

## Micropropagation of English Yew, an Ornamental-Medicinal Tree

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An *in vitro* propagation method of English yew (*Taxus baccata* L.) through organogenesis method using kinetin (Kin) and indole butyric acid (IBA) as plant growth regulators and apical bud as explant is presented. Apical buds excised from mother plants were inoculated on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of Kin (0.00, 0.50, 1.00 and 2.00 mg l<sup>-1</sup>) as a cytokinin and IBA (0.00, 0.50, 1.00 and 2.00 mg l<sup>-1</sup>) as an auxin. Results showed that the highest number of node (6.75) was obtained on MS medium containing 2.00 mg l<sup>-1</sup> Kin. The maximum shoot number (5.00) was obtained on MS medium supplemented with 1.00 mg l<sup>-1</sup> Kin together with 1.00 mg l<sup>-1</sup> IBA. The largest number of root (6.50) was produced on explants grown on medium enriched with 2.00 mg l<sup>-1</sup> Kin together with 1.00 mg l<sup>-1</sup> IBA. Plantlets were transferred to pots filled with perlite and peat moss in equal proportions for acclimatization. These plantlets were acclimated and successfully established in cultivation beds.

Abstract

**Keywords:** Forest trees, *In vitro* multiplication, Plant growth regulators, Threatened ornamental plant.

## INTRODUCTION

*Taxus* is a genus of 5-10 species of broadly rounded to upright, dioecious, evergreen, coniferous, large shrubs or small trees found in forest extending from N. temperate areas to the Philippines and Central America. Yews are grown for their linear, dark green leaves, often paler beneath; these are spirally arranged but often appear 2-ranked. On the female plants, single-seeded, oblong-ovoid fruits are produced in open, fleshy arils. Grow as specimen plants or use as hedges and topiary; the prostrate forms make a good groundcover, even in dense, dry shade. Most tolerate coastal exposure, dry soils, and urban pollution. All parts (except the fleshy red seed coats) are highly toxic if ingested. Grow in any well-drained, fertile soil, including alkaline or acidic soils, in sun or deep shade. Trim hedge in summer and early autumn (Brickell and Zuk, 1997). *Taxus baccata* L. (English yew) is broadly conical tree with spreading, horizontal branches, scaly, purple-brown bark, and shoots that remain green for several years. Linear, glossy or matte, dark green leaves, 2-3 cm long, paler beneath, are 2-ranked and patted either side of the shoots. Yellow male cones are borne in spring. Fruit consist of single green seeds with juicy, sweet, usually red arils (1.5 cm) across, 10-20 m height, 8-10 diameter (Brickell and Zuk, 1997).

*In vitro* propagation is a suitable technique for the regeneration of large numbers of plants in a relatively short period and, without seasonal restrictions. *In vitro* propagation of trees has been recognized as an important and efficient method for large-scale propagation and overcoming problems caused by heterogeneous seed production (Campbell *et al.*, 2003; Nunes *et al.*, 2018). Commercial use of propagating woody plants through tissue culture is limited to some species due to high variability and plantlets' survival during acclimatization. Due to the slow propagation of yew under natural conditions, micropropagation is a suitable method for mass production of this plant. Some methods for micropropagation of different yew species were developed (Chang *et al.*, 1998; Majada *et al.*, 2000; Anderson and Owens, 2001; Chang *et al.*, 2001; Metaxas *et al.*, 2004). The number of published reports on micropropagation of *Taxus* is limited. Study of Abbasin *et al.* (2010) on micropropagation of *T. baccata* using bud explants showed that the best result in shoot multiplication and root induction was obtained with 2.00 mg l<sup>-1</sup> of BAP and 8.00 mg l<sup>-1</sup> IBA, respectively. Similar results were reported by some other researchers (Chang *et al.*, 2001). The results of various studies showed that different types of plant growth regulators (PGRs) in different concentrations have a significant role in increasing the micropropagation of ornamental plants. Study on *Buxus hyrcana* showed the most shoot number was obtained in treatment of 1.00 mg l<sup>-1</sup> BAP together with 0.50 mg l<sup>-1</sup> IBA (Kaviani and Negahdar, 2017). These researchers also showed that the most root number was counted in treatment of 1.00 mg l<sup>-1</sup> BAP together with 1.50 mg l<sup>-1</sup> IBA. Maximum number of shoot and root in *Mespilus germanica* were regenerated on MS medium supplemented with 0.50 mg l<sup>-1</sup> BA together with 1.00 mg l<sup>-1</sup> NAA (Adibi Baladeh and Kaviani, 2021).

