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SOME FACTORS AFFECTING MICROPROPAGATION OF *TAXODIUM DISTICHUM* L.

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ABSTRACT

Shoot tips of 1.5 cm long of *Taxodium distichum* L. sterilized with mercuric chloride 0.2% for 10 *min* followed by immersing in 5, 10 or 20% Clorox for 20 or 40 min. did not show contamination, while survival percentage decreased down to 70% with the highest Clorox concentration of 20%, and was 100% with low Clorox concentration of 5% for 5-20 *min*. MS medium free of growth regulators showed a good sprouting of the buds and considered good establishment medium. During the multiplication of the obtained shoots from the establishment stage, MS medium supplemented with 2, 4 or 8 µg/ml kinetin, benzyle adenine or 2-ip showed that the highest shoot number/explant (5.2 shoots) was obtained by 8 µg/ml 2-ip, followed descendingly by 2 or 4 µg/ml BA (3.0 – 3.06 shoots), while the least number was due to control or kinetin treatments. All cytokinin concentrations decreased shoot length and did not show any callus formation on explant base. Using progesterone in multiplication stage at 0, 2.5, 5 or 10 µg/ml did not show multiplication. Also, using diphenyl urea at 0.25, 0.5 or 1 µg/ml plus 8 µg/ml 2-ip was uneffective compared with 8 µg/ml 2-ip alone. In rooting stage using IAA, IBA, NAA as *rooting* agents in full or 1/2 MS strength and activated charcoal or low as well as high sucrose concentration, as rooting percentage reached 14% with 1 µg/ml NAA in 1/2 MS medium. Also, pulsing shoot bases in 0.5, 1, 2 or 4 µg/ml NAA for 24 h. then culturing them in 1/2 or full MS did not show rooting, moreover, continuous film of 0.5, 1, 2 or 4 µg/ml of NAA on MS medium surface containing the same concentrations did not show rooting response. The rooted plantlets could be acclimatized on peat moss and sand medium (1:1 v/v).

Keywords: Tissue culture, *Taxodium distichum* L., growth regulators, shooting, rooting.

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INTRODUCTION

Microprogation is a vegetative plant propagation *In vitro* through aseptic cell, tissue or organ culture in which regeneration occurs via organogenesis or somatic embryogenesis. Today, it is used worldwide by the plant propagation industry, with the majority of propagated species being ornamental plants, (Haapala, 2004; Winkelman *et al.,* 2006; Read, 2007). Besides, mass propagation, is applied in the production of genetically modified plant material, in plant breeding, in cryopreservation of valuable plant genotypes and in production of *In vitro*-derived plant secondary metabolites, (Qiaochun *et al.,* 2005; Häggman *et al.,* 2007; Read, 2007).

The main methods used to propagate conifers include seeds, cuttings and grafting as well as micropropagation techniques, organogenesis and somatic embryogenesis. Propagation from seed is the principal method used and is important for the mass production of plants for forestry. Vegetative propagation techniques have traditionally been developed and used for the production of horticultural cultivars, but these methods are increasingly being used for the mass production of genetically improved planting stock for forestry, (Jinks, 2003). In general, there are 3 broad pathways that allow *In vitro* vegetative propagation of conifers: somatic embryogenesis; use of adventitious meristems; and via proliferation or enhancement of bud break of existing meristems. The major strategies using these pathways are discussed, (Schwarz *et al.,* 1994).

Despite major advances in forest biotechnology, clonal regeneration by somatic embryogenesis or organogenesis is still difficult for many woody species and is often limited to the use of juvenile explants, (Bonga *et al.,* 2010).

This study was carried out on an ornamental woody tree *Taxodium distichum* L. (Taxodiaceae). *Taxodium distichum* L. is also known as bald-cypress or swamp cypress, is specie of deciduous conifer native to the Southeastern USA. It is popular in southern landscapes and quite tolerant of flooding, salt, alkalinity and hurricanes. Bald-cypress has been noted for its high wood merchantable yields. In virgin stands, yields from 112 to 196 m^3/ha were common, and some

stands might exceed 1000 m^3/ha.The tree is pollution-tolerant and excels in compacted, low-oxygen or swampy conditions. It stands strong in the face of hurricanes, is amazingly long lived (1000 years) and, with time, can become quite large. Therefore, the objectives of this study were outlined as follows:

1. To assess the best sterilizing treatment to obtain the lowest contamination rate and the highest survival percentage.
2. To study the effect of some factors affecting multiplication and growth rate of shoots in order to obtain high multiplication rate.
3. To determine the best conditions for plantlets rooting.

MATERIALS AND METHODS

This study was carried out in Plant Tissue Culture Laboratory of Horticulture Department, Faculty of Agriculture, Zagazig University during the period from 2009 to 2011.

This work was conducted to study the effect of some treatments affecting *In vitro* micropropagation of *Taxodium distichum* L.

Explants (shoot tips) were obtained from young branches of *Taxodium distichum* L. trees of 10 years old growing in the garden of Agriculture Faculty, Zagazig University. Shoot tips (1.5 cm long) were used as explants.

