocorm bodies of *Rhynchostylis gigantean* were found in medium gelled with gellan gum, brown rice flour and corn flour. He added that the seedlings of *Vanda lilacina* grew best on the medium containing brown rice flour. Anderson (1991) recorded that the seedlings of *Spiranthes magnicamporum* grew well on oat medium (based on oat flour). Bhattacharya *et al.* (1994) found that the shoots of *Chrysanthemum* plantlets produced on agar medium were significantly taller than those grown on medium gelled with isubgol or sago. Varags-Suarez *et al.* (1996) mentioned that using starch as a carbon source in the culture medium promoted the chlorophyll accumulation in maize callus. Zapratan (1996) stated that MS medium supplemented with corn extract was considered the best to give microplants of *Leontopodium alpinum.*

The aim of this work is to study the effect of some natural products (flour of barley, wheat, faba bean and chickpea) as culture media, in comparison with the MS medium on shootlet proliferation behaviour of *Ruscus hypoglossum* and *Aspidistra elatior* explants *in vitro.* Also the chemical composition of the obtained shootlet tissues was determined.

MATERIALS AND METHODS

The expermintal work of this study was carried out in the tissue culture laboratory in El-Zoharya Garden at Cairo and Faculty of Agriculture Cairo University at Giza, during the years 2003 and 2004.

Nine month-old *Ruscus hypoglossum* and *Aspidistra elatior* seedlings, grown under plastic house conditions of El- Zoharya Botanic Garden, were used as a source of nodals explants.

Seedlings were washed with running tap water for two hours, then soaked in mercuric chloride solution at 0.1 (w/v) with few drops of tween-20 for 10 minutes. Then they were washed 3 times with sterile distilled water. To obtain the sufficient sterilization, the explants were soaked another time in 50% sodium hypochlorite solution (NaOCl) provided with few drops of tween-20 for 10 min and subsequently washed 3 times with a sterile distilled water.

MS basal medium (Murashige and Skoog, 1962) was used at full, half and quarter strength. At the same time, different natural media made from flours of wheat, barley, faba bean and chickpea at concentrations of 50, 100 and 150 g/l were prepared. Also, the flours of wheat, barley, faba bean and chickpea were mixed at the rate of 1:1:1:1 and used for preparing media used at concentration of 100 and 150 g/l. According to Complell *et al.*, (1963) the components of different flours were presented in Table (1). All natural media were solidified with 5.0 g/l agar and the pH was adjusted to 5.7±1 before autoclaving. 50 ml of the prepared media were dispensed into 350 ml glass jars and closed with polypropylene lids. Then they were autoclaved at 121°C and 1.5 kg/cm^2 for 20 min.

Nodal explants (1-2 cm), consisting of axillary buds, were excised from sterilized seedlings under laminar air flow hood and placed into the culture vessels. Five explants cultured per jar in 5 replicates were used for each treatment. All cultures were incubated at 26±2°C under 16-hours photoperiod light and 8-hours dark with white fluorescent light (3000 lux).

After four weeks of culturing, the obtained shootlets from each treatment, were aseptically recultured into fresh media. This

procedure was repeated three times at four weeks intervals.

At the end of each reculture, the following characters were measured: number of shoots per explant, shootlet length (cm) and number of leaves per explant. At the end of experiments (after three months of culturing) the chemical contents of shootlet tissues were measured; chlorophyll A and B and carotenoids were determined according to Saric *et al.* (1967); total indoles were measured according to the methods mentioned by Salim *et al.* (1978) and total soluble phenols were determined as described by William *et al.* (1965).

Data were statistically analyzed and the Least Significant Difference (LSD) test was used to compare the means of treatments according Steel and Torrie (1980).

Table (1): Composition of different flours used in this study for in vitro culture of Ruscus hypoglossum and Aspidistra elatior (after Complell et al., 1963).

