Establishment of an *in vitro* propagation protocol for *Taxodiumdistichum* and *Taxodium distichum* var. 'distichum'

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Abstract: This study was carried out in cooperation between Plant Biotechnology Department, Genetic Engineering and Biotechnology Division, National Research Center, Giza, Egypt, and the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the period from 2004 to 2008. The aim of the study is investigating the factors affecting rooting and acclimatization, and establishing an effective protocol for propagation of *Taxodium distichum* and *Taxodium distichum* var. 'distichum', using tissue culture techniques. Nodal explants from young branches were sterilized by using different concentration of Clorox and mercuric chloride (MC), and then cultured on different concentrations of Benzyl adenine (BA) and different types of media. The recorded data showed that the best results in sterilization of nodal explants were recorded with using 20% Clorox for 5 min., followed by 0.2% MC for 5 min. Half strength B_5 medium supplemented with 0.4 mg BA/l gave better shoot lets multiplication, as compared with half strength MS medium. The longest shoot lets were recorded with woody plant medium (WPM) at full salt strength. Half strength WPM medium + 1.0 g activated charcoal (AC)/l + 0.5 mg IBA/l was the best medium for *in vitro* rooting percentage and root number/shoot let. The maximum percentage of acclimatized plantlets survival (86.5%) was recorded with the mixture of sand and peat (1:1, v/v). [Nature and Science 2010;8(9):216-227]. (ISSN: 1545-0740).

Keywords: acclimatization; propagation; *Taxodium distichum; Taxodium distichum;* tissue culture;

1. Introduction:

Forests are important renewable natural resources, because they provide several important products, including fuel, timber, lumber, paper, and fodder. They are the main wildlife habitat, and also serve other purposes such as recreation, and as air and water sheds. Forests regulate the level of rainfall necessary for the existence of vegetation on earth. They also help in recycling moisture. In Egypt, it is important to raise the mass production of various woody trees by cultivating them in different arid and semi-arid regions. There are many tree species grown in various areas of the country, but cultivation of woody trees (including *Taxodium*) in Egypt has been faced with several problems, like a failure to provide the necessary provision for plantation, and lack of interest by farmers and crop growers. *Taxodium* could be one of the promising crop trees in Egypt. There are only four *Taxodium distichum* trees in all of Egypt.

Taxodium wood has a multitude of uses and is well known for its ability to resist decay. Oil extracted from the wood is believed to give bald cypress high decay resistance. For this reason, cypress wood has long been favored in building construction, fences, planking in boats, river pilings, furniture, interior trim, cabinetry, sills, rafters, siding, flooring and shingles, garden boxes, greenhouses, and many other uses (Choong *et al.*, 1986).

Some forest trees are propagated vegetative by cuttings, grafting, layering, etc. However, only a limited number of plants can be produced by this way, and it takes years to build up enough stock for planting in fields or forests. Sometimes, vegetative propagation proves to be impossible. Moreover, there are some problems that face the propagation of forest trees from seeds. Storage of seeds for long periods is not feasible to preserve the germplasm.

A number of reports concerning isolation and characterization of bioactive compounds from various parts of *Taxodium distichum* have appeared in the literature. Such compounds include cytotoxic diterpenoid quinine methides, 2-furaldehyde, tannins, flavone and its derivatives, sterols and fatty acids, and proanthocyanidin (Kupchan *et al*., 1969). Hirasawa *et al*. (2007) isolated two new abietane-type diterpenes, taxodistines A and B, from the fruits of *Taxodium distichum* by the guidance of their inhibitory effect on tubulin polymerization, and the structures were elucidated by using 2D NMR data. Taxodistine B showed inhibition of tubulin polymerization.

This study was conducted with the aim of establishing an *in vitro* propagation protocol for *Taxodium distichum* and *Taxodium distichum* var. 'distichum'

2. Materials and Methods:

This study was carried out in the Plant Biotechnology Department, Genetic Engineering and Biotechnology Division, National Research Center, Giza, Egypt, and the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the period from 2004 to 2008.

The aim of the study was to investigate the effects of some factors influencing the different stages of micropropagation of *Taxodium distichum* and *Taxodium distichum* var. 'distichum' from nodal explants, as follows:

3. Results and Discussion:

Experiment (1): Effect of different sterilization treatments on explants survival during the establishment stage.

Nodal explants (as starting plant materials were taken from young branches of *Taxodium distichum* and *Taxodium distichum* var. 'distichum' trees growing in Orman Garden, Giza, Egypt. The explants were rinsed in soapy water using septol soap, then agitated in a Savlon disinfectant solution (3%) for 20 min., washed with running tap water for one hour, and soaked for 1 min. in 70% ethanol under aseptic conditions in a laminar air-flow cabinet. The explants were then immersed for 5 min. in different solutions containing commercial Clorox (NaOCl, 5.25% free chlorine) at concentrations of 20, 40, or 80% (v/v) with a few drops of Tween-20, and/or mercuric chloride (MC) at concentrations of 0.1 or 0.2 (w/v) with a few drops of Tween-20. Clorox and MC were used either separately (at the above concentrations), or in different combinations of the tested concentrations of the two chemicals. A total of 11 disinfection treatments (3 Clorox concentrations + 2 MC concentrations + 6 Clorox/MC combinations) were tested in this study. Each treatment consisted of 10 jars, each jar containing two nodal explants. After receiving the disinfection treatments, the explants were rinsed three times with sterile distilled water. The explants receiving the different disinfection treatments were cultured on a basal MS medium (Murashige and Skoog, 1962) at half salt strength. The culture medium was solidified by the addition of 0.7 % agar prior to autoclaving at 1.2 kg/cm^2 for 15 min. The pH of the culture medium was adjusted to 5.8 by addition of 0.1 N KOH. Culturing was done in 300 ml glass jars containing 50 ml of the medium.