In the whole work Murashige and Skoog (1962) basal medium with 30 g/l sucrose was used as shown in Table 1 and supplemented with the other tested additives according to aim of the experiment.

After the preparation of the medium and addition of all the above-mentioned components of each group and prior to addition of agar (6.0 g/l), the pH was adjusted at 5.8 by using a few drops of either sodium hydroxide (0.1 *N*) or hydrochloric acid (0.1 *N*). The medium was poured into jars (370ml) filled with 50 ml medium and plugged with polypropylene closure. The medium was autoclaved at 121°C and 1.1 kg/cm^2 for 20 minutes. All cultures of different experiments were maintained in a growth room at 25 ± 2°C and exposed to 16 h day photoperiod at an intensity of 2000 Lux from cool white fluorescent lamps.

This work included four stages; establishment, multiplication, rooting and acclimatization of plantlets.

Table 1. Composition of basal medium of Murashige & Skoog (1962) with some additives

Constituents	Concentrations (mg/l)
Macro-nutrients:	
NH_4NO_3	1650.00
KNO_3	1900.00
$CaCl_2.2H_2O$	440.00
$MgSO_4.7H_2O$	370.00
KH_2PO_4	170.00
Micro-nutrients:	
$MnSO_4.4H_2O$	22.30
$ZnSO_4.4H_2O$	8.60
H_3BO_3	6.20
Kl	0.83
$NaMoO_4.2H_2O$	0.25
$CuSO_4.5H_2O$	0.025
$CoCL_2.6H_2O$	0.025
Iron:	
Na_2EDTA	37.25
$FeSO_4.7H_2O$	27.85
Vitamins:	
Nicotinic acid	0.5
Pyridoxine-HCl	0.5
Thiamine-HCl	0.10
Myo-inositol	100.00
Sucrose **	30 g/l

**Sucrose concentration was changed in sucrose experiments.

Establishment Stage

Effect of Clorox concentration and exposure time on contamination

Shoot tips (1.5 cm long) were rinsed in a soapy water for 10 min., then washed with a running tap water for 30 min., and soaked for 30 min. in risolex antifungal solution at a concentration of 2g/l. The explants were then washed three times with a sterilized water. The explants were then immersed in mercuric chloride (MC) at concentrations of 0.2% for 10 min. followed by washing three times with a sterilized distilled water under aseptic conditions in a laminar air-flow cabinet. The explants were then immersed in commercial Clorox solution (NaOCl, 5.25% free chlorine) at concentrations of 5, 10 or 20% for 5, 10, 20 or 40 min followed by washing three times with a sterilized distilled water under the same conditions. Explants were inoculated in glass tubes (25 ×150 mm) containing Murashige and Skoog (1962) basal medium with 30 g/l sucrose, free from growth regulators. Each treatment was consisted of 10 tubes. Contamination and survival percentages were recorded after four weeks. The produced shoots were individually separated and used in multiplication stage.

Multiplication Stage

Experiment (1): Effect of low concentrations of BA, ki and 2, ip on multiplication

Shoots produced *In vitro* from establishment stage were individually separated and cultured on half salt strength MS medium supplemented with BA, ki or 2,ip (0 and 0.4 mg/l).

Experiment (2): Effect of BA, ki and 2, ip on multiplication

Shoots produced *In vitro* from establishment stage were individually separated and cultured on MS medium supplemented with different concentrations of BA (0, 2, 4 and 8 mg/l), ki (0, 2, 4 and 8 mg/l) or 2,ip (0, 2, 4 and 8 mg/l).

Experiment (3): Effect of progesterone on multiplication

Shoots produced *In vitro* from establishment stage were individually separated and cultured on MS medium supplemented with different concentrations of progesterone (0, 2.5, 5 and 10 mg/l).

Experiment (4): Effect of 2-ip and diphenyl urea, adenine sulphate or malt extract on multiplication

Shoots produced *In vitro* from establishment stage were individually separated and cultured

on MS medium containing 8 2-ip with diphenyl urea at 0, 0.25, 0.5 or 1.0 mg/l, adenine sulphate at 0, 40, 80 or 120 mg/l or malt extract at 0, 2.0, 4.0 or 8.0 g/l.

Experiment (5): Effect of 2-ip and IAA

Shoots produced *in vitro* from establishment stage were individually separated and cultured on MS medium containing 8 mg/l 2- ip with 0, 0. 5, 1 or 2 mg/l IAA .

The following data were recorded after 6 weeks:

1. Number of shoots/explant,
2. Shoot length (cm),
3. Callus percentage, and
4. Callus size

Rooting Stage

Effect of growth regulators on rooting of *Taxodium distichum*

Experiment (1): Effect of IAA and NAA on rooting

This experiment was designed to test the effect of different concentrations of IAA or NAA (0, 1.0, 2.0, and 4.0 mg/l) in half salt strength MS medium supplemented with 2g/l AC on rooting of shoots obtained from multiplication stage.