The aim of the present study was to evaluate the effect of different concentrations of Kin and IBA, individually and in combination, on *in vitro* propagation of *Taxus baccata* L. using apical buds' explants by the direct organogenesis method.

## MATERIALS AND METHODS

### Experiment conditions

In this experiment, English yew (*Taxus baccata* L.), an ornamental tree species, was used as mother plants (Fig. 1). Apical buds are used as explants. The experiments were performed in a greenhouse and laboratory of the Hyrcan Agriculture and Biotechnology Research Institute, Amol city, Mazandaran province, the northern part of Iran, on 2021.



Fig. 1. English yew (*Taxus baccata*) shoot with mature and immature cones (arils).

### Explant sterilization

Shoots were washed thoroughly under running tap water for 10 min. with a few drops of washing liquid followed by fully rinsed with running tap water. Apical buds were transferred to aseptic condition in a laminar air flow cabinet and were surface sterilized by dipping into 70% (v/v) ethanol for 2 min. followed by agitation for 10 min. in a sodium hypochlorite (NaOCl) solution containing 5% available chlorine and 0.05% (v/v) Tween-20, after which the buds were then rinsed five times with sterile distilled water.

### Culture medium, treatments and measured parameters

The surface sterilized apical buds were inoculated on MS (Murashige and Skoog, 1962) medium supplemented with 0.00, 0.50, 1.00 and 2.00 mg l<sup>-1</sup> of Kin and IBA, individually and in combination. Sucrose (3%) was used as carbon source and media were solidified with Agar-agar at a concentration of 0.7% (w/v). The pH of the media was adjusted to 5.7 ± 0.10 using 1 N NaOH or 0.1 N HCl prior to autoclaving for 20 min. at 15 psi, 121 °C. The cultures were maintained in a plant growth chamber at 20 ± 2 °C under a 12-h photoperiod, with photosynthetic flux density (PFD) of approximately 50 μmol m<sup>-2</sup> s<sup>-1</sup> provided by cool white fluorescent lamps and 75–80% relative humidity (RH). After 90 days of culture, some growth parameters including shoot number, leaf number, root length and root number were measured.

### Plantlets acclimatization

Plantlets with well-developed roots were taken out from culture vessels and washed with sterilized distilled water to remove adherent nutrient. They were then planted in a mixture of perlite and peat moss in 1:1 (v/v) ratio. Plantlets were covered by thin plastic bags and transferred to a greenhouse at a temperature of 25 ± 1°C with 70-80% RH. These plantlets were watered regularly. Plantlets were exposed gradually to external environment by removing the plastic bags of the pots.

### Experimental design and data analysis

The experiment was done in a completely randomized design (CRD) with 4 replicates. The results were expressed as mean ± SD of the experiments conducted thrice. The statistical analysis of data was performed using Statistical Package for Social Sciences (SPSS). Least Significant Difference (LSD) test at P < 0.05 was used to find out the significance of differences among the mean values.

**RESULTS****Shoot length**

There was statistically significant difference among different concentrations of IBA and Kin ( $P < 0.01$ ). There was no significant difference between different concentrations of IBA in combination with Kin (Table 1). The longest root length (6.50 cm per explant) was recorded in explants treated with 2.00 mg l<sup>-1</sup> Kin without IBA. The lowest average of the root length (3.07 cm) was measured in explants treated with 0.50 mg l<sup>-1</sup> Kin in combination with 1.00 mg l<sup>-1</sup> IBA (Table 2).

Table 1. Analysis of variance of the effect of different concentrations of IBA and Kin on measured parameters of *Taxus baccata* L. grown *in vitro* condition.

S.o.V	df	MS						
		Stem length	Shoot number	Node number	Root length	Root number	Callus number	Viability
IBA	3	2.07**	1.93 <sup>ns</sup>	2.93**	3.98**	17.37**	418.27**	520.83**
Kin	3	11.75**	11.68**	17.18**	0.98**	7.70 <sup>ns</sup>	42.52 <sup>ns</sup>	595.83**
IBA × Kin	9	0.71 <sup>ns</sup>	2.40**	6.00**	1.90**	1.61**	69.17**	433.33**
Error	45	0.38	0.75	0.538	10.43	0.94	22.06	115.27

\*, \*\*: Significant at the 0.05 and 0.01 probability level, respectively, <sup>ns</sup>: Not significant at  $P < 0.05$ .

Table 2. Mean comparison of the effect of different concentrations of IBA and Kin on measured parameters of *Taxus baccata* L. grown *in vitro* condition.