All cultures were incubated for 4 weeks under controlled conditions in the growth chamber. The incubation temperature was 24±2° C, controlled by a "Power" air conditioner. The photoperiod was 16 hours light/8 hour darkness, controlled automatically. Illumination intensity was 3000 lux from cool fluorescent lamps (120 cm long). The explant survival percentage was recorded after one month of culturing.

The explants surviving from the establishment stage were used as a source for the plant material used for the following *in vitro* experiments, which were conducted during the multiplication stage.

Experiment (2) Effects of Benzyladenine (BA) and MS salt strengths during the multiplication stage

This experiment was designed to study the effect of different combinations of BA concentrations (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 mg/l) and MS salt strengths (full, 3/4 and 1/2 strength) on a shoot let development on the explants of *Taxodium distichum* and *Taxodium distichum* var. 'distichum'. The experiment included 18 treatments (6 BA concentrations X 3 MS strengths). Each treatment combination was replicated 5 times (one jar/replicate) in a completely randomized design, and the explants were resutured three times, at 30 day intervals. One month after the third subculture (after three months from the first subculture, or starting the experiment), the number of shootlets / explant and shoot let length (cm) were recorded.

Experiment (3) Effect of different types of media during the multiplication stage

The experiment was designed to study the effect of four different types of culture media, as well as medium salt strength, on shoot let development on the explants. The nodal explants were cultured on MS (Murashige and Skoog, 1962), LS (Linsmaier and Skoog, 1965), B_5 (Gamborg *et al.*, 1968) and WPM **(**Lloyd and McCown, 1980) media at full and half salt strength. All the culture media were amended with 0.4 mg/l BA. The composition of the culture media is presented in Table (1). Thus, the experiment included 8 treatments (4 media X 2 salt strengths), with 4 replicates per treatment, arranged in a completely randomized design. The explants were resutured three times at 30 day intervals.

One month after the third subculture, the number of shootlets / explant and shoot let length (cm) were recorded.

Experiment (4): Effect of culture media, activated charcoal (AC), silver nitrate and IBA during the rooting stage.

This experiment was conducted to investigate the effects of culture media, activated charcoal (AC), silver nitrate and IBA during the rooting stage. Uniform shoot lets produced *in vitro* from the multiplication stage were individually separated and cultured on MS or WPM media, each

at half salt strength. Each of the tested media was either unmodified, or was amended using activated charcoal (AC) at 1.0 g/l, combined with $AgNO_3$ at 0.0, 5.0 or 7.0 mg/l, or combined with IBA at 0, 0.5, 1.0, 2.0 or 3.0 mg/l. The shoot lets were incubated for 8 weeks, and then the rooting response to the treatments was recorded, in terms of rooting percentage (%), number of roots / shoot, and root length (cm).

Table 1: Chemical components of MS, LS, B5, and WPM media.

Chemical components	Culture media			
	MS	LS	B5	WPM
Macro elements (mg/l):				
NH_4NO_3	1650.00	1650.00	-	400.00
KNO_3	1900.00	1900.00	2500.00	-
$CaCl_2$	440.00	332.02	113.23	96.00
$MgSO_4.7H_2O$	370.00	180.54	121.56	370.00
KH_2PO_4	170.00	170.00	-	170.00
$(NH_4)_2SO_4$	-	-	134.00	-
$NaH_2PO_4.H_2O$	-	-	-	-
$Ca(NO_3)_2.4H_2O$	-	-	-	556.00
K_2SO_4	-	-	-	990.00
Micro elements (mg/l):				
H_3BO_3	6.200	6.200	3.000	6.200
$MnSO_4.H_2O$	16.900	16.900	10.000	22.300
$ZnSO_4.7H_2O$	8.600	8.600	2.000	8.600
KI	0.830	0.830	0.750	-
$Na_2MoO_4.2H_2O$	0.250	0.250	0.250	0.025
$CuSO_4.5H_2O$	0.025	0.025	0.025	0.025
$COCl_2.6H_2O$	0.025	0.025	0.025	-
$FeSO_4.7H_2O$	27.800	-	-	27.800
Na_2 EDTA ($2H_2O$)	37.300	36.700	36.700	37.300
Organic components (mg/l):				
Myo-inositol	100.00	100.000	100.00	100.00
Nicotinic acid	0.50	-	1.00	0.50
Thiamine HCl	0.10	0.400	10.00	1.00
Pyridoxine HCl	0.50	-	1.00	0.50
Glycine	-	-	-	2.00

Experiment 5. Effect of different growing media during the plantlet acclimatization stage100%

Table 2. Effect of different Clorox and mercuric chloride (M.C.) concentrations on survival percentage of nodal of *Taxodium distichum* and *T. distichum* var. 'distichum' explants.