Experiment (2): Effect of low concentration of IAA, NAA and IBA on rooting

This experiment was designed to test the effect of different concentrations of IAA, NAA and IBA (0, 0.25, 0.5, 0.75and 1.0 mg/l) in half salt strength MS medium supplemented with 2g/l AC on rooting of shoots obtained from multiplication stage.

Experiment (3): Effect of NAA, AC and sucrose on rooting

This experiment was designed to test the effect of different concentrations of NAA (0 , 0.5, 1.0 and 2.0 mg/l) in half salt strength MS medium supplemented with different concentrations of sucrose (10 , 20 g/l) and AC (1.0 , 2.0 g/l) on rooting for shoots obtained from multiplication stage.

Experiment (4): Effect of IBA, AC and sucrose on rooting

This experiment was designed to test the effect of different concentrations of IBA (0 , 0.5 , 1.0 and 2.0 mg/l) in half salt strength MS medium supplemented with different concentrations of sucrose (10 , 20 g/l) and AC (1.0 , 2.0 g/l) on rooting of shoots obtained from multiplication stage.

Experiment (5): Effect of soaking of shoot bases in auxins on rooting

This experiment was designed to test the effect of soaking shoot bases in different concentrations of NAA (0.5, 1.0, 2.0 and 4.0 mg/l) for 12 h and 1000 mg/l for 5 min. in full and half salt strength MS medium on rooting of shoots obtained from multiplication stage.

Experiment (6): Effect of using two treatments of NAA in rooting medium

This experiment was designed to test the effect of using two treatments of NAA on rooting of shoots obtained from multiplication stage. The first treatment was full and half salt strength MS solid medium which contains NAA treatment, the second treatment was a liquid solution of different concentrations of NAA (0.5, 1.0, 2.0, 4.0 and 1000 mg/l) on media surface.

The following data were recorded after 12 weeks:

1. Rooting (%),
2. Root length (cm),
3. Root number,
4. Branches length (cm),
5. Branches number, and
6. Plantlet length (cm)

Acclimatization Stage

The objective of this stage was to adapt the plantlets, obtained *In vitro* before transferring to the open filed. The produced plantlets were washed with a tap water, and then disinfected by immersion of the roots in Benlate solution (1.0 g/l) for 10 minutes. The plantlets were then transferred to plastic pots (9×7 cm) containing peat moss: sand (1:1 v/v). The cultured pots were covered with polyethylene bags to keep high relative humidity around the plants and incubated at 25 ± 2°C in a growth room for six weeks.

Statistical analysis

The statistical layout of all the above mentioned experiments during the whole work was simple completely randomized design. The obtained data were statistically analyzed and the means were compared using the Duncan's multiple range test according to Duncan (1955) and Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Explants Sterilization

Data in Table 2 indicate the effect of immersing shoot tips explants in mercuric chloride (0.2%) for 10 min. followed by immersing in

Table 2. Effect of Clorox concentrations and exposure time on contamination

Treatments	Contamination (%)	Survival (%)*
5% Clorox 5 min.	30	100
10% Clorox 5 min.	30	100
20% Clorox 5 min.	20	100
5% Clorox 10 min.	20	100
10% Clorox 10 min.	10	100
20% Clorox 10 min.	10	100
5% Clorox 20 min.	10	100
10% Clorox 20 min.	0	100
20% Clorox 20 min.	0	90
5% Clorox 40 min.	0	90
10% chloral 40 min.	0	90
20% Clorox 40 min.	0	70

* Calculated for uncontaminated explants

Clorox solution in different concentrations and soaking time of *Taxodium disticum* L.

The data clear that soaking explants in mercuric chloride 0.2% for 10 min followed by immersing in 5 or 10% Clorox resulted in 30% contamination. However, increasing concentration of Clorox to 20% for 5 min. recorded 20% contamination, also increasing Clorox concentration to 10 or 20% for 10 min. decreased contamination percentage to 10%, while increasing soaking time to 20 or 40 min. with 5, 10 or 20% Clorox concentrations nullified the contamination.

The survival percentage as calculated from uncontaminated explants was 100% with the low concentration of Clorox and short soaking time and decreased to be 70% with the highest Clorox concentrations (20%) concomitant with the largest soaking time of 40 min.

Those results are convenient since increasing sterilization agent concentration or soaking time refluxed in less contamination and convenient survival percentage of explants.

Establishment Stage

Shoot tips (1.5 cm long) obtained from 10 years old growing trees were sterilized and cultured on MS medium free from growth regulators. The sprouted shoots of 1.5 cm long were used in multiplication stage.

Multiplication Stage

Effect of cytokinin concentration on multiplication of *T. distichum*

Data in Table 3 reveal that there was no any significant effect of kinetin, 2-ip or BA at 0.4 mg/l on either shoot number or shoot length.

On the other side, data in Table 4 and Fig. 1 show the multiplication response of *T. distichum* cultured on MS medium supplemented with 2, 4 or 8 μg/ml of kinetin, benzyl adenine or 2-ip. The data clear that survival percentage was the highest with 8 μg/ml kinetin and was decreased as kinetin concentration decreased to 4 or 2 μg/ml. On the other side, both BA and 2-ip at all concentrations recorded less survival percentage ranged between 23 – 50%. However, 4 μg/ml 2-ip was nearly analogous to control where 2 or 8 μg/ml of BA or 2-ip recorded less values than control.