IBA + Kin (mg l <sup>-1</sup> )	Stem length (cm)	Shoot number	Node number	Root length (cm)	Root number	Callus number	Viability (%)
0.00 + 0.00	3.55	1.50 <sup>c</sup>	2.50 <sup>cd</sup>	2.75 <sup>c</sup>	1.75 <sup>f</sup>	15.50 <sup>c</sup>	70.00 <sup>c</sup>
0.00 + 0.50	3.55	1.50 <sup>c</sup>	2.50 <sup>cd</sup>	2.37 <sup>cd</sup>	2.75 <sup>d</sup>	14.25 <sup>cd</sup>	77.50 <sup>b</sup>
0.00 + 1.00	3.87	3.25 <sup>c</sup>	2.50 <sup>cd</sup>	2.62 <sup>c</sup>	2.25 <sup>e</sup>	13.75 <sup>d</sup>	80.00 <sup>b</sup>
0.00 + 2.00	6.50	1.25 <sup>e</sup>	6.75 <sup>a</sup>	2.90 <sup>bc</sup>	3.25 <sup>bc</sup>	12.25 <sup>d</sup>	62.50 <sup>d</sup>
0.50 + 0.00	3.15	1.75 <sup>de</sup>	2.00 <sup>cd</sup>	3.15 <sup>b</sup>	2.00 <sup>e</sup>	24.00 <sup>b</sup>	72.50 <sup>c</sup>
0.50 + 0.50	3.27	2.00 <sup>d</sup>	2.00 <sup>cd</sup>	3.00 <sup>b</sup>	2.75 <sup>d</sup>	36.50 <sup>a</sup>	77.50 <sup>b</sup>
0.50 + 1.00	3.07	4.00 <sup>b</sup>	3.25 <sup>b</sup>	3.62 <sup>b</sup>	3.00 <sup>e</sup>	24.50 <sup>b</sup>	92.50 <sup>a</sup>
0.50 + 2.00	4.62	1.75 <sup>de</sup>	6.00 <sup>a</sup>	3.30 <sup>b</sup>	2.50 <sup>de</sup>	20.25 <sup>b</sup>	100.00 <sup>a</sup>
1.00 + 0.00	3.12	1.75 <sup>de</sup>	2.00 <sup>cd</sup>	5.55 <sup>a</sup>	3.00 <sup>e</sup>	21.25 <sup>b</sup>	80.00 <sup>b</sup>
1.00 + 0.50	3.65	2.00 <sup>d</sup>	2.75 <sup>c</sup>	3.12 <sup>b</sup>	5.00 <sup>ab</sup>	21.25 <sup>b</sup>	70.00 <sup>c</sup>
1.00 + 1.00	3.52	5.00 <sup>a</sup>	3.25 <sup>b</sup>	3.07 <sup>b</sup>	4.00 <sup>b</sup>	19.00 <sup>bc</sup>	90.00 <sup>ab</sup>
1.00 + 2.00	4.62	2.00 <sup>d</sup>	3.00 <sup>bc</sup>	3.27 <sup>b</sup>	6.50 <sup>a</sup>	24.00 <sup>b</sup>	90.00 <sup>ab</sup>
2.00 + 0.00	3.70	2.00 <sup>d</sup>	3.25 <sup>b</sup>	2.30 <sup>cd</sup>	2.25 <sup>e</sup>	16.75 <sup>c</sup>	85.00 <sup>ab</sup>
2.00 + 0.50	3.75	2.50 <sup>cd</sup>	3.00 <sup>cd</sup>	2.85 <sup>bc</sup>	2.75 <sup>d</sup>	18.75 <sup>bc</sup>	65.00 <sup>d</sup>
2.00 + 1.00	3.30	2.25 <sup>cd</sup>	1.75 <sup>d</sup>	2.90 <sup>bc</sup>	2.00 <sup>e</sup>	19.50 <sup>bc</sup>	82.50 <sup>b</sup>
2.00 + 2.00	4.92	3.25 <sup>c</sup>	2.75 <sup>c</sup>	3.10 <sup>b</sup>	2.50 <sup>de</sup>	23.00 <sup>b</sup>	75.00 <sup>b</sup>

\*Means with different letters on the same column are significantly different ( $P < 0.05$ ) based on LSD test.

**Shoot number**

Analysis of variance (Table 1) showed that shoot number is affected by treatments of Kin and Kin along with IBA ( $P < 0.01$ ). The highest shoot number (5.00 per explant) was calculated on medium supplemented with 1.00 mg l<sup>-1</sup> IBA in combination with 1.00 mg l<sup>-1</sup> Kin (Table 2). There was no positive correlation between enhancing IBA and Kin concentrations and increasing the number of shoot. The lowest shoot number (1.25 per explant) was calculated on medium containing 2.00 mg l<sup>-1</sup> Kin without IBA.