Composition	Unit	Wheat	Barley	Faba bean	Chickpea
Water	%	8.56	7.86	8.18	7.28
Ash	%	0.79	3.03	3.6	2.56
Protein	%	9.59	8.87	18.34	16.75
N	%	1.68	1.55	3.21	2.93
Fat	%	3.25	3.04	2.64	6.57
Ca	mg / 100g	38.16	44.08	50.33	90.71
Mg	mg / 100g	106.21	154.55	179.06	142.29
Fe	mg / 100g	3.11	5.48	17.42	7.96
Zn	mg / 100g	1.66	2.30	2.81	2.64
K	mg / 100g	732.65	735.00	1023.74	1219.32
Na	mg / 100g	31.29	49.95	58.49	33.43
P	mg / 100g	0.356	0.366	0.383	0.335
Thiamine	mg / 100g	0.57	0.38	0.53	0.46
Riboflavin	mg / 100g	0.12	0.20	0.30	0.16
Niacin	mg / 100g	4.3	7.2	2.5	1.7
Vit. C	mg / 100g	0.0	0.0	6.0	1.0

RESULTS AND DISCUSSION

Ruscus hypogolossum

Shootlets per explant

The data in Table (2) on *Ruscus hypoglossum* illustrated that using the full strength MS-medium produced the highest number of shootlets per explant (12.58). While using media of mixed flours at 50 and 150 g/l concentrations and barley at 50 g/l gave the minimum number of shootlets/explant (2.58, 2.58 and 2.33 respectively).

Concerning the effect of the number of subcultures on shootlets/explant, the data in Table (2) indicated that with increasing the number of subcultures from the first through third subculture, the number of shootlets per explant was significantly increased (from 2.86 to 8.14, respectively).

For the interaction between different media and number of subcultures, the maximum number of shoots was recoreded when using full strength MS medium at the third subculture (18.00).

Shootlet length

According to data illustrated in Table (2), using half strength MS medium resulted in the longest shootlet (5.29cm) in Ruscus hypoglosum.

Concerning the effect of subculture number, as the subculture number increased the shootlet length significantly increased (from 1.97cm at the 1st to 3.25 cm at the 3rd subculture).

It was observed that using half strength MS medium at the third subculture produced the longest shoot (6.50 cm).

Leaves / shootlet

Reffering to the effect of type of culture media on number of leaves/shootlet, data in Table (2) clearly indicated that using full strength MS medium produced the highest number of leaves per shootlet (11.50) in *Ruscus hypoglosum*. Whereas, using the flour mixture medium at 50 g/l resulted in the smallest number of leaves (3.91).

It was found that, increasing the number of subcultures from 1st to 2nd and 3rd subcultures significantly increased the number of leaves from 3.70 to 5.88 and 8.15, respectively.

As for the interaction between different media and number of subcultures, the highest number of leaves per shoolet (13.00) was counted in case of using full strength MS medium at the third subculture.

From the above mentioned data, repeating the subculture three times significantly allowed the explants to produce the greatest number of shootlets/explant which were more pronounced in case of culturing on full strength MS medium, but culturing on half strength MS-medium significantly increased the shootlet length. This means that with reducing the salt concentration of MS salt to half strength, the biological processes controlling shootlet elongation became at their highest activity, but using the salts of MS-medium at full concentration clearly promoted both of formation of more shootlets and more leaves in *Ruscus hypoglossum*. It is worthy to note that the tested organic media did not prove any superiosity over MS media at full and half strength concerning the 3 studied shootlet traits. In contrast, these organic media resulted in the reduction of all studied traits as compared to MS media. Opposite results were reported by Hosni and Abou-Dahab (2002) on *Solidago altissima*. They found that using natural media of barley, wheat and faba bean flours gave the greatest number of shootlets, the longest shootlets and the greatest number of leaves as compared to MS-medium.

Table (2): Effect of different natural culture media on shootlet growth behaviour of Ruscus hypoglossum in vitro.