As for shoot number/explant, the data clear that the highest shoot number (5.2 shoot) was belonged to 8 μg/ml 2-ip followed descendingly by 2 or 4 μg/ml BA (3.00 – 3.06 shoots), where the least number was belonged to either control or 2 and 4 μg/ml kinetin (1.53 – 2.20 shoot). As for shoot length, all cytokinen concentrations significantly decreased shoot length comparing to control. The least shoot length was belonged to BA concentration (2, 4 and 8 μg/ml). The data also clear that applying cytokinens did not induce callus on explants base.

The obtained results concerning the effect of BA on shoot multiplication are in harmony with those found by Yin *et al.* (2004) on *Taxodium mucronatum.* They found that modifying MS medium supplemented with 10 mg/l BA could induce the differentiation of adventitious buds high- frequently and multiplication coefficient reached 6.52. However, the more efficiency of 2-ip than BA in inducing multiple shoots is un line with the findings of Margaret and Colin (1985) on many ornamental cultivars of Ericaceae, also Al-Sulaiman and Barakat (2010) mentioned that BA and 2-ip were better than kinetin for production of lateral branches of *Ziziphus spinachristi.*

Table 3. Effect of low concentrations of cytokinin on multiplication of *T. distichum* after 6 weeks

Treatments (µg/ml)	Shoot length (cm)	Shoot number/explant
Control 1/2 MS	0.53 a	3.06 a
0.4 KI 1/2 MS	0.53 a	3.33 a
0.4 2-ip 1/2 MS	0.60 a	2.80 a
0.4 BA 1/2 MS	0.50 a	2.53 a

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

Table 4. Effect of cytokinine on multiplication of *Taxodium distichum* after 6 weeks

Treatments (µg/ml)	Survival (%)	Shoot number /explant	Shoot length (cm)	Callus (%)
control	47	1.53 d	3.16 a	0
2 ki	50	1.60 cd	2.28 b	0
4 ki	63	2.20 cd	2.25 b	0
8 ki	85	2.00 cd	1.63 c	0
2 BA	23	3.06 bc	0.98 cd	0
4 BA	35	3.00 bc	0.87 d	0
8 BA	26	3.60 b	0.85 d	0
2 2ip	28	2.40 c	1.58 c	0
4 2ip	50	2.26 cd	1.62 c	0
8 2ip	35	5.20 a	1.41 c	0

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

Fig. 1. Multiplication stage of *T. distichum* (1: Control ; 2: 8 µg/ml 2-ip)

Effect of progesterone on multiplication of *T. distichum*

Data in Table 5 indicate that progesterone at 2.5, 5 and 10 µg/ml did not cause any significant effect on shoot number, and shoot length of *Taxodium distichum*. Moreover, progesterone concentration did not induce any callus formation on explants base. The obtained results in the herein work did not prove the efficiency of progesterone in multiplication of *Taxodium distichum*. Although, it showed good multiplication of *Bougainvillea buttina* "Mrs But", (El-Shamy, 2002) and *Dieffenbachua picta* var. tropica, (Abass, 2011), so another investigation is needed to assess the efficiency of progesterone on multiplication of *Taxodium distichum*.

Effect of 2-ip, DPU and ME on multiplication of *T. distichum*

Data in Table 6 indicate that 8 µg/ml 2-ip resulted in the highest shoot number/explant (4.7 shoot).On the other side, addition of DPU to 8 µg/ml 2-ip at 0.25, 0.5 or 1 µg/ml and/or adenine sulphate at 40, 80 or 120 µg/ml and/or malt extract at 2, 4 or 8 gm/l significantly decreased shoot number /explant comparing to 2-ip alone. All additions resulted in a nearly similar response to control which mean that all additions suppressed the enhancing effect of 8 µg/ml 2-ip on shoot number.

As for shoot length, the data clear that 8 µg/ml 2-ip alone or combined with DPU concentrations significantly decreased shoot length by a similar magnitude comparing

Table 5. Effect of progesterone on multiplication of *T. distichum* after 6 weeks

Treatments (µg/ml)	Main shoot length (cm)	Shoot number/explant	Callus (%)
Control	2.33 a	1.66 a	0
2.5 pro	2.05 a	1.73 a	0
5 pro	2.33 a	1.8 a	0
10 pro	2.632 a	1.93 a	0

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

Table 6. Effect of 2-ip, DPU and ME on multiplication of *T. distichum* after 6 weeks

Treatments	Shoot number /explant	Shoot length(cm)
Control	2.00 bc	2.97 a
8 µg/ml 2ip	4.70 a	1.31 bc
8 µg/ml 2ip+ 0.25µg/mlDPU	2.30 bc	1.54 bc
8 µg/ml 2ip+ 0. 5µg/mlDPU	2.60 b	1.55 bc
8 µg/ml 2ip+ 1µg/mlDPU	2.30 bc	0.89 c
8 µg/ml 2ip+ 40µg/mlAds	1.85 bc	1.9 b
8 µg/ml 2ip+ 80µg/mlAds	1.70 bc	2.35 ab
8 µg/ml 2ip+ 120µg/mlAds	2.50 bc	1.61 bc
8 µg/ml 2ip+ 2g/l ME	2.50 bc	1.63 bc
8 µg/ml 2ip+ 4g/l ME	1.40 c	2.45 ab
8 µg/ml 2ip+ 8g/l ME	1.45 c	1.56 bc

