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IN VITRO PROPAGATION METHOD OF *Taxodium distichum* AND *Taxodium mucronatum*

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ABSTRACT

The experiments have been achieved to examine a method to produce multiple shoots from piece of stem (needle) to obtain a lot of seedlings of this unique tree *Taxodium distichum* and *Taxodium mucronatum* Family Taxodiaceae as well as studying the effect of different culture media composition on shoots formation . The statistical analysis of data revealed the following findings: Sterilized needles from basal sprouts of the trunk were cultured at ½ MS medium supplemented with agar at 8g/L. In the first experiment (The effect of different levels of growth regulators on shoots production), the level of 0.5 mg/L BAP increased the number of *T. distichum* shoots per explant and the absence of both BAP and NAA significantly increased the shoot length. In the second experiment in these stage, the highest significant number of *T. mucronatum* shoots value was obtained as a result of modifying MS medium with1.0 mg/L BAP and (1.0 mg/L IAA+1.0 mg/L Kinetin), significantly increased shoot length. In subculturing stage, 0.5 mg/L BAP increased the number of *T. distichum* shoots per explant and the shoot length also. On the other hand 1.0 mg/L BAP increased the number of *T. mucronatum* shoots per explant also increased the shoot length compared with the control or (0.5mg/l TDZ). As for shoot elongation stage MS ½ strength medium supplemented with 30g/L sucrose, 8g/L agar, and 20g/L activated charcoal induced the highest significant diameter of plant length and shoots number in *T. distichum* and *T. mucronatum* compared with the control. Applying (AC) to the *T. distichum* culture medium increased plant length compared with *T. mucronatum* culture medium on contrast *T. mucronatum* culture medium increased shooting compared with *T. distichum.*

Keywords: *In vitro*, propagation, growth regulators, activated charcoal, *Taxodium distichum* and *T. mucronatum*

INTRODUCTION

Taxodium distichum (L.) Rich Family (Taxodiaceae), a genus of three species of deciduous coniferous trees (Vashishta, 1983).

A. Pond cypress (*T. ascendens*), also known as *T. distichum* var. nutans. This species has many slender branchles with appressed awl-shaped leaves that branch (ascend) from larger branchlets (red arrow). It apparently does not cross pollinate with the closely related *T. distichum*.

B. Bald cypress or Swamp cypress (*T. distichum*). This species is native to swamplands of the southeastern United States along with *T. ascendens*. It is also known as " Tide water red cypress "; tall deciduous coniferous tree becoming 50 m high with trunk 1-1½ m, but sometimes 4 m in diameter.

C. Montezuma bald cypress (*T. mucronatum*), a Mexican species native from Sonora and Coahuila south to Guatemala. It is planted in the

United States and grows well in areas that are not inundated by water. Taxodium grows best in sheltered areas with wet or permanently moist soil. Height 35 ft; spread 15 ft. a long-lived hardy species of moderately slow growth and suitable as a specimen tree in large gardens or parks.

The leaves of *T. distichum* are linear and pointed, about 1cm long, 0.2 cm width and light green in color; in autumn they change in color to orange-brown. The alternate shoots (deciduous branchlets), on which the leaves are arranged in two rows, do not lengthen the branches but fall together with the leaves. The unisexual flowers (cones) are carried on the same plant on separate branches (monoecious plant). The male cones are present in pendulous, branching clusters, and the female cones in little strobiles Anonymous (1982). Taxodium considered as one of the most important medicinal tree in the world because of its biological activity:

1- Antitumor activity.
2- HIV-1PR inhibitory activity.
3- Vasoactive effect.
4- Promotion of menstruation.
5- Inhibition of silkworm enteric microorganisms.

This study aims to an in vitro propagation method enhancing shoot proliferation and elongation.

MATERIALS AND METHODS

This study was carried out in the tissue culture laboratory of Vegetable and Ornamental Department, Faculty of Agriculture, Mansoura University during the seasons of 2005-2008.

Sterilization method:

Needles must be chosen from basal sprouts of the trunk then washing in tap water containing soap and small drops of tween 20 (a wetting agent which lower the surface tension , allowing better surface contact .) , 1min in ethanol 70% , washed with sterile distilled water and immersed in 50% commercial Clorox solution for 20 min . Needles were washed three times with sterile distilled water in laminar air flow hood to remove the residuals.

Experiment 1. Effect of different levels of growth regulators on shoots production of *T. distichum* and *T. mucronatum* .