Culture media	Shootlets /explant				Shootlet length (cm)				leaves/shootlet			
	Subculture			Mean (B)	Subculture			Mean (B)	Subculture			Mean (B)
	1	2	3		1	2	3		1	2	3	
Full MS medium	7.50	12.25	18.00	12.58	3.75	4.75	5.12	4.54	10.00	11.50	13.00	11.50
Half MS medium	5.75	9.25	13.50	9.50	3.87	5.50	6.50	5.29	6.00	8.25	10.75	8.33
Quarter MS medium	4.25	5.75	8.50	6.17	3.37	3.62	4.00	3.66	5.75	6.50	7.25	6.50
50 g/l Wheat	2.50	3.25	4.25	3.33	1.87	2.37	3.50	2.58	3.25	5.75	7.25	5.41
100 g/l Wheat	3.25	6.75	12.50	7.50	1.75	2.12	3.00	2.29	3.75	8.75	11.50	8.00
150 g/l Wheat	3.25	4.50	6.25	4.66	1.50	2.00	2.75	2.08	3.25	6.25	7.75	5.75
50 g/l Barley	1.50	2.25	3.25	2.33	1.50	1.75	2.00	1.75	3.00	4.00	6.00	4.33
100 g/l Barley	1.75	3.00	6.25	3.66	1.62	2.00	2.50	2.04	2.75	4.25	5.75	4.25
150 g/l Barley	1.75	2.25	4.00	2.66	1.50	1.87	2.25	1.87	2.25	3.75	6.25	4.08
50 g/l Faba bean	2.00	3.50	5.25	3.58	1.87	2.50	3.37	2.58	2.75	5.00	6.25	4.66
100 g/l Faba bean	3.50	9.75	15.00	9.42	1.75	2.25	3.50	2.50	3.25	7.75	12.75	7.91
150 g/l Faba bean	2.25	4.25	7.75	4.75	1.62	2.00	2.75	2.12	2.50	6.00	8.00	5.50
50 g/l Chickpea	1.75	3.00	5.75	3.50	1.62	2.37	3.50	2.50	3.50	5.50	8.25	5.75
100 g/l Chickpea	3.50	9.00	12.75	8.41	1.50	2.25	2.87	2.20	3.00	6.00	11.50	6.83
150 g/l Chickpea	2.50	4.50	8.75	5.25	1.50	2.00	2.62	2.04	2.75	5.00	7.25	5.00
50 g/l Flour mixture	1.50	2.50	3.75	2.58	1.87	2.37	3.12	2.45	3.25	3.75	4.75	3.91
100 g/l Flour mixture	1.75	4.50	7.00	4.41	1.62	2.12	2.50	2.08	3.25	3.50	6.00	4.25
150 g/l Flour mixture	1.25	2.50	4.00	2.58	1.50	2.00	2.62	2.04	2.50	4.50	6.50	4.50
Mean (A)	2.86	5.15	8.14		1.97	2.54	3.25		3.70	5.88	8.15	
L.S.D 0.05 for subcultures (A) =	**0.6297**				**0.2710**				**0.6480**			
L.S.D 0.05 for media (B) =	**0.2571**				**0.1107**				**0.2646**			
L.S.D 0.5 for (A× B) =	**1.0900**				**0.4695**				**1.1220**			

Table (3): Effect of different natural culture media on chemical composition (in mg/100g fw)of Ruscus hypogolossum shootlet tissues.

Treatment	Chlorophyll. A	Chlorophyll. B	Caroteniods.	Indoles	Phenols
Full MS medium	110.9	10.18	26.13	0.398	2.079
Half MS medium	127.2	13.01	24.65	0.393	2.686
Quarter MS medium	126.7	12.69	42.11	0.545	2.101
50 g/l Wheat	33.46	17.35	35.05	0.534	2.761
100 g/l Wheat	20.98	15.46	33.40	0.751	2.228
150 g/l Wheat	27.41	17.15	36.45	0.648	2.607
50 g/l Barley	27.04	15.37	35.29	0.505	2.641
100 g/l Barley	12.42	12.82	15.77	0.410	1.711
150 g/l Barley	21.78	14.51	26.91	0.599	2.281
50 g/l Faba bean	12.22	9.00	27.53	0.519	2.239
100 g/l Faba bean	36.07	13.49	30.68	0.542	1.579
150 g/l Faba bean	14.06	10.50	28.64	0.375	1.320
50 g/l Chickpea	12.49	12.64	23.28	0.542	2.779
100 g/l Chickpea	8.90	10.30	20.74	0.720	4.507
150 g/l Chickpea	11.32	11.80	20.42	0.663	3.261
Flour mixture 50 g/l	7.48	8.04	18.88	0.634	2.400
Flour mixture 100 g/l	9.37	8.54	17.71	0.464	1.763
Flour mixture 150 g/l	8.47	9.94	18.16	0.652	2.079
LSD at 0.05	14.89	6.27	7.54	0.287	0.881

The differences between our results and those previously reviewed may be attributed to the genetic background of the different genera tested in different studies.

