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In vitro Propagation of *Thuja occidentalis* Through Apical Shoot Culture

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Abstract

In vitro plant regeneration of *Thuja occidentalis* was obtained in apical shoot cultures from field grown plants. Hormone free MS medium 100 % explants produced shoots. The average number of shoots per explant was 6.57 ± 0.45 and the average shoot length of 4.5 ± 0.27 cm were recorded in this medium. Shoots rooted well when they were transferred into half strength MS with 1.0 mg/l IBA. The average number of root per shoot was 3.92 ± 0.28 and the average root length of 3.64 ± 0.38 cm were observed in this medium. No morphological variants were observed during the passage of *in vitro* culure.

Introduction

Thuja occidentalis is an attractive landscape tree that belongs to the family Cupressaceae and native to North America (Hosie 1979). It is an important group of conifers that have a lot of demand as ornamental plants in Bangladesh and abroad. It is mainly grown in front of offices and along the highways for beautifying places. It is also found to grow in front of the private houses. Moreover, it has some medicinal value as well. But, there is no traditional process to propagate this plant as they do not produce seeds in Bangladesh environment and as such almost 100% seedlings are imported from India to meet the demand. Cutting, grafting and budding also have not proved very successful. Therefore, tissue culture technique is an attractive alternative to propagate this valuable or ornamental plant. In fact, Thorpe and Coleman (1977) reported *in vitro* culture and Thorpe and Nour (1993) mentioned *in vitro* shoot multiplication of *Thuja occidentalis*. Beside this, so far in our knowledge, no other reports are available for *in vitro* propagation of this plant. Thus, the present study was conducted to establish a reproducible protocol for large scale *in vitro* production of *Thuja occidentalis* using apical shoots as explants.

Materials and Methods

Apical shoots of field grown *Thuja occidentalis* were collected from AERE Campus, Savar, Dhaka. The explants were washed thoroughly using household detergent, Trix for 15 minutes under running tap water. Subsequent sterilization was carried out in the laminar air flow cabinet under aseptic conditions. Shoots were sterilized in 40% Clorox for 5 minutes followed by 0.1% mercuric chloride for 5 minutes. Rinsing was done three times with sterile distilled water. Apical shoots of about 2 cm long were excised and inoculated into media containing half strength MS, MS0 and MS media supplemented with different concentrations of BA and Kn alone or BA in combination with NAA for shoot induction. Subculture was done 30 days interval on the same medium for promoting strong and healthy multiple shoots. Morphologically healthy shoots were transferred to half strength MS supplemented with different concentrations of IBA, IAA and NAA for root induction. The sucrose concentration was used 30 g/l and the pH of the media adjusted to 5.8 prior to autoclaving. Cultures were incubated at 26 ± 2°C with a 16 hour illumination of 21.8 μmol/cm^2/s provided by cool white fluorescent tubes. The experiment was conducted with 30 explants per medium component. Data were collected on different characters at day 90 for multiple shooting and at day 60 for rooting of shoots. Observation on cultures were carried out daily.

Results and Discussion

Explants grew up and produced new shoot buds and leaves within two weeks of culture on medium containing MS devoid of plant growth regulators. On the other hand, the rest of the media explants responded poorly. However, shoot proliferation differed according to media component used (Table 1). Among the media component used, MS medium devoid of plant growth regulators was found to be the best for multiple shoot formation, in which 100% explatns produced multiple shoot (Fig. 1). The average number of shoots per explant was 6.50 ± 0.45 and the average shoot lengths of 4.50 ± 0.27 cm were observed in this medium. On the other hand succeulent shoots with stunted growth observed in the hormone supplemented media, suggests that phytohormones inhibited normal shoot growth and its multiplication. Unlike, previous reports in which BA and Kn either alone or in combination promoted shoot formation and its multiplication as in sugarcane (Lal and Singh 1994) and other plants, the two hormones were ineffective in our study. It was observed that there is a tendency to produce callus at the base of the explants in the media combinations containing NAA, which might be due to the effect of the presence of NAA in the media. It was also observed that the explants started to rot at the base in BA supplemented media at subculture three indicating that BA is toxic to explant tissues. It was also noted that *in vitro* raised shoots died when it was excised and

transferred to the shoot multiplication medium, MS0. It was thought that this might be due to the lacking of mother tissue with the excised shoots or the imbalance media composition the identical media explants stopped their growth

Table 1. Effects of different media components on the *in vitro* shoot regeneration of *T. occidentalis* at 90 days.

Hormone supplements in mg/l added to MS	% of shoot forming explants	Av. No. of shoots/explant (Mean ± SE)	Av. shoot lengths/ explant (cm) (Mean ± SE)	Response of nature
Half strength MS	80	4.50 ± 0.35	3.25 ± 0.25	Moderate shoot growth
No supplement (MS0)	100	6.52 ± 0.45	4/5 ± 0.27	Vigorious shoot growth
1.0 BA	30	2.54 ± 0.65	2.45 ± 0.25	Poor shoot growth
2.0 BA	-	-	-	Explants became brown and died
1.0 Kn	10	1.50 ± 0.40	2.60 ± 0.20	Poor shoot growth
2.0 Kn	-	-	-	Explant became brown and died
1.0 BA + 0.1 NAA	20	1.56 ± 0.45	1.45 ± 0.26	Poor shoot growth with callusing at the base
1.0 BA + 0.5 NAA	10	1.42 ± 0.29	1.32 ± 0.26	Poor shoot growth with callusing at the base