Sterilized explants were cultured in half strength Murashige and Skoog (1962) (MS) medium as illustrated in Table (1). The medium was supplemented with Benzylamino purine (BAP), at (0.0,0.5,0.75 and 1) mg/L, combined with (0.0 and 0.01 mg/L) Naphthalene acetic acid (NAA), also [1mg/L indole acetic acid (IAA) +1mg/L Kinetin], agar was used at 8g/L, and sucrose at 30 g/L (as a carbon source). The media were distributed into clean jars. Each of which contained 30 ml of nutrient media. Afterwards, jars were autoclaved for 15 min. at 121ºC, 1.5 Kg/ cm^3

The experiment consisted of nine treatments; each treatment included ten jars (each contained three explants)

Exp. 2: Effect of subculturing of*T. distichum* and *T. mucronatum* multiplication.

New sprouts growth on needles obtained from experiment 1 (which resembled the best result). In *Taxodium distichum* was (BAP 0.5mg/L + 0.0mg/L NAA) but, in *T. mucronatum* was (BAP 1mg/L + 0.0mg/L NAA).

Explants were cultured in ½ strength (MS) medium as illustrated in Table (1). The medium of *T. distichum* was supplemented with 0.5 mg/L Benzylamino purine (BAP) and TDZ 0.5 mg/L but, in *T. mucronatum* it was 1mg/L BAP and TDZ 0.5 mg/L , agar was used at 8 g/L and sucrose at 30 g/L (as a carbon source). L-ascorbic acid 2.5 mg/L and citric acid 3.75 mg/L. The media were distributed into clean jars, each of which contained 30ml of nutrient media. Afterwards, jars were autoclaved for 15 min. at 121 ºC, 1.5 kg/cm^3. The experiment consisted of one treatment of each type of Taxodium every treatment included ten jars.

Exp. 3: Effect of activated charcoal on shoot elongation of *T. distichum* and *T. mucronatum* .

Clusters of dwarfing shoots obtained from experiment 1. Explants were cultured in ½ strength (MS) medium. The medium was supplemented with 20g/L activated charcoal, 30g/L sucrose and 8g/L agar. The media were distributed into clean jars, each of which contained 30 ml of nutrient media jars were autoclaved for 15 min. at 121 ºC, 1.5 kg/cm^3. The experiment consisted of one treatment of each type of Taxodium every treatment included ten jars.

Culture conditions (for all experiments):

The media was adjusted to pH 6.2 by adding suitable amount of 0.1 N HCl or 0.1 Na OH using the pH meter before autoclaving for 15 min. at 121 ºC, 1.5 kg/cm^3. All treatments were incubated in the growth chamber at 26±2 ºC and exposed to 16 hr. light/day photoperiod under constant fluorescent light of 1500 Lux.

Experimental design and statistical analysis:

A complete randomize design was used throughout the research. The obtained data were subjected to analysis of variance and the treatment means were compared using L.S.D. test as outlined by Snedecor and Cochran (1975).

Data recorded:

The following data were recorded after 4 weeks from culturing on media for:

1- Average length of shoots (cm).
2- Average number of shoots per explant.

RESULTS AND DISCUSSION

Exp. 1: (A) The effect of different levels of growth regulators on shoots production of *T. distichum*:

a- Shoot length (cm):

Data of shoot-tips grown in MS medium including different concentrations of growth regulators were as shown in Table (1). It was clear that the shoot length of *T. distichum* was (3.65 cm) in the absence of both BAP and NAA. In case of adding (0.5 mg/L BAP+0.01 mg/L NAA), the shoot

length was significantly decreased to 2.49 cm. Culturing bald cypress on MS medium supplemented with 0.01 mg/L NAA, 0.75 mg/L BAP and (1.0 mg/L BAP+0.01 mg/L NAA) also reduced shoot length but the reductions were not significant when compared with control. Among all BAP concentrations, MS medium containing 0.01 mg/L NAA was the best in increasing shoot length than the other concentrations used but still less than control since it reached (3.17 cm). Concerning the level of (1.0 mg/L IAA+1.0 mg/L Kinetin), the shoot length was significantly decreased to (2.51cm). The levels of 0.5 mg/L BAP, 1.0 mg/L BAP and(0.75 mg/L BAP+0.01 mg/L NAA) shoot length was significantly decreased to(2.9cm) and (2.8cm). In contrast, Boulay (1989) found that, IAA induced elongation of the redwood (*Sequoia sempervirens*) meristem with in 1 month.

Table (1): The effect of different levels of growth regulators on shoots production of *T. distichum*.