M.C. % (w/v)	*T. distichum*				*T. distichum* var. 'distichum'			
	Clorox %				Clorox %			
	0.0	20	40	80	0.0	20	40	80
	Survival percentage				Survival percentage			
0.0	N.D.	50	65	40	N.D.	60	65	35
0.1	15	80	75	35	15	85	75	20
0.2	25	100	80	25	30	95	50	10

Where: N.D. = Not determined.

Survival in case of *T. distichum* and 95% in *T. distichum* var. 'distichum'. Furthermore, it was observed that *T. distichum* var. 'distichum' was generally more susceptible to damage caused by the sterilization chemicals, compared to *T. distichum*, especially with the elevated levels of both disinfectants.

The obtained result may be explained by the sensitivity of plant tissues of *T. distichum* and *T.*

distichum var. 'distichum' to excessive surface sterilization with $HgCl_2$, as stated by Russel and Chopra (1990). Moreover, Rice *et al.* (1992) reported that Chlorox is a powerful antimicrobial agent. Furthermore, Arafa *et al.* (1999) and Hussein (2002) reported that surface sterilization with $HgCl_2$ followed by Chlorox resulted in the highest decontamination and survival percentage of *Dieffenbachia exotica* cv. Tropic-Snow and *Aglaonema spp.*, respectively.

Experiment 2. Effects of Benzyladenine (BA) and MS salt strengths during the multiplication stage

1. Shoot lets number per explants

Data tabulated in Table (3) and Fig. (1) clearly showed that, in both varieties, significant increases in number of shoot lets /explants were recorded with BA at the different concentrations (with basal MS at full, ¾ and ½ salt strength). In this concern, BA at 0.4 mg/l gave the highest mean shoot lets number/explant (13.50, 13.80 and 19.20, with full, ¾, and ½ MS salt strength, respectively). Among the different strengths of MS, half the salt strength was the best MS strength compared with the full and ¾ salt strength.

The recorded data also showed that in general, *T. distichum* gave significantly more shootlets /explant than *T. distichum* var. 'distichum'. Concerning the interaction between BA concentrations and MS salts strengths, the highest number of shootlets /explant in the two varieties (19.60 and 18.80 shootlets /explant in *T. distichum* and *T. distichum* var. 'distichum', respectively) were recorded with ½ MS supplemented with 0.4 mg/l BA. On the other hand, the lowest number of shootlets formed on *T. distichum* explant (2.40 shootlets/explant) were recorded with ¾ MS basal medium, while the lowest number of shootlets formed on *T. distichum* var. 'distichum' explants (2.20 shootlets/explant) was recorded with the full strength MS medium.

The positive effect of BA and MS medium in promoting the proliferation of shootlets number was also detected by **Boulay (1989)**, who indicated that the best shoot proliferation of *Sequoia sempervirens* occurred on MS medium with half of macroelements, modified with 0.5-1.0 mg BA /L and 0.02 mg NAA /. Also, **Koriesh *et al.* (2003)** found that the highest shoot multiplication rate was obtained when *Eucalyptus citriodora* explants were cultured on MS medium supplemented with 0.5 mg BA /L. Also, **Aloufa *et al.* (2003)** cultured nodal explants of *Ximenia americana* on an MS medium containing 2.5 to 15.0 µM BA and found that the number of shoots per explant was increased with the increase in the level of cytokinin.

Table 3. Effect of different BA concentrations and MS salt strengths on number of shootlets formed on nodal explants of *T. distichum* and *T. distichum* var. 'distichum'.

Treatments (A)	Plant variety (B)		Mean (A)
	T. distichum	*T. distichum* var. 'distichum'	
Full MS (basal)	3.20	2.20	2.70
Full MS + 0.2 mg BA /L	8.80	7.40	8.10
Full MS + 0.4 mg BA /L	14.20	12.80	13.50
Full MS + 0.6 mg BA /L	10.40	8.40	9.40
Full MS + 0.8 mg BA /L	10.20	8.80	9.50
Full MS + 1.0 mg BA /L	7.00	6.20	6.60
3/4 MS (basal)	2.40	2.80	2.60
3/4 MS + 0.2 mg BA /L	7.80	6.20	7.00
3/4 MS + 0.4 mg BA /L	14.80	12.80	13.80
3/4 MS + 0.6 mg BA /L	11.40	10.20	10.80
3/4 MS + 0.8 mg BA /L	10.40	7.80	9.10
3/4 MS + 1.0 mg BA /L	8.00	7.20	7.60
1/2 MS (basal)	3.40	3.80	3.60
1/2 MS + 0.2 mg BA /L	9.60	8.40	9.0
1/2 MS + 0.4 mg BA /L	19.60	18.80	19.20
1/2 MS + 0.6 mg BA /L	16.80	14.80	15.80
1/2 MS + 0.8 mg BA /L	10.20	9.800	10.00
1/2 MS + 1.0 mg BA /L	7.80	7.80	7.80
Mean (B)	9.77	8.67	_____
LSD $_{0.05}$	A = 1.027		
	B = 0.3423		
	AB = 1.452		