Chemical composition of shootlet tissues

Data presented in Table (3) showed that MS medium at half and quarter strength showed the highest amounts of chlorophyll-A (127.2 and 126.7 mg/100g fw, respectively), while wheat medium at 50 and 150 g/l gave the highest amount of chlorophyll-B (17.35 and 17.15 mg/100g fw, respectively). The carotenoids contents reached to the maximum values (42.11 mg/100g fw) when the quarter strength MS medium was used. The total amounts of indoles were at their lowest values (0.398, 0.398, 0.505 0.410, 0.375 and 0.464 mg/100g fw) in the shootlet tissues when using full and half strength MS media, 50 and 100 g/l barley, 150 g/l faba bean and 100 g/l of flour mixture media, respectively. The highest values of indoles resulted in case of using either wheat or chickpea media at 100 or 150 g/l respectively. The phenols contents were significantly increased to 4.507 and 3.26 mg/100g fw in case of using 100 and 150 g/l chickpea-media, respectively.

These results may be explained by that reducing the salt concentration of MS-medium to the half or quarter strength may have accelerated the biochemical activity leading to higher rates of forming chlorophyll-A and carotenoids anabolism, while using the above mentioned natural media promoted the biochemical pathways of forming chlorophyll-B, indoles and phenoles to maximum amounts. Similar results were reported by Vargas-Suarez *et al.* (1996) in maize, Saadawy (2000) in *Laelia anceps* and *Cymbidium devonianum*, and Hosni and Abou-Dahab (2002) in *Solidago altissima*.

Table (4): Effect of different natural culture media on shootlet growth behaviour of Aspidistra elatior in vitro.

Culture media	Shootlets /explant				Shootlet length (cm)				leaves/shootlet			
	Subculture			Mean (B)	Subculture			Mean (B)	Subculture			Mean (B)
	1	2	3		1	2	3		1	2	3	
Full MS medium	4.25	5.75	11.00	7.00	1.75	2.37	3.12	2.41	4.75	7.00	12.50	8.08
Half MS medium	4.25	5.25	10.00	6.50	2.12	2.87	3.75	2.91	4.50	5.25	10.50	6.75
Quarter MS medium	3.25	4.75	6.25	4.75	2.87	3.62	4.75	3.75	4.25	6.00	8.50	6.25
50 g/l Wheat	1.00	1.75	2.25	1.66	1.75	2.00	3.12	2.29	3.00	3.50	4.75	3.75
100 g/l Wheat	1.50	2.75	3.75	2.66	1.87	2.62	4.25	2.91	3.50	4.00	5.50	4.33
150 g/l Wheat	1.00	2.00	2.50	1.83	1.87	2.62	3.50	2.66	3.00	3.25	4.50	3.58
50 g/l Barley	1.00	1.25	1.75	1.33	1.75	2.25	3.12	2.37	3.00	3.25	4.00	3.41
100 g/l Barley	1.00	2.25	2.50	1.91	2.00	3.50	5.00	3.50	3.00	4.00	6.25	4.41
150 g/l Barley	1.00	2.00	2.25	1.75	1.75	2.87	3.87	2.83	3.00	4.25	5.00	4.08
50 g/l Faba bean	1.00	2.00	2.50	1.83	1.50	2.37	3.00	2.29	3.00	3.75	4.50	3.75
100 g/l Faba bean	1.75	2.75	4.00	2.83	1.87	2.00	2.87	2.25	3.50	5.00	6.25	4.91
150 g/l Faba bean	1.00	2.25	2.75	2.00	1.62	2.00	3.00	2.20	3.00	4.25	5.00	4.08
50 g/l Chickpea	1.00	1.00	1.00	1.00	1.75	2.37	3.37	2.50	3.00	3.00	3.50	3.16
100 g/l Chickpea	1.00	1.50	1.50	1.33	2.00	3.25	5.25	3.50	3.0	3.75	4.25	3.66
150 g/l Chickpea	1.0	1.25	1.25	1.16	1.75	2.62	3.87	2.75	3.00	3.25	3.75	3.33
50 g/l Flour mixture	1.0	1.50	2.00	1.50	1.50	2.37	2.87	2.25	3.00	3.50	4.25	3.58
100 g/l Flour mixture	1.00	1.50	2.50	1.66	1.75	2.12	2.37	2.08	3.00	3.75	5.00	3.91
150 g/l Flour mixture	1.00	1.75	2.00	1.58	1.75	2.37	2.25	2.12	3.00	3.75	4.25	3.66
Mean (A)	1.55	2.40	3.43		1.84	2.56	3.52		3.30	4.13	5.68	
L.S.D 0.05 for subcultures (A) =		0.3892				0.2511				0.4372		
L.S.D 0.05 for media (B) =		0.1589				0.1025				0.1785		
L.S.D 0.5 for (A× B) =		0.6589				0.4349				0.7572		

Aspidistra elatior

Shootlets /explant

Data presented in Table (4) revealed that the number of shootlets/explant for *Aspidistra elatior* was the highest (7.0) when using full strength MS medium.