### Node number

The data clearly showed that node number is strongly affected by treatments of IBA, Kin and IBA along with Kin ( $P < 0.01$ ) (Table 1). The highest number of node (6.75 and 6.00 per explant) was obtained in explants grown on media fortified with 2.00 mg l<sup>-1</sup> Kin without IBA and 0.50 mg l<sup>-1</sup> IBA together with 2.00 mg l<sup>-1</sup> Kin, respectively (Table 2). The lowest number of node (1.75 per explant) was obtained with treatment of 2.00 mg l<sup>-1</sup> IBA together with 1.00 mg l<sup>-1</sup> Kin.

### Root length

The maximum root length (5.55 cm per explant) was recorded in explants treated with 1.00 mg l<sup>-1</sup> IBA without Kin. The lowest average of the root length (2.37 cm) was measured in explants treated with 0.50 mg l<sup>-1</sup> Kin without IBA (Table 2). There was statistically significant difference among different concentrations of Kin, IBA, also IBA in combination with Kin and root length ( $P < 0.01$ ).

### Root number

Maximum number of roots (6.50 per shoot) were produced in medium containing 1.00 mg l<sup>-1</sup> IBA together with 2.00 mg l<sup>-1</sup> Kin (Table 2). Shoots cultured on medium without PGRs produced the least roots (1.75). Statistically significant difference was observed between the mean for root number and IBA, and IBA together with Kin.

### Callus number

The data showed that callus number is strongly affected by treatments of IBA, and IBA along with Kin ( $P < 0.01$ ) (Table 1). The highest callus number (36.50) was obtained in explants grown on medium fortified with 0.50 mg l<sup>-1</sup> IBA together with 0.50 mg l<sup>-1</sup> Kin (Table 2). The least callus number (12.25) was obtained with treatment of 2.00 mg l<sup>-1</sup> Kin without IBA.

### Viability

Viability percentage was full for plantlets treated with 2.00 mg l<sup>-1</sup> Kin without IBA and 0.50 mg l<sup>-1</sup> IBA. Viability percentage was over than 90% in plantlets grown on medium supplemented with 1.00 mg l<sup>-1</sup> Kin together with 0.50 mg l<sup>-1</sup> IBA. Viability percentage was relatively low (62%) for plantlets treated with 2.00 mg l<sup>-1</sup> Kin without IBA. Effect of Kin and IBA, individually or in combination on viability percentage was significant ( $P < 0.01$ ) (Table 1).

### Establishment of plantlets

Well-developed plantlets were transferred to small plastic pots containing peat moss and perlite in 1:1 (v/v) ratio for *ex vitro* establishment. Then, plantlets were transferred to big plastic pots filled with the same medium. The acclimatized plantlets were covered by thin plastic bags and transferred to the greenhouse with 90% establishment rate.

## DISCUSSION

Production of true-to-type plants within a short period of time is the first step in successful study on micropropagation. Current study reports a mass propagation system for *T. baccata*, a valuable ornamental-medicinal tree. Shoot multiplication was strongly influenced by both Kin and IBA. Successful use of cytokinins such as BAP, zeatin, Kin, TDZ and (N<sup>6</sup>-( $\Delta^2$ -isopentenyl) adenine (2-iP) were reported for shoot multiplication and growth in this genus (Ewald, 2007; Abbasin *et al.*, 2010). Maximum shoot multiplication in most plants particularly trees and shrubs has been obtained on media containing low concentrations of cytokinins (Alvarez *et al.*, 2009;

Abbasin *et al.*, 2010; Kaviani and Negahdar, 2017; Adibi Baladeh and Kaviani, 2021). Optimum concentration for maximum shoot proliferation is different between each species. This can be due to the content of endogenous PGRs in each species. Also, the response to exogenous PGRs varies with species and the percentage of response varies with PGRs treatments and type of explants. The combination of Kin and IBA improved the shoot proliferation. Combination of a cytokinin and an auxin for successful *in vitro* propagation of some ornamental trees have been shown (Kaviani and Negahdar, 2017; Sharma, 2017; Dinesh *et al.*, 2019; Adibi Baladeh and Kaviani, 2021). Similar to our findings, some studies have shown that in a medium containing cytokinins individually or in combination with auxins, apical and axillary buds produced a suitable number of shoots (Kaviani and Negahdar, 2017; Sharma, 2017; Sharma *et al.*, 2017; Dinesh *et al.*, 2019). In many woody species, cytokinins especially BAP and Kin were effective for shoot multiplication (Sharma, 2017). Higher concentrations of cytokinins are inhibitory in some woody plants (Kaviani and Negahdar, 2017). Positive effect of other cytokinins like BA, Zeatin and TDZ on shoot multiplication were reported in some woody plants (Yıldırım, 2012; Kereša *et al.*, 2012; Fan *et al.*, 2017; Sharma *et al.*, 2017). In medlar, simultaneous use of BA and NAA increased the number of leaf and node (Adibi Baladeh and Kaviani, 2021). The highest number of shoots in *Couroupita guianensis*, a medicinally tree, were regenerated on MS medium enriched with 1.00 mg l<sup>-1</sup> each of BAP and Kin together with 0.50 mg l<sup>-1</sup> NAA (Mahipal *et al.*, 2016). Type and optimum concentration of PGRs for maximum shoot induction and multiplication depends on the type of species, the type of explants, and the content of indigenous PGRs.