Treatments	Shoot length/plant (cm)	Number of shoots/plant
0.0 mg/L BAP+ 0.0 mg/L NAA (control)	3.65	1.98
0.0 mg/L BAP + 0.01 mg/L NAA	3.17	1.71
0.5 mg/L BAP + 0.0 mg/L NAA	2.90	2.11
0.5 mg/L BAP + 0.01 mg/L NAA	2.49	1.58
0. 75 mg/L BAP + 0.0 mg/L NAA	3.14	1.58
0.75 mg/L BAP + 0.01 mg/L NAA	2.82	1.24
1.0 mg/L BAP + 0.0 mg/L NAA	2.83	0.92
1.0 mg/L BAP + 0.01 mg/L NAA	3.10	1.18
1.0 mg/L IAA+ 1.0 mg/L Kinetin	2.51	0.90
L.S.D at 5%	0.63	0.55

b- Number of branchs:

As for number of shoots per explant results in the same table showed that the level of 0.5 mg/L BAP increased the number of shoots per explant than the control. The control increased the number of shoots per explant than the other concentrations. Follow the control the levels of 0.01 mg/L NAA, (0.5 mg/L BAP+0.01 mg/L NAA) and 0.75 mg/L BAP significantly increased the number of shoots per explant to(1.71) and (1.58) than the levels of 1.0 mg/L BAP and (1.0 mg/L IAA+1.0 mg/L Kinetin) which was a matter of interest to notice that shoots production was inhibited .

Fouret (1984) has found similar results studying the in vitro rejuvenation of three clones of *Sequoia sempervirens* (hormone equilibrium BAP1mg/l and NAA0.01mg/l). She found that the more frequent the transfer onto cytokinin medium were, the more leaves were able to form adventitious buds. In this case, the leaves used for adventitious budding originated from in vitro-propagated sterile shoots.

Exp. 1: (B) The effect of different levels of growth regulators on shoots production of *T. mucronatum*:

a- Shoot length (cm):

In this work, results in Table (2) indicated that using MS medium supplemented with (1.0 mg/L IAA+1.0 mg/L Kinetin), significantly increased

shoot length than the control 3.42 cm. Similarly, using 0.01 mg/L NAA and (1.0 mg/L BAP+0.01 mg/L NAA) significantly increased shoot length to3.12cm and 2.27cm. Adding 0.5mg/l BAP decreased significantly the shoot length to 2.22cm compared to the level (1.0 mg/L IAA+1.0 mg/L Kinetin). While using (control),(0.75mg/l BAP),(0.75 mg/L BAP+0.01 mg/L NAA) and(1.0 mg/L BAP) significantly decreased shoot length to 2.34, 2.30, 2.60, and 2.43cm .

Table (2): The effect of different levels of growth regulators on shoots production of *T. mucronatum*.

Treatments	Shoot length/plant (cm)	Number of shoots/plant
0.0 mg/L BAP+ 0.0 mg/L NAA (control)	2.34	2.18
0.0 mg/L BAP + 0.01 mg/L NAA	3.12	2.07
0.5 mg/L BAP + 0.0 mg/L NAA	2.22	1.42
0.5 mg/L BAP + 0.01 mg/L NAA	2.69	1.58
0. 75 mg/L BAP + 0.0 mg/L NAA	2.30	2.58
0.75 mg/L BAP + 0.01 mg/L NAA	2.60	1.11
1.0 mg/L BAP + 0.0 mg/L NAA	2.43	2.84
1.0 mg/L BAP + 0.01 mg/L NAA	2.72	1.56
1.0 mg/L IAA+ 1.0 mg/L Kinetin	3.42	2.26
L.S.D at 5%	0.47	0.68

Gabr *et al.* (2008) found that the longest shootlet in *Taxodium distichum* was recorded with basal (free of growth regulators) of MS medium. Supporting results were obtained by Bouza *et al.* (1994) reported that, the cytokinin used in shooy multiplication of *Paeonia suffriticosa* was associated with further shootlet elongation and rooting stages, and that both were related to the accumulation of endogenous levels of indole acetic acid and the subsequent decrease in the cytokinin levels at the end of multiplication cycle .

b- Number of branchs:

Concerning the effect of different levels of growth regulators on shoots production, it was examined as shown in Table (2). It was obvious that1.0 mg/L BAP increased significantly the number of shoots per explant than the control it was (2.84). Follow the level of 0.75 mg/L BAP increased the number of shoots per explant than the other concentrations it was (2.58). The levels of (0.57 mg/L BAP) and (1.0 mg/L IAA+1.0 mg/L Kinetin) increased the number of shoots per explant to (2.58 and 2.26) and the difference was not significant when compared with control.