A

B

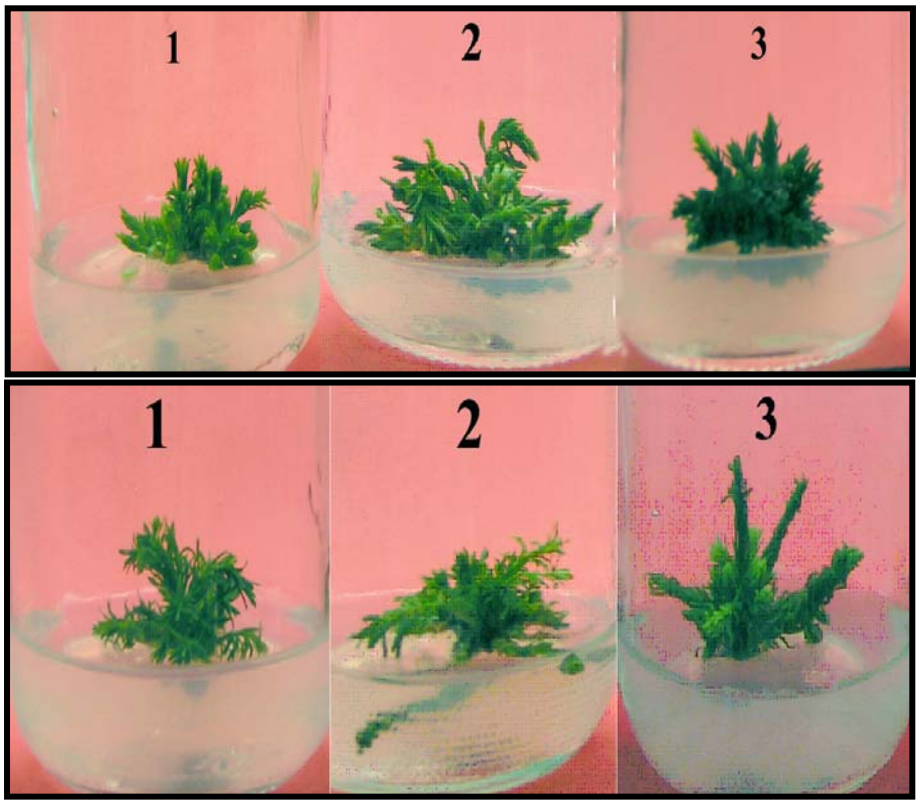

Fig. 1. Shoot proliferation on nodal explants of *T. dustichum* (A), and *T. dustichum* var. 'distichum' (B), after three subcultures.

1- Full MS + 0.4 mg BA /L
2- ¾ MS + 0.4 mg BA /L
3- ½ MS + 0.4 mg BA /L

2. Shootlet length

Data in Table (4) revealed that the length of shootlets formed on *T. distichum* and *T. distichum* var. 'distichum' nodal explants was inversely related to BA concentration and MS salt strength. The longest shootlets (with a mean length of 4.20 cm) were recorded with the basal MS medium (free of growth regulators) at ½ strength. Within each MS medium salt strength, the shortest shootlets (1.65, 1.95 and 1.90 cm with full, ¾ and ½ MS, respectively) were recorded when the medium was supplemented with the highest BA concentration (1.0 mg/L), with no significant differences between these values. Furthermore, the data showed that *T. distichum* var. 'distichum' gave significantly longer shoot lets (3.0 cm) then *T. distichum* (2.80 cm).

The general decrease in shoot let the length in both *T. distichum* and *T. distichum* var. 'distichum' pursuant to increasing BA concentration in the culture medium is in agreement with the findings of Hunter and Donnell (1988) on *Sequoia sempervirens*, Mantell *et al*. (1998) and Boggetti *et al*. (1999) on cashew (*Anacardium occidentals*), Cuenca *et al*. (1999) on *Centaura paui*, and Meghwal *et al*. (2003) on guava.

Experiment 3. Effect of different types of media during the multiplication stage

1. Shootlets number

Data in Table (5) and Fig. (2), show the effect of different types of nutrient media (MS, LS, B_5 and WPM), at full or half salt strength, supplemented with 0.4 mg BA /L on the number of shoot lets /explant in *T. distichum* and *T. distichum* var. 'distichum'. The highest mean number of shoot lets/explant (31.25) was recorded with B_5 at half salt strength, as compared with full B_5 salt strength (25.88) or other types of media. On the other hand, the lowest number of shoot lets/explant (13.75) was recorded with the full strength LS medium. Concerning the differences between *T. distichum* and *T. distichum* var. 'distichum', the obtained data indicated that *T. distichum* gave more shoot lets (20.47 shoot lets/plant) than *T. distichum* var. 'distichum' (19.09 shoot lets/plant).