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p_ 0.05)

to control. On the other side, control plants, or the concentrations of 8 μg/ml 2-ip + 80 μg/ml ADS and/or 8 μg/ml 2-ip + 4 gm/l ME did not cause reduction in shoot length and were similar to control, where increasing ME concentration to 8 gm/l combined with 8 μg/ml 2-ip significantly reduced shoot length.

The effect of DPU when added with 2-ip in decreasing shoot length are in harmony with the results of Genkov *et al.* (1997) on carnation plant shoot number who mentioned that phenyl urea at 0.4μM resulted in short stems. Also, DPU at 1μg/ml combined with 16 μg/ml 2-ip +1 μg/ml IAA recorded less significant values of shoot length and leaf number of *Diffenbachia picta* var. tropica, (Abass, 2011).

The addition of 2-ip plus adenine sulphat suppressed the effect of 2-ip on multiplication of *Taxodium distichum*. In this regard a similar effect was found when adenine sulphate combined with BA were tested on *Bougainvillea buttina* "Mrs But" (El-Shamy, 2002). Similarly, poor multiplication and shoot length were found when malt extract (ME) was used at 8 g/l on multiplication stage of *Diffenbachia picta* (Abass, 2011).

Effect of 2-ip + IAA on multiplication of *T. distichum* L.

Data in Table 7 clear that the highest shoot number /explant (4.7) was belonged to 8 μg/ml 2-ip where 8 μg/ml 2-ip plus 0.5, 1 or 2 μg/ml IAA did not affect shoot number comparing to control, and resulted in less significant shoot number comparing to 8 μg/ml 2-ip alone. The results mean that IAA suppressed the enhancing effect of 2-ip on shoot initiation.

All applied treatments as 2-ip alone or combined with IAA decreased shoot length comparing to control. In these regard, Burkhart (1991) found that when shoots of *Thuja occidentalis* were treated with 10 μM BA, several basal axillary shoots were produced, but inclusion of 10 μM NAA suppressed the BA stimulation of basal shoots, while the addition of 0.1 μM NAA to BA stimulated it.

Rooting Stage

Effect of auxins on rooting of *T. distichum*

Data in Table 8 & 9 and Fig. 2 indicate that the highest shoot number resulted during rooting stage was belonged to 1 μg/ml IAA (4.0) where the least shoot number was belonged to the low concentrations of IAA as 0.25, 0.5

Table 7. Effect of 2-ip + IAA on multiplication of *T. distichum* after 6 weeks

Treatments	Shoot number /explant	Shoot length(cm)
Control	2.00 b	2.97 a
8 µg/ml 2ip	4.70 a	1.31 b
8 µg/ml 2ip+0.5 µg/ml IAA	2.40 b	1.25 b
8 µg/ml 2ip+ 1µg/ml IAA	2.50 b	1.56 b
8 µg/ml 2ip+ 2 µg/ml IAA	2.55 b	1.08 b

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test ($p= 0.05$)

Table 8. Effect of auxins and activated charcoal on rooting of *T. distichum* after 12 weeks

Treatments	Rooting (%)	Root length (cm)	Root number	Main shoot length (cm)	Shoot number/ explant
1/2 MS+2g/L AC	4.00	1.60 c	4	2.50 bc	2.20 c
1 NAA 1/2 MS+2g/L AC	14.8	5.66 a	1	2.56 bc	7.73 a
2 NAA 1/2 MS+2g/L AC	0.0	0.00	0	4.03 b	5.00 b
4 NAA 1/2 MS+2g/L AC	0.0	0.00	0	5.13 a	4.86 b
1 IAA 1/2 MS+2g/L AC	6.66	4.00 b	2	3.23 b	2.06 c
2 IAA 1/2 MS+2g/L AC	0.00	0.00	0	2.90 bc	2.00 c
4 IAA 1/2 MS+2g/L AC	0.00	0.00	0	2.16 c	2.06 c

Means followed by the same letter (s) within the same column are not significantly different according to Duncan,s multiple range test ($p= 0.05$)

Table 9. Effect of low concentration of auxins on rooting of *T. distichum* after 12 weeks