While the level of (0.75 mg/L BAP+0.01 mg/L NAA) decreased significantly the number of shoots to (1.11) compared with the control and the other levels. Similarly using (0. 5 mg/L BAP+0.01 mg/L NAA) or (1.0 mg/L BAP+0.01 mg/L NAA) gives the same result which reduced number of shoots (1.5) and, difference was significant which compared with control . These results were similar to the results achieved by Gad *et al.* (2006) on *Sequoia sempervirens* who observed that, half-MS supplemented with BA, (up to1.0mg/l) and repeat subculture up to three times increased the shootlets proliferated number per microcutting . The length of shootlets was decreased with increasing BA concentration.

Exp. 2: (A) The effect of sub-culturing of *T. distichum* multiplication:

a- Shoot length (cm):

Data in table (3) showed that control increased the length of shoots to (3.65cm) and the differ was significant when compared with BAP and TDZ levels. In addition it was a matter of importance to note that the presence of a significant decrement between the two concentrations of (0.5mg/l BAP and 0.5mg/lTDZ) and the control.

b- Number of branchs:

As for the effect of BAP on number of branches, it was found that culturing on (MS) medium supplemented with (0.5mg/l BAP) stimulated branches production, and it is was a significant increment (5.0) when compared with the control or (0.5mg/L TDZ). TDZ 0.5 mg/l also gives a significant increase of branches number to (3.7) than control. This may closely consist with hand; Ramanayake *et al.* (2006) showed that shoots of *Bambusa vulgaris* were treated with different levels of BA or TDZ. The highest mean shoot number was obtained in the presence of 4mg/l BA. This did not differ significantly from shoots in 6 or 0.1 mg/l TDZ. Shoot length was significantly less in the presence of TDZ than in 4mg/l BA. Repeated subculture in the higher BA of 6.0 mg/l, caused the shoots became shorter and vitrified. Therefore, 4.0 mg/l BA was selected as the optimum level for continuously proliferating axillary shoots.

Table (3): The effect of sub-culturing on *T. distichum* multiplication.

Treatments	Shoot length/plant (cm)	Number of shoots/plant
Control	3.65	1.98
BAP (0.5 mg/L)	2.66	5.0
TDZ (0.5 mg/L)	2.09	3.7
L.S.D at 5%	0.39	1.48

Exp. 2: (B) The effect of sub-culturing of *T. mucronatum* multiplication:

a- Shoot length (cm):

Dealing with the effect of BAP and TDZ on shoot multiplication, data in Table (4) showed that the presence of BAP in the level of 1mg/L significantly gave the longest shoot length (3.67cm) compared with control and TDZ in the level of (0.5) which gives the lowest shoot length (1.75cm) compared with other levels and the decrement was significant. Concerning the control it was better than the level of (0.5mg/l TDZ) which gives a significant increment in shoot length (2.34cm).

b- Number of branchs:

As illustrated in Table (4) the presence of BAP in the medium increased the average of branches number. For instance average number of branches raised to (5.8) when 1mgL BAP was added to the medium compared to (2.18) for control. This promotion had a high significant increment effect in average number of branches than the control and than the level of 0.5 mg/l TDZ. On the other hand the number of branches (3.0) was obtained when the level of 0.5 mg/l TDZ was added whish significantly increased the number of branches than the control.

Table (4): The effect of subculturing on *T. mucronatum* multiplication.

Treatments	Shoot length/plant (cm)	Number of shoots/plant
controls	2.34	2.18
BAP (1 mg/L)	3.67	5.8
TDZ (0.5 mg/L)	1.75	3.0
L.S.D at 5%	0.72	2.7

Chalupa *et al.* (2002) may agree with our data as he found that the fast axillary bud proliferation and shoot multiplication of mature trees of *Sorbus aucuparia* was achieved on modified MS medium supplemented with a low concentration of cytokinin (BA or TDZ) plus auxin (IBA).

Exp. 3: Effect of activated charcoal on shoot elongation of *T. distichum* and *T. mucronatum*:

a- Shoot length (cm):

Regarding to the effect of MS medium supplemented with 20g/L activated charcoal (AC), data recorded in Table (5) showed that adding (AC) increased plant length of the two species to 2.95cm compared with control (without AC) 0.73cm. Concerning the effect of plant species, data at the same table disclosed that *T. distichum* length was significantly higher than *T. mucronatum* with values of 2.22 cm for the former and 1.45 cm for the later. Dealing with the interaction between two factors, data in the same table indicated that applying (AC) to the *T. distichum* culture medium increased plant length to 3.49 cm compared with *T. mucronatum* culture medium which gives plant length about 2.41 cm.