Current investigation showed the positive effect of both Kin and IBA in combination on root induction. Similar to our finding, some researchers revealed successful rooting on the base of shoots using a combination of auxin and cytokinin (Kaviani and Negahdar, 2017; Adibi Baladeh and Kaviani, 2021). In *Buxus hyrcana*, the longest root length was obtained in medium containing 1.00 mg l<sup>-1</sup> BAP along with 1.00 mg l<sup>-1</sup> NAA (Kaviani and Negahdar, 2017). Explants cultured on MS medium enriched with 0.50 mg l<sup>-1</sup> BAP in combination with 1.00 mg l<sup>-1</sup> NAA produced the largest number of root per plantlet (Kaviani and Negahdar, 2017). Auxin concentration and type significantly influenced rooting percentage and root length. IBA is more effective for rooting as compared with other auxins as reported for many woody species (Onay, 2000; Romano *et al.*, 2002; Chand and Singh, 2004a; Prakash *et al.*, 2006; Kalinina *et al.*, 2007). Maximum root production was obtained using 8.00 mg l<sup>-1</sup> IBA in culture medium of *T. baccata* (Abassin *et al.*, 2010). The better impact of IBA compared with NAA and IAA in growth of roots has been reported in *T. baccata* L. (Ewald, 2007), *T. brevifolia* Nutt. (Mitchell, 1997) and *T. mairei* (Chang *et al.*, 2001). IBA and NAA promoted rooting of different trees and shrubs species (Dhar *et al.*, 2000; Takihira *et al.*, 2007; Noroozi Sharaf *et al.*, 2011).

In *Medlar germanica*, 1.00 mg l<sup>-1</sup> NAA, individually or in combination with 0.50 mg l<sup>-1</sup> BA, successfully induced rooting (Adibi Baladeh and Kaviani, 2021). In many species, maximum roots were formed when medium was supplemented with IBA (Yıldırım, 2012; Kereša *et al.*, 2012; Sulusoglu and Cavusoglu, 2013; Sharma and Vashistha, 2015c; Mahipal *et al.*, 2016; Fan *et al.*, 2017; Dinesh *et al.*, 2019). Castillón and Cornish (2000) revealed that IBA was most effective auxin for induction of roots than IAA and NAA in *Parthenium argentatum*, a woody desert shrub. Venkatachalam *et al.* (2015) showed that among the three auxins (IAA, NAA and IBA) used for *in vitro* rooting of the cultured shoots of *Bambusa arundinacea*, IBA was the most suitable followed by NAA. In *Couroupita guianensis*, the multiplied shoots were rooted on medium supplemented with 2.50 mg l<sup>-1</sup> IBA (Mahipal *et al.*, 2016). The nature of auxin required, the needed concentration of exogenous auxins and the content of endogenous auxins for *in vitro* root regeneration are species-specific (Rathore *et al.*, 2004). In some woody plants, the presence of both cytokinin and auxin

stimulated better rooting than when only one auxin was used (Savita *et al.*, 2010; Kaviani and Negahdar, 2017; Sharma, 2017).

## CONCLUSION

*In vitro* propagation of trees and shrubs (woody plants) is difficult due to some problems. We tried to propagate *Taxus baccata* L. *in vitro* using apical bud as explant and Kin and IBA as PGRs through direct organogenesis method. In the present study, the highest shoot multiplication was obtained in medium enriched with 1.00 mg l<sup>-1</sup> Kin together with 1.00 mg l<sup>-1</sup> IBA. Also, the highest root number was produced in medium enriched with 2.00 mg l<sup>-1</sup> Kin together with 1.00 mg l<sup>-1</sup> IBA.

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