In conclusion, it can be stated that B_5 at half salt strength was the best nutrient medium for shoot lets multiplication on explants of both *T. distichum* and *T. distichum* var. 'distichu.

Table 4. Effect of different BA concentrations and MS salt strengths on shootlet length (cm) in of *T. distichum* and *T. distichum* var. 'distichum' explants.

Treatments (A)	Plant variety (B)		Mean (A)
	T. distichum	*T. distichum var. 'distichum'*	
Full MS	3.70	4.0	3.85
Full MS + 0.2 mg BA /L	2.90	3.40	3.15
Full MS + 0.4 mg BA /L	2.90	2.80	2.85
Full MS + 0.6 mg BA /L	2.90	2.70	2.80
Full MS + 0.8 mg BA /L	2.50	2.40	2.45
Full MS + 1.0 mg BA /L	1.60	1.70	1.65
3/4 MS	3.50	3.60	3.55
3/4 MS + 0.2 mg BA /L	3.70	3.80	3.75
3/4 MS + 0.4 mg BA /L	2.60	2.80	2.65
3/4 MS + 0.6 mg BA /L	2.50	2.80	2.70
3/4 MS + 0.8 mg BA /L	2.30	2.70	2.50
3/4 MS + 1.0 mg BA /L	1.80	2.10	1.95
1/2 MS	4.00	4.40	4.20
1/2 MS + 0.2 mg BA /L	3.90	3.30	3.60
1/2 MS + 0.4 mg BA /L	3.50	3.50	3.50
1/2 MS + 0.6 mg BA /L	3.20	3.40	3.30
1/2 MS + 0.8 mg BA /L	2.20	2.60	2.40
1/2 MS + 1.0 mg BA /L	1.80	2.00	1.90
Mean (B)	2.86	3.00	—

LSD $_{0.05}$ A = 0.3526; B = 0.1175; AB = 0.4986

Table 5. Effect of different types of culture media, medium salt strength, and addition of BA to the medium, on number of shoot lets formed on nodal explants of *T. distichum* and *T. distichum* var. 'distichum'.

Treatments (A)	Plant variety (B)		Mean (A)
	T. distichum	*T. distichum* var. 'distichum'	
Full MS	17.00	14.25	15.63
Half MS	22.25	22.75	22.50
Full LS	14.00	13.50	13.75
Half LS	18.00	16.25	17.13
Full B5	27.50	24.25	25.88
Half B5	32.50	30.00	31.25
Full WPM	15.00	14.75	14.88
Half WPM	17.50	17.00	17.25
Mean (B)	20.47	19.09	—

LSD $_{0.05}$ A = 1.698; B = 0.8490; AB = 2.401

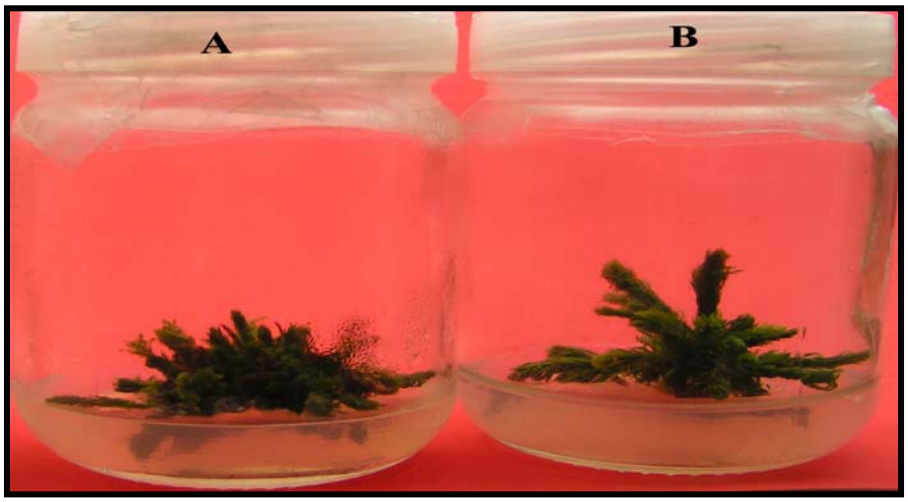

Fig. 2. Shoot proliferation on nodal explants of *T. dustichum* (A), and *T. dustichum* var. 'distichum' (B), after three subcultures on B_5 medium at half salt strength

2. Shoot let length

Data presented in Table (6) show the effect of different types of nutrient media, viz., MS, LS, B_5 and WPM at full or half of salt strength, supplemented with 0.4 mg BA /L , on the shoot let length in *T. distichum* and *T. distichum* var. 'distichum' explants. Results obtained clearly showed that shoot let length were significantly affected by the two factors (type and strength of the culture media). The longest shoot lets (4.0 cm) were obtained with WPM medium at full salt strength, while the shortest shoot lets (2.188 cm) were recorded with LS medium at half salt strength. No significant difference was observed between the shoot let lengths in *T. distichum* and *T. distichum* var. 'distichum'.

The results obtained are in agreement with those obtained by Sakr *et al.* (1999) on *Magnolia grandiflora*, Khatri *et al.* (2001) on *Tectona grandis*, Nassar *et al.* (2001) on *Bixa orellana*, Sebastian *et al.* (2002) on *Rotula aquatic,* and Brum *et al.* (2003) on *Ficus carica*.