Table (5): Effect of activated charcoal (AC) on shoot length of *T. distichum* and *T. mucronatum*

Treatments	*T. distichum*	*T. mucronatum*	Means of AC conc.
Control (0.0 AC)	0.95	0.51	0.73
20g/l AC	3.49	2.41	2.95
Means of plant species	2.22	1.45	
L.S.D at 5%	Plant species× AC conc. 0.72 Plant species 0.51 AC conc. 0.51		

b- Number of shoots:

Data in Table (6) showed that the use of MS medium supplemented with 20g/L significantly increased shooting of plant to 4.67 compared to control 2.34. In the same Table, data revealed that *T. mucronatum* was the best in number of branches of to 4.16 compared with *T. distichum* applying 2.85. Studying the response of the interaction between two factors, data in the same table illustrated that the best number of shoots was 5.39, this result was obtained with *T. mucronatum* treated with MS medium supplemented with 20g/L (AC), but *T. distichum* in the same treatment its number of shoots was 3.95.

Table (6): Effect of activated charcoal (AC) on branching of *T. distichum* and *T. mucronatum*

Treatments	*T. distichum*	*T. mucronatum*	Means of AC conc.
Control (0.0 AC)	1.74	2.94	2.34
20g/l AC	3.95	5.39	4.67
Means of plant species	2.85	4.16	
L.S.D at 5%	Plant species× AC conc. 1.45 Plant species 1.03 AC conc. 1.03		

These results were similar to the results achieved by Abd El- Kafie (2004) on *Nephrolepis exaltata* who observed that the presence of activated charcoal (AC) significantly increased gametophyte colonies diameter compared with control (without AC). The beneficial effects of activated charcoal may be from the darkening of the medium which stimulated the natural soil characteristics. In addition activated charcoal has been used as a light adsorbance in agar nutrient substrates to prevent light which induced growth inhibition of tissues. Moreover, some plants have the unpleasant characteristics of exudating brown / black pigments upon wounding (Usually oxidized poly phenol, like compound and tannins), which often making growth and development impossible. Also (AC) adsorbs gases and perhaps stimulates growth of injured explants probably due to ethylene absorption.

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إكثار نبات تاكسوديوم المتهدل والمخروطي بإستخدام مزارع الأنسجة

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أجري هذا البحث بمعمل زراعة الانسجة بقسم الخضر والزينة بكلية الزراعة- جامعة المنصورة خلال الفترة من أكتوبر ٢٠٠٥-أكتوبر ٢٠٠٨ بهدف الحصول علي أفضل تفريع وطول الأفرع لنبات التاكسوديوم بصنفيه المتهدل والمخروطي عن طريق بعض المعاملات لأنتاج أعداد كبيرة من النباتات في فترة قصيرة. وأوضحت النتائج أنه فى الصنف المتهدل كانت أفضل زيادة معنوية فى التفريع (٢.١١) عند مستوى ٠.٥مجم/لتر من البنزيل أمينو بيورين و أفضل طول (٣.٦ سم) فى حالة عدم استخدام منظمات النمو٠ بينما كان أفضل معدل تفريع للصنف المخروطى (٢.٨٤) في مستوي ١.٠ مجم/لتر من البنزيل أمينو بيورين وأعلي زيادة معنوية للطول (٣.٤٢ سم) عند مستوي ١.٠ مجم/لتر اندول اسيتك اسيد+١.٠ مجم/لتر من الكينيتين. وعند دراسة تأثير نقل النباتات علي أفضل تركيز من الهرمون ٠.٥مجم/لتر من البنزيل أمينو بيورين وتركيز ٠.٥ مجم/لتر من هرمون الثياديازيرون في الصنف المتهدل كانت أفضل نتائج التفريع وطول النبات عند المعاملة الأولي، كذلك عند التركيز ١.٠ مجم/لتر من البنزيل أمينو بيورين و٠.٥ من هرمون الثياديازيرون كانت أفضل النتائج المتحصل عليها بالنسبة للتفريع وطول النبات من المعاملة الأولي. وعند دراسة تأثير الفحم النشط علي استطالة النباتات كانت أفضل النتائج المتحصل عليها لطول النبات (٣.٤٩سم) والتفريع (٣.٩٥) بالنسبة للمتهدل و (٢.٤١ سم) وتفريع (٥.٣٩) للصنف المخروطي عند الزراعة علي بيئة تحتوي علي تركيز ٢٠جم/لتر مقارنة بالكنترول (٠.٠ جم/لتر).

قام بتحكيم البحث

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