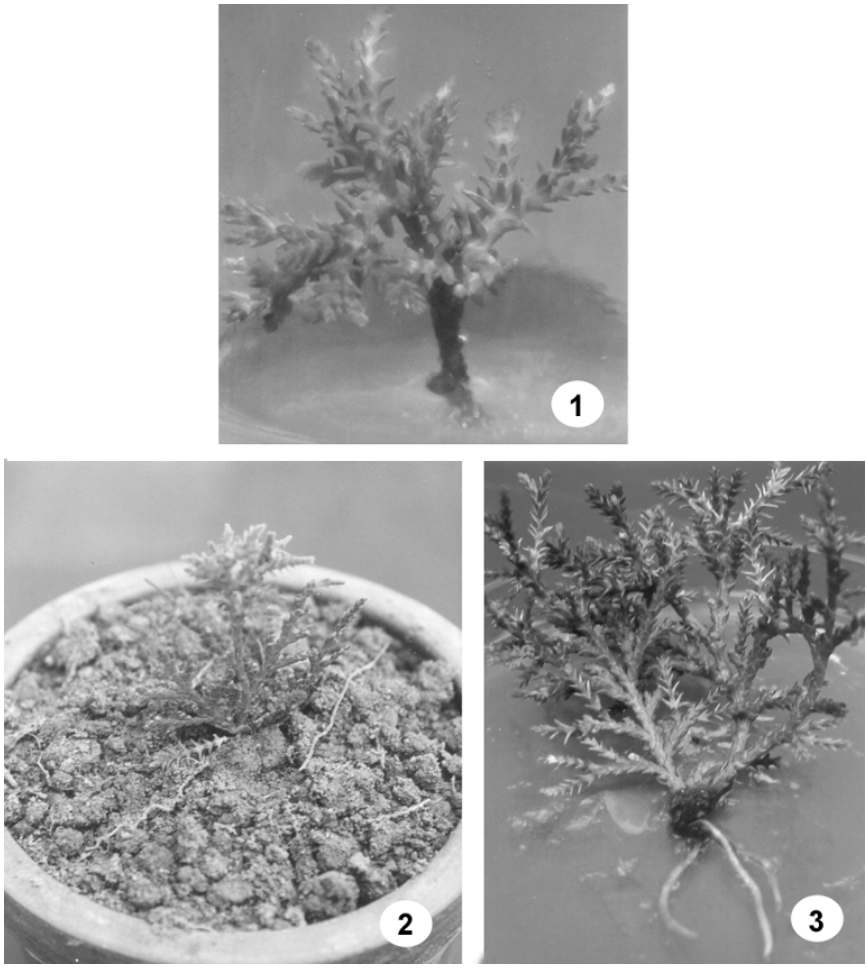
- = Indicates no response of the culture.

Table 2. Effects of IBA, IAA and NAA on half strength MS media in root induction of *in vitro* raised shoots of *T. occidentalis* at 60 days.

Name of hormone	Concentrations (mg/l)	% shoots responding to root induction	Averge number of roots induced/shoot	Average/root length/shoot (cm) (Mean ± SE)
IBA	0.5	10	1.92 ± 0.25	2.62 ± 0.48
	1.0	100	3.92 ± 0.28	3.64 ± 0.38
	1.5	20	1.52 ± 0.42	2.42 ± 0.32
	2.0	10	1.25 ± 0.72	2.20 ± 0.36
IAA	0.5	-	-	-
	1.0	-	-	-
	1.5	-	-	-
	2.0	-	-	-
NAA	0.5	-	-	-
	1.0	-	-	-
	1.5	-	-	-
	2.0	-	-	-

- = Indicates no response of the culture.

at subculture four even of they were subcultured in the same growing medium. This indicates that both shoot multiplication and its growth reach optimum level with the passage of fourth subculture.



Figs. 1-3. *In vitro* plant regeneration through apical shoot culture. 1. Multiple shoot formation on half strength MS devoid of plant growth regulators. 2. Rooted formation of *in vitro* raised shoots of half strength MS + 1.0 mg/l IBA. 3. *In vitro* raised plants in on earthen pot.

The rooting responses differed among the auxins used (Table 2). IBA was found responsive for root induction and 1 mg/l IBA was found optimum, in which 100% shoot rooted (Fig. 2) within 15 days of culture. The average number of roots per shoot was 3.92 ± 0.28 and the average root length of 3.84 ± 0.38 cm were observed in this medium. Shoots did not respond for rooting in the media containing IAA or NAA and they eventually died. The superiority of IBA for rooting over other auxins has also been reported (Jaiswal and Amin 1987, Amin

et al. 1992, Amin and Akhter 1993 and Grewal et al. 1994). Morphologically strong and healthy rooted about 5.0 cm tall shoots were taken out from the culture vessels and washed gently under running tap water to get rid of agar. The *in vitro* raised plantlets were then transferred to a small earthen pot (Fig. 3) containing a mixture of soil and compost (2 : 1) and acclimated following the usual procedures shade and mixed twice a day. About 60% plantlets resumed new growth within 30 days of acclimation period. The study showed that it is possible to successfully regenerate complete plantlets from apical shoots of filed grown plants of *Thuja occidentalis*. Thus this protocol might be used for *Thuja* propagation commercially.

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