Table 6. Effect of different types of culture media, medium salt strength, and addition of 0.4 mg/l BA on shootlet length of *T. distichum* and *T. distichum* var. 'distichum'.

Treatments (A)	Plant variety (B)		Mean (A)
	T. distichum	*T. distichum* var. 'distichum'	
Full MS	2.750	2.750	2.750
Half MS	3.625	3.500	3.563
Full LS	2.500	2.625	2.563
Half LS	2.250	2.125	2.188
Full B5	3.000	3.125	3.063
Half B5	3.000	3.000	3.00
Full WPM	4.250	3.750	4.00
Half WPM	3.500	3.750	3.625
Mean (B)	3.109	3.078	—
$LSD_{0.05}$ A = 0.3700;	B = 0.1850;	AB = 0.5233	

Experiment 4. Effect of culture media, activated charcoal (AC), silver nitrate and IBA during the rooting stage

Data represented in Tables (7) and Fig. (3) illustrated the effect of different types of media on root formation and their characteristics. In preliminary studies, we found no difference between the effects of full and half strength of either MS or WPM on *in vitro* root formation of *T. distichum* and *T. distichum* var. 'distichum'. Therefore, half strength of both media was used in this part of the study. Such observation was in agreement with several reports on *in vitro* root formation of different woody plants.

1. Rooting percentage

The percentage of root formation was significantly affected by the different treatments tested on *T. distichum* and *T. distichum* var, 'distichum' (Table 7). The highest percentage of root formation (50%) was observed as a result of amending the half-strength WPM medium with 1.0 g AC /L + 0.5 mg IBA /L, while the percentage was sharply decreased to 10%, 9% and 10% by using (1) ½ MS medium + 1.0 g AC/L + 1.0 mg IBA/L, (2) ½ MS medium + 1.0 g AC/L + 2.0 mg IBA/L, or (3) 1/2 WPM medium + 1.0 g AC /L + 5.0 mg $AgNO_3$/L, respectively. However, the shoot lets completely failed to form roots in the control media (i.e., on ½ MS and ½ WPM), as well as in the ½ MS medium + 1.0 g AC /L (with or without 5.0 mg $AgNO_3$/L), and in the ½ WPM medium + 1.0 g AC /L. The data also showed that WPM medium gave significantly better results on root formation than MS medium.

Table 7. Effect of culture medium type (MS or WPM), and addition of IBA, AC or $AgNO_3$ on *in vitro* root formation (%) on *T. distichum* and *T. distichum* var. 'distichum'.

Treatments (A)	Plant variety (B)		Mean
	T. distichum	*T. distichum* var. 'distichum'	(A)
½ MS (basal)	0.0	0.0	0.0
½ MS + 1.0 g AC /L	0.0	0.0	0.0
½ MS + 1.0 g AC /L + 5.0 mg $AgNO_3$/L	0.0	0.0	0.0
½ MS + 1.0 g AC /L + 7.0 mg $AgNO_3$/L	16	20	18
½ MS + 1.0 g AC /L + 0.5 mg IBA /L	20	20	20
½ MS + 1.0 g AC /L+ 1.0 mg IBA /L	12	8	10
½ MS + 1.0 g AC /L + 2.0 mg IBA /L	10	8	9
½ MS + 1.0 g AC /L+ 3.0 mg IBA /L	22	18	20
½ WPM (basal)	0.0	0.0	0.0
½ WPM + 1.0 g AC /L	0.0	0.0	0.0
½ WPM + 1.0 g AC /L + 5.0 mg $AgNO_3$/L	10	10	10
½ WPM + 1.0 g AC /L+ 7.0 mg $AgNO_3$/L	20	16	18
½WPM + 1.0 g AC /L + 0.5 mg IBA /L	48	52	50
½ WPM + 1.0 g AC /L + 1.0 mg IBA /L	28	16	22
½WPM + 1.0 g AC / L+ 2.0 mg IBA /L	20	12	16
½WPM + 1.0 g AC /L + 3.0 mg IBA /L	40	38	39
Mean (B)	17.57	15.57	—
LSD $_{0.05}$ A= 4.270; B= 1.614; AB= 6.038			