The number of shootlets per explant in this genus had significantly increased from 1.55 to 2.40 and 3.43 as the number of subcultures increased from 1st to 2nd and 3rd subculture, respectively.

For 1st, 2nd and 3rd subculture studied, culturing the explants on full and half strength MS medium gave the greatest number of shootlets 4.25 and 4.25 for the 1st subculture, and 5.75 and 5.25 shootlet in the 2nd subculture, respectively. While in the 3rd subculture full strength MS medium produced the maximum shootlet number (11.0).

Shootlet length

Data in Table (4) showed that different media resulted in a different shootlet length of *Aspidistra elatior*. Using quarter strength MS medium produced the longest shootlet (3.75 cm), while using 100 and 150 g/l mixed flours-media resulted in the shortest one (2.08 and 2.12 cm, respectively).

Regarding the effect of subculture number on shootlet length, the data in Table (4) revealed that with increasing the subculture number from 1 to 2 and 3, the shootlet length accumulatively increased significantly from 1.84 to 2.56 and 3.52 cm, respectively.

Data in Table (4) showed that, in case of the first subculture, using quarter strength MS-medium produced the tallest shootlet (2.87 cm). In case of the second subculture, using quarter strength MS medium, 100g/l barley and 100 g/l chickpea-media gave the maximum length of shootlet (3.62, 3.50 and 3.25 cm, respectively). In case of third subculture, using 100 g/l barley and 100 g/l chickpea media gave the maximum shootlet length (5.00 and 5.25 cm, respectively).

Leaves /shootlet

As shown in Table (4) the number of leaves/shootlet was highest (8.08) when using full strength MS medium, while all tested natural media produced the lowest number (less than 4.41).

For the 1st subculture, the maximum number of leaves per shootlet (4.75, 4.5) resulted when using full and half strength MS-medium, respectively. For the 2nd and 3rd subcultures, full strength MS-medium gave the highest number of leaves (7.00 and 12.5, respectively).

Chemical composition of shootlet tissues

For pigments contents of shootlet tissues of *Aspidistra elatior*, data in Table (5) revealed that using full MS medium resulted in the maximum amount of chlorophyll-A and carotenoids (91.18 and 57.89 mg/100g fw, respectively), while using 50g/l faba bean - medium gave the highest amount of chlorophyll-B (27.23 mg/100g fw).

Moreover, using 150 g/l chickpea medium produced the greatest amount of total indoles (9.628 mg/100g fw), while using 100 g/l wheat medium gave the lowest amount (3.370 mg/100g fw).

Fig. (1): Shootlet proliferation in Ruscus hypoglossum using natural media:

1. *100 g/l Wheat flour.*
2. *100 g/l Faba bean flour.*
3. *100 g/l Barley flour.*
4. *100 g/l Chickpea flour*
5. *100 g/l Mixture of Wheat, Faba bean, Barley and Chickpea flours.*

Fig. (2): Shootlet proliferation in Aspidistra elatior using natural media:

1. *100 g/l Wheat flour.*
2. *100 g/l Faba bean flour.*
3. *100 g/l Barley flour.*
4. *100 g/l Chickpea flour*
5. *100 g/l Mixture of Wheat, Faba bean, Barley and Chickpea flours.*

As for phenols, the maximum amount (1.236 mg/100g fw) was recorded when using 50 g/l faba bean medium and the minimum amount (0.361 mg/100g) was observed when using 150 g/l flour mixture medium.

Table (5): Effect of different natural culture media on chemical composition in mg/100g fw of Aspidistra elatior.