Treatments	Shoot number /explant	Shoot length (cm)	Rooting (%)	Root number /explant	Root length (cm)
1/2 MS +2g/L AC	2.00 b	1.75 a	4.00	4	1.60
0.25 µg/ml IAA	1.08 c	2.45 a	0.00	0	0.00
0.5 µg/ml IAA	1.08 c	2.45 a	10.00	3	11.00
0.75 µg/ml IAA	1.08 c	1.95 a	0.00	0	0.00
1.0 µg/ml IAA	4.00 a	1.75 a	6.66	2	4.00
0.25 µg/ml IBA	1.58 bc	2.35 a	0.00	0	0.00
0.5 µg/ml IBA	1.66 bc	2.30 a	0.00	0	0.00
0.75 µg/ml IBA	2.33 b	2.10 a	0.00	0	0.00
1.0 µg/ml IBA	2.58 b	1.95 a	0.00	0	0.00
0.25 µg/ml NAA	1.41 bc	2.35 a	0.00	0	0.00
0.5 µg/ml NAA	1.66 bc	1.75 a	0.00	0	0.00
0.75 µg/ml NAA	2.08 b	1.80 a	0.00	0	0.00
1.0 µg/ml NAA	2.16 b	2.00 a	14.8	1	5.66

Means followed by the same letter (s) within the same column are not significantly different according to Duncan,s multiple range test (p= 0.05)

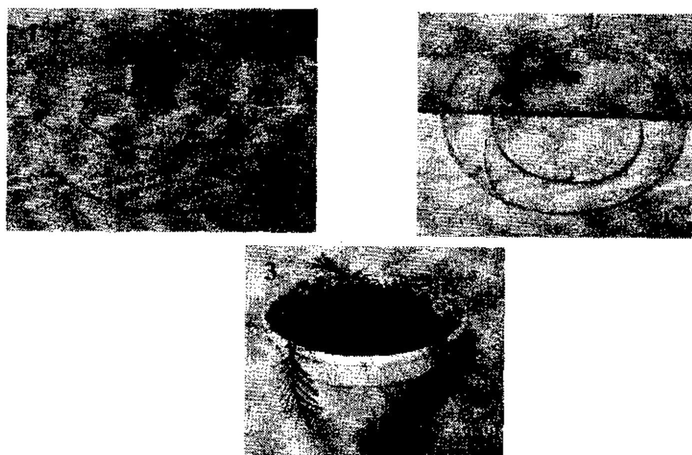

Fig. 2. Rooting and acclimatization stages of *T. distichum* (1: 1/2 MS+2g/L AC; 2: 1 NAA 1/2 MS+2g/L AC ; 3: acclimatized plant of 1 NAA)

and 0.75 μg/ml as they resulted in less shoot number comparing to control. On the other side, both IBA and NAA at 0.25, 0.5, 0.75 or 1 μg/ml resulted in a similar shoot number as control. No significant effect on shoot length was detected with all auxin concentrations.

As for rooting percentage, it is clear that control treatment (1/2 MS) resulted in 4% rooting followed ascendingly by 1 μg/ml IAA (6.66%), 0.5 μg/ml IAA (6.66%) and the highest percentage (14%) was belonged to 1 μg/ml NAA. The other auxin concentrations did not cause rooting.

As for root number /shoot, the highest shoot length (4 cm) was belonged to control followed descendingly by 0.5 μg/ml and 1 μg/ml IAA, and the least number (1) was belonged to 1 μg/ml NAA.

As for root length, the largest root was belonged to 0.5 μg/ml IAA (11cm) where the least root length (1.6 cm) was belonged to control (1/2 MS). The other resulted values were 4 cm belonged to 1 μg/ml IAA and 5.66 cm belonged to 1 NAA.

The general conclusion is that the best treatments for rooting was 0.5 μg/ml IAA which resulted in 10 % rooting with average of 3 roots/ shoot and average of 11 cm length and / or 1μg/ml NAA which resulted in 14.8% rooting with average 1 root/shoot and 5.66 cm root length.

Effect of sucrose, activated charcoal and NAA on rooting of *T. distichum* L.

Data in Table 10 indicate that rooting percentage was 10% with average 2 roots/ shoot and 5.5 cm root length was obtained by 10% sucrose combined with 2g/l activated charcoal and 0.5 μg/ml NAA. All other combinations of sucrose as 20 or 1g/l activated charcoal and 0.5, 1and 2 μg/ml NAA did not show any response for rooting. However, an enhancement of shoot number was obtained with 10% sucrose combined with 1g/l activated charcoal and 0.5 μg/ml NAA and raising the concentrations of sucrose and NAA decreased shoot number. While, all applied concentrations tented to increase shoot length.

Effect of sucrose, activated charcoal and IBA on rooting of *T. distichum*

Data in Table 11 indicate that rooting percentage was 10% on MS medium supplemented with 10% sucrose combined with 1g/l activated charcoal and 0.5μg/ml IBA

Table 10. Effect of sucrose, activated charcoal (AC) and NAA on rooting of *T. distichum* after 12 weeks