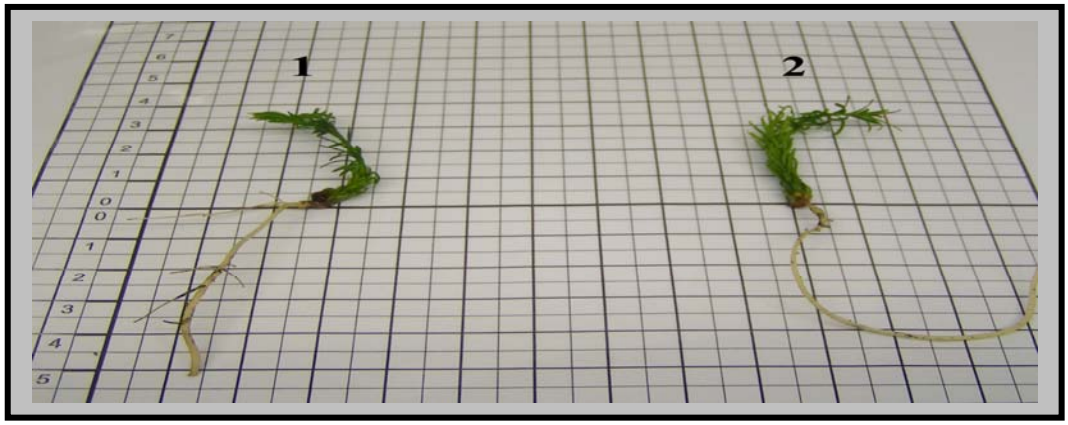

Fig. 3. Rooting of *T. distichum* (1) and *T. distichum* var. 'distichum' (2) on ½ WPM+ 1.0 g AC /L + 0.5 mg IBA / L.

Concerning the difference between the rooting abilities of *T. distichum* and *T. distichum* var, 'distichum' shootlets, the data in Table (7) showed that *T. distichum* gave a significantly higher rooting percentage (17.57%) compared to *T. distichum* var, 'distichum' (15.57%).

In conclusion, it can be stated that the half strength WPM medium + 1.0 g AC /L + 0.5 mg IBA /L, was the best medium for *in vitro* root formation of both *Taxodium distichum* and *Taxodium distichum* var. 'distichum'

2. Root number

As shown in Table (8), the recorded data indicated that there was a significant difference in the number of roots formed on shootlets of *T. distichum* and *T. distichum* var, 'distichum' as a result of tested treatments. The highest number of roots/shootlet (2.10) was recorded with half WPM medium + 1.0 g AC /L + 0.5 mg IBA /L , while the lowest number of root/shootlet let (0.80) was recorded on the half strength WPM medium + 1.0 g AC /L + 5.0 mg $AgNO_3$/L. Regarding the difference between the root numbers on *T. distichum* and *T. distichum* var, 'distichum' shootlets, the recorded data showed that there was no significant difference.

In conclusion, half WPM medium + 1.0 g/l AC + 0.5 mg/l IBA, was the best medium for root number/shootlet of both *T. distichum* and *T. distichum* var, 'distichum'.

Table 8. Effect of culture medium type, and addition of IBA, AC or $AgNO_3$ on *in vitro* root number/shootlet on *T. distichum* and *T. distichum* var. 'distichum'.

Treatments (A)	Plant variety (B)		Mean (A)
	T. distichum	*T. distichum var. 'distichum'*	
½ MS (basal)	0.0	0.0	0.0
½ MS + 1.0 g AC /L	0.0	0.0	0.0
½ MS + 1.0 g AC / L + 5.0 mg $AgNO_3$/L	0.0	0.0	0.0
½ MS + 1.0 g AC / L + 7.0 mg $AgNO_3$/L	1.0	1.0	1.0
½ MS + 1.0 g AC / L + 0.5 mg l IBA / L	1.60	1.20	1.40
½ MS + 1.0 g AC / L + 1.0 mg IBA / L	1.0	1.0	1.0
½ MS + 1.0 g AC / L + 2.0 mg IBA / L	1.0	1.0	1.0
½ MS + 1.0 g AC / L + 3.0 mg IBA / L	1.40	1.0	1.20
½ WPM (basal)	0.0	0.0	0.0
½ WPM + 1.0 g AC / L	0.0	0.0	0.0
½WPM + 1.0 g AC / L + 5.0 mg $AgNO_3$/L	0.6	1.0	0.8
½WPM + 1.0 g AC / L + 7.0 mg $AgNO_3$/L	1.80	1.60	1.70
½ WPM + 1.0 g AC L /+ 0.5 mg IBA / L	2.20	2.0	2.10
½ WPM + 1.0 g AC / L + 1.0mg IBA / L	1.60	1.60	1.60
½ WPM + 1.0 g AC L / + 2.0 mg IBA / L	1.20	1.0	1.10
½ WPM + 1.0 g AC / L + 3.0 mg IBA / L	1.9	2.00	1.95
Mean (B)	1.092	1.029	—
$LSD_{0.05}$ A= 0.2831;	B= 0.1070;	AB= 0.4004	

3. Root length (cm)

Data in Table (9) revealed that the highest mean root lengths (3.20 and 3.15 cm) were formed due to amending the WPM medium by the addition of 1.0 g AC /L + 0.5 or 3.0 mg IBA /L, respectively, while the shortest roots (1.40 cm) were formed on the half-strength WPM medium + 1.0 g AC /L + 5.0 mg $AgNO_3$/L. Data in Table (9) also showed that there was no significant difference between the root lengths in *T. distichum* and *T. distichum* var, 'distichum'. The longest root (3.50 cm) was recorded in *Taxodium distichum* var. 'distichum' with WPM supplemented with 1.0 g AC/L + 0.5 mg IBA /L, followed by root lengths of *T. distichum* plantlets cultured on WPM medium supplemented with 1.0 g AC/L + 1.0, 2.0 or 3.0 mg IBA/L (giving root lengths of 3.20, 3.10 and 3.00 cm, respectively). On the other hand, the shortest roots formed in *Taxodium distichum* (1.60 cm) were obtained on ½ MS medium + 1.0 g AC /L + 3.0 mg IBA /L, while the shortest roots formed in *Taxodium distichum* var. 'distichum' (1.60 cm) were obtained on the ½ MS medium + 1.0 g AC /L + 7.0 mg $AgNO_3$/L, or the ½ MS medium + 1.0 g AC /L + 2.0 mg IBA /L.