Treatment	Chlorophyll A	Chlorophyll B	Carotenoids	Indoles	Phenols
Full MS medium	91.18	2.35	57.89	5.265	0.490
Half MS medium	47.48	6.49	46.80	4.685	0.614
QuarterMS medium	52.01	10.63	47.09	4.673	0.614
50 g/l Wheat	23.98	24.92	36.52	5.765	0.577
100 g/l Wheat	22.06	16.76	31.44	3.370	0.691
150 g/l Wheat	33.96	20.32	50.39	6.666	0.901
50 g/l Barley	24.02	20.02	32.99	6.419	0.915
100 g/l Barley	16.62	14.28	33.40	6.057	0.869
150 g/l Barley	22.77	17.95	32.87	4.524	0.898
50 g/l Faba bean	24.34	27.23	42.16	4.663	1.236
100 g/l Faba bean	12.72	10.58	23.39	4.358	0.754
150 g/l Faba bean	13.60	13.51	20.54	4.553	0.691
50 g/l Chickpea	23.23	14.94	32.93	5.196	0.677
100 g/l Chickpea	18.30	12.54	27.56	7.899	0.800
150 g/l Chickpea	15.71	12.09	28.59	9.628	0.874
Flour mixture 50 g/l	10.03	13.53	15.65	8.595	0.855
Flour mixture 100 g/l	10.27	9.65	21.02	7.728	0.679
Flour mixture 150 g/l	8.15	8.81	18.02	6.250	0.361
LSD at 0.05	14.83	9.124	5.675	1.859	0.296

From the previour results on *in vitro* shootlet proliferation of *Aspidistra elatior*, the increased capabilily of explant tissue to produce the maximum number of shootlets/explant and/or leaves/shootlet and form the greatest amount of chlorophyll-A and carotenoids may be attributed to the highest physological activity of the full strength MS components. As for increase of shootlet elongation and total indoles formation at maximum values could be explained by the biochemical effect of natural media of chickpea or barley at concentration of 100 or 150 g/l. The biochemical activity controlling the formation of chlorophyll-B and phenols were at high rate when 50 g/l faba bean medium was used. In this respect, the previous results of Janthranee-Kittipuriwong (1991) reported that the largest protocorm bodies of *Rhynchostylis gigantean* were found on medium gelled with gellan gum, brown rice flour and corn flour. He added that the seedlings of *Vanda lilacina* grew best on the medium containing brown rice flour. Anderson (1991) recorded that the seedlings of *Spiranthes magnicamporum* grew well on oat medium (based on oat flour). Bhattacharya *et al.* (1994) found that the shoot length of *Chrysanthemum* plantlets produced on agar medium was significantly longer than those grown on medium gelled with isubgol or sago. They concluded that using different natural or chemical media had a different effect on the growth characters and chemical composition of the obtained plantlets.

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الملخص العربي

تأثير بعض بيئات الزراعة العضوية على استيلاد الافرع لنباتات السفندر والاسبدسترا بمزارع الانسجة

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تم اجراء هذا البحث خلال الاعوام 2003 ، 2004 بمعمل الزهرية بهدف دراسة تأثير بعض البيئات الزراعية العضوية على سلوك تضاعف الافرع لنباتات السفندر والاسبدسترا بمزارع الانسجة. ويمكن تلخيص النتائج فيما يلى:

بالنسبة لنبات السفند: زراعة الفصلة النباتية لثلاث فترات متتالية (كل فترة شهر واحد) ادى الى زيادة واضحة فى معدل تكلثر الافرع وذلك عند استخدام نصف تركيز لبيئة موراشيج وسكوج MS ولكن اطول الفريعات واكبر عدد من الاوراق المتكونة لكل فريعة تم الحصول عليها عند استخدام التركيز الكامل لبيئة MS وقدرت اكبر كمية من الكلوروفيل (أ) والكاروتين فى انسجة الافرع النامية على 1/2، 1/4 تركيز املاح بيئة MS وكانت اعلى كمية من كلوروفيل (ب) قد وجدت فى حالة الزراعة على بيئة القمح بتركيز 50، 100 جرام/لتر. وادى استخدام 50، 100 جرام/لتر من كل من الشعير والحمص الى انتاج اعلى كمية من الاندولات والفينولات فى انسجة الافرع .

بالنسبة لنبات الاسبدسترا: بتكرار الزراعة لثلاث فترات (كل فترة شهر واحد) وجد ان اكبر عدد من كل من الافرع للفصلة النباتية والاوراق/فريعة قد تم الحصول عليها عند استخدام التركيز الكامل لبيئة MS. بينما اطول الفريعات وجدت فى حالة استخدام 100 جرام/لتر من بيئات دقيق الشعير والحمص . ووجد ان الزراعة على تركيز كامل لبيئة MS اعطى اعلى كمية من كلوروفيل (أ) والكاروتين ولكن الزراعة على بيئة الفول البلدى (50 جرام/لتر) زادت من كمية كلوروفيل (ب) والاندولات لاعلى مستوى. ووجد ان الزراعة على 150 جرام/لتر من بيئة دقيق الحمص انتجت اعلى قيمة من الفينولات الكلية.