Treatments			Means				
Sucrose concentration (%)	(AC) concentration (g/l)	NAA Concentration (µg/ml)	Shoot number /explant	Shoot length (cm)	Rooting (%)	Root number /explant	Root length (cm)
0	0.0	0.0	2.20 b	1.55 b	0.0	0.0	0.0
10	1.0	0.5	3.14 a	2.20 ab	0.0	0.0	0.0
10	1.0	1.0	2.00 b	2.20 ab	0.0	0.0	0.0
10	1.0	2.0	1.50 bc	3.05 a	0.0	0.0	0.0
10	2.0	0.5	2.40 ab	2.90 a	10.0	2.0	5.5
10	2.0	1.0	1.50 bc	2.70 a	0.0	0.0	0.0
10	2.0	2.0	1.50 bc	2.80 a	0.0	0.0	0.0
20	1.0	0.5	1.40 bc	2.20 ab	0.0	0.0	0.0
20	1.0	1.0	1.00 c	3.10 a	0.0	0.0	0.0
20	1.0	2.0	1.00 c	3.00 a	0.0	0.0	0.0
20	2.0	0.5	2.35 ab	2.10 ab	0.0	0.0	0.0
20	2.0	1.0	1.95 bc	1.95 ab	0.0	0.0	0.0
20	2.0	2.0	1.90 bc	2.05 ab	0.0	0.0	0.0

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

Table 11. Effect of IBA, activated charcoal and sucrose concentration on rooting of *T. distichum* after 12 weeks

Treatments			means				
Sucrose concentration (%)	(AC) concentration (g/l)	IBA Concentration (µg/ml)	Shoot number /explant	Shoot length (cm)	Rootig (%)	Root number /explant	Root length (cm)
0	0.0	0.0	2.2 cd	1.55 c	0.0	0.0	0.0
10	1.0	0.5	6.0 a	3.94 a	10.0	1.0	1.5
10	1.0	1.0	3.7 bc	2.66 b	0.0	0.0	0.0
10	1.0	2.0	2.4 cd	2.65 b	0.0	0.0	0.0
10	2.0	0.5	2.6 cd	4.73 a	10.0	6.0	2.4
10	2.0	1.0	2.7 cd	3.10 ab	0.0	0.0	0.0
10	2.0	2.0	1.9 d	2.45 bc	0.0	0.0	0.0
20	1.0	0.5	5.9 a	2.40 bc	0.0	0.0	0.0
20	1.0	1.0	4.7 b	2.35 bc	0.0	0.0	0.0
20	1.0	2.0	4.9 ab	2.17 bc	10.0	7.0	4.35
20	2.0	0.5	4.7 b	2.53 b	0.0	0.0	0.0
20	2.0	1.0	3.4 c	2.20 bc	0.0	0.0	0.0
20	2.0	2.0	1.9 d	2.40 bc	0.0	0.0	0.0

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

with the lowest root length (1.5 cm) and root number (1) per explant. The increase of activated charcoal concentration to 2 g/l in the same medium increased the root length (2.4 cm) and root number (6) per explant with the same root percentage (10%).The highest root length (4.35 cm) was obtained with MS medium supplemented with 20% sucrose, 1g/l activated charcoal and 2 mg/l IBA. All other combinations of sucrose, activated charcoal and IBA did not show any response for rooting. Also, it was found that the shoot number and length increased with 10% sucrose combined with 1g/l activated charcoal and 0.5 µg/ml IBA.

Effect of MS strength and pulsing of NAA on rooting of *T. distichum*

Data in Table 12 show that MS strength (1/2 or full) combined with shoots basis pulsing for 24 hours in NAA at 0, 0.5, 1, 2 or 4 µg/ml did not show any rooting response, whereas, all NAA treatments decreased survival percentage with 1/2 or full MS strength as NAA increased to 4 µg/ml.

Effect of MS strength and two addition methods of NAA in the media and liquid phase of media surface on rooting of *T. distichum*

Data in Table 13 indicate that using 1/2 or full MS strength combined with NAA at 0, 0.5, 1, 2 or 4 µg/ml in the solid medium in addition to film of the same NAA concentration on medium surface did not cause any rooting response. On the other side, NAA in the two phases significantly decreased shoot number comparing to 1/2 MS without NAA but significantly enhanced shoot length.

The obtained poor rooting results of *T. distichum* in the herein work reflex the problem of conifer plants rooting as affected by many factors. In this regard, when IAA plus NAA were used, 30% Douglas fir rooted, (Boulay, 1979) and IAA plus IBA caused 25% rooting of Norway spruce (*Picea abies*) shoots to root, (Eriksson, 1984 and Von Arnold, 1982).

The herein work did not show rooting responses with pulse treatment of NAA although this method was effective in other conifers, as it resulted in 60% rooting for radiate pine shoots; and 100% rooting for rewood (*Seqouia sempervirens*) shoots, (Poissonnier *et al.*, 1980). Moreover, continuous and pulse auxin exposures on agar medium have been compared for western white pine (*Pinus monticola* Dougl) as the shoots continuously treated with 0.54 µM NAA did not form roots.