In conclusion, it is clear that the half salt strength WPM medium, supplemented with

different concentrations of IBA, was more effective for promoting root elongation in both *T. distichum* and *T. distichum* var, 'distichum', compared to the MS medium. Similar results were obtained by Manisha *et al.* (2001) on *Alnus nepalensis*, and Gad *et al.* (1999) on *Khaya ivorensis*.

Table 9. Effect of culture medium type (MS or WPM) and addition of IBA, AC or $AgNO_3$ on *in vitro* root length (cm) on *T. distichum* and *T. distichum* var. 'distichum'.

Treatments (A)	Plant variety (B)		Mean
	T. distichum	*T. distichum* var. 'distichum'	(A)
½ MS (basal)	0.0	0.0	0.0
½ MS + 1.0 g AC /L	0.0	0.0	0.0
½ MS + 1.0 g AC /L + 5.0 mg $AgNO_3$/L	0.0	0.0	0.0
½ MS + 1.0 g AC /L + 7.0 mg $AgNO_3$/L	2.0	1.60	1.80
½ MS + 1.0 g AC /L + 0.5 mg $AgNO_3$/L	1.80	1.70	1.75
½ MS + 1.0 g AC /L + 1.0 mg $AgNO_3$/L	1.80	1.70	1.75
½ MS + 1.0 g AC /L + 2.0 mg $AgNO_3$/L	1.80	1.60	1.70
½ MS + 1.0 g/l AC + 3.0 mg IBA /L	1.60	1.70	1.65
½ WPM (basal)	0.0	0.0	0.0
½ WPM + 1.0 g AC /L	0.0	0.0	0.0
½WPM + 1.0 g AC /L + 5.0 mg $AgNO_3$/L	0.90	1.90	1.40
½ WPM + 1.0 g AC /L + 7.0 mg $AgNO_3$/L	2.10	2.50	2.30
½WPM + 1.0 g AC /L + 0.5 mg IBA /L	2.90	3.50	3.20
½WPM + 1.0 g AC /L + 1.0 mg IBA /L	3.20	2.50	2.85
½ WPM + 1.0 g AC /L + 2.0 mg IBA /L	3.10	2.40	2.75
½ WPM + 1.0 g AC /L + 3.0 mg IBA /L	3.00	3.30	3.15
Mean (B)	1.729	1.743	__
$LSD_{0.05}$ A= 0.3293; B= 0.1245; AB= 0.4657			

Experiment 5. Effect of different growing media during the plantlet acclimatization stage

In this stage, soil mixture markedly influenced the characteristics of the acclimatization, as demonstrated in Table (10). The highest survival percentages of acclimatized plantlets (86.5, 86 and 85%) were recorded in pots filled with sand and peat (1:1 v/v), sand + peat + vermiculite (1:1:1 v/v), and sand + peat + perlite (1:1:1 v/v), respectively, with no significant difference between these treatments.

The increase in shoot length during the acclimatization stage confirmed the promotive effect of the soil mixture consisting of sand, peat and perlite (1:1:1 v/v) which resulted in the tallest plantlets (7.45 cm), which were insignificantly different than plantlets produced in the mixture of sand and peat (1:1, v/v), with a length of 7.30 cm. In contrast, the mixture of sand, peat and vermiculite (1:1:1 v/v) gave significantly shorter plantlets (6.60 cm). Similar results were obtained by Mereti *et al.* (2002), who acclimatized *Arbutus unedo* in a growing medium containing peat and perlite at a 1:1 ratio. The same growing medium was used and gave best results with *Rubus arcticus* (Shalupaev and Yatsyna, 2002). Furthermore, Hussein (2002) obtained favourable results when three species of *Aglaonema* plants were acclimatized on a medium containing perlite, peatmoss and vermiculite (0.5:1:1 v/v).

Table 10: Effect of soil mixture on acclimatization of *T. distichum* and *T. distichum* var. 'distichum' transplants.

Treatments	Survival % (B)		Mean (A)	Shoot length (cm) (B)		Mean (A)
	T. distichum	T. distichum var. 'distichum'		T. distichum	T. distichum var. 'distichum'	
Sand : Peat (1:1)	87	86	86.5	7.0	7.60	7.30
Sand : Peat : Perlite (1:1:1)	87	85	86	6.50	6.70	6.60
Sand : Peat : Vermiculate (1:1:1)	86	84	85	7.40	7.50	7.45
Mean (B)	86.67	85	__	6.967	7.267	___
LSD0.05 A= 4.191			0.6072			
B = 3.422			0.4958			
AB= 5.927			0.8588			

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