Table 12. Effect of soaking shoot bases in NAA on rooting of *T. distichum* after 12 weeks

Treatments		Survival (%)	Rooting (%)
Strength MS media	**NAA concentration (µg/ml)**		
1/2 MS	0.0	100	0
	0.5	50	0
	1.0	50	0
	2.0	50	0
	4.0	33.3	0
Full MS	0.0	100	0
	0.5	50	0
	1.0	50	0
	2.0	50	0
	4.0	33.3	0

Table 13. Effect of MS strength and two addition methods of NAA in the medium and liquid phase of media surface on rooting of *T. distichum*

Treatments		Survival (%)	Shoot number /explant	Shoot length (cm)	Rooting (%)
Strength MS medium	**NAA concentration (µg/ml)**				
1/2 MS	0.0	100.00	3.00 a	0.54 c	0.0
	0.5	100.00	1.50 b	3.16 ab	0.0
	1.0	100.00	1.66 b	2.70 ab	0.0
	2.0	91.66	1.58 b	2.83 ab	0.0
	4.0	88.88	1.50 b	2.37 b	0.0
Full MS	0.0	100.00	1.58 b	3.12 ab	0.0
	0.5	100.00	1.66 b	3.54 a	0.0
	1.0	100.00	1.75 b	2.83 ab	0.0
	2.0	91.66	1.66 b	2.75 ab	0.0
	4.0	50.00	1.41 b	2.75 ab	0.0

Means followed by the same letter (s) within the same column are not significantly different according to Duncan's multiple range test (p= 0.05)

This was the case of the obtained results in the herein work as auxins pulsing treatment or continuous film of low or high auxin concentration on 1/2 or full MS medium surface did not induce rooting of *T. distichum*. Also, low or high sucrose concentration and using activated charcoal did not induce high rooting percentage. However, this poor rooting percentage may be due to the previous high cytokinin used concentration in shoots proliferating medium. In this regard, it was found that, when maple (*Acer sp*) shoots was proliferated at high BA concentration, they had reduced rooting compared to shoots proliferation at lower BA concentration (Kerns, 1988).

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بعض العوامل المؤثرة علي الاكثار الدقيق للسرو المتساقط

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أظهرت القمم النامية لنبات السرو المتساقط بطول ١،٥ سم و المعقمة باستخدام كلوريد الزئبق (٠,٢%) لمدة ١٠ ق ثم الغمر في الكلوركس بتركيزات ٥ و ١٠ او ٢٠% لمدة ٢٠ او ٤٠ ق عدم وجود تلوث عند الأكثار الدقيق، في حين ان نسبة البقاء تناقصت الي ٧٠% عند التركيز المرتفع من الكلوركس (٢٠%) وكانت النسبة ١٠٠% عند التركيزات المنخفضة من الكلوركس (٥% لمدة ٥-٢٠ ق)، وقد أظهرت بيئة موراشيج وسكوج الخالية من منظمات النمو أفضل إنبات للبراعم واعتبرت علي انها افضل بيئة لمرحلة البدء، اثناء تضاعف الأفرخ الناتجة من مرحلة البدء اظهرت بيئة موراشيج و سكوج المدعمة بالكينتين (٢ و ٤ أو ٨ جزء في المليون) والبنزايل ادنين أو 2-ip أن أعلى عدد من الأفرخ لكل جزء نباتي (٥,٢ فرخ) تم الحصول عليها من تركيز ٨ جزء في المليون 2-ip متبوعة تنازلياً بـ ٢ أو ٤ جزء في المليون بنزايل ادنين (٣ - ٣,٠٦ فرخ) في حين نتج أقل عدد افرخ من الكنترول أو من المعاملة بالكينتين، وأنقصت جميع تركيزات السيتوكينين طول الفرخ ولم تظهر أى تكوين للكالس على قاعدة الجزء النباتى، كما لم يظهر استخدام البروجسترون فى مرحلة التضاعف بتركيزات صفر و ٢,٥ و ٥ أو ١٠ جزء في المليون أي تضاعف، وأيضاً استخدام الداى فينيل يوريا (٠,٢٥ و٠,٥ أو ١ جزء في المليون) إضافة إلى ٨ جزء في المليون 2-ip كان غير فعالا مقارنة بـ ٨ جزء في المليون 2-ip بمفرده. وفي مرحلة التجذير عند استخدام اندول حمض الخليك وإندول حمض البيوتيرك ونفثالين حمض الخليك كعوامل مشجعة علي التجذير في بيئة موراشيج وسكوج كاملة القوة أو نصف القوة مع اضافة الفحم النشط، وباستخدام تركيزات مرتفعة أو منخفضة من السكروز وجد ان نسبة التجزير وصلت الي ١٤% مع ١ جزء في المليون من نفثالين حمض الخليك في بيئة موراشيج وسكوج نصف القوة، كذلك فإن غمر قواعد الأجزاء النباتية في محلول نفثالين حمض الخليك بتركيزات ٠,٥ و ١ و ٢ أو ٤ جزء في المليون لمدة ٢٤ ساعة قبل الزراعة علي بيئة موراشيج وسكوج كاملة القوة أو نصف القوة لم يحدث تجذير، وعلاوة علي ذلك فإن استخدام فيلم رقيق من محلول نفثالين حمض الخليك علي سطح البيئة الصلبة و بنفس التركيزات السابقة لم يظهر أي استجابة للتجذير، وقد تمت أقلمة النبيتات التي تم تجذيرها علي بيئة تحتوي علي مخلوط الرمل و البيت موس بنسبة ١:١ حجماً.