Effects of donor tree age, cutting collection time and K-IBA application on rooting ability of *Taxus baccata* L. stem cuttings: preliminary results

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Abstract

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The present study investigates the effect of donor tree age (juvenile, adult), collection time (March, November) and the K-IBA (indole-3-butyric acid potassium salt; 0, 3, 6, 12 g L^{-1} K-IBA) application on rooting of *T. baccata* stem cuttings. Terminal hardwood leaf stem cuttings were collected of the year 2013 from individuals growing in their natural habitat in the Cholomontas mountains in the northern part of Greece. In cuttings taken from adult individuals, the application of K-IBA only significantly improved the rooting percentage. However, in cuttings taken from juvenile individuals, the collection time and K-IBA application as well as their interaction were statistically significant. In November collection, the cuttings treated with 12 g L^{-1} of K-IBA exhibited the highest rooting percentage (98.3%), while in March collection, the cuttings treated with 3 g L^{-1} of K-IBA exhibited higher rooting percentages (61.7%) than those of control (41.7%). In cuttings taken from juvenile individuals, the K-IBA application significantly improved the rooting of cuttings collected in November compared with those collected in March. The cuttings taken from juvenile individuals, exhibited significantly higher rooting percentages than those taken from adult individuals.

Keywords

European yew, indole-3-butyric acid, mist system, vegetative propagation

Introduction

The genus *Taxus* L. (Taxaceae) includes 12 species (including two artificial hybrid species) distributed in the northern hemisphere (Cope, 1998). *Taxus baccata* L. (known as European yew) is a native species in most of Europe, northern Africa and west Asia (Browicz, 1982; Cope, 1998). It is a very slow growing and long-lived evergreen tree which is characterised by the ability to grow under the canopy of other higher trees (Browicz, 1982). In Greece, *T. baccata* belongs to the group of rare and potentially endangered species which mainly occurs in dispersed localities of the mountains of central and northern continental parts of the country (Kassioumis et al.,

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2004). It is usually grown singly or in small groups found in the lower storey of cool temperate forests (Boratynski et al., 1992).

Taxus baccata is a valuable species. There is a great demand for this species in the market due to its horticultural value and as a result numerous cultivars have been created which are being cultivated in parks, gardens and arboreta (Voliotis, 1986). Furthermore, in recent years the genus *Taxus* has received increased attention due to taxane diterpenoids, particularly taxol, contained in its leaves and bark (Bayraktar et al., 2018). Taxol is well-known for its anticancer properties and was first isolated from the bark of *Taxus brevifolia* and the report of its isolation and chemical structure was first published in 1971 (Wani et al., 1971). The harvesting of twigs and leaves from *T. baccata*, as an alternative sustainable source for taxol production, may cause a pressure to wild populations of species and consequently to loss of genetic resources. Taking into account the above and the fact that *T. baccata* grows in small isolated populations, great attention has to be given for the conservation of the species.

Information about the technique of propagation is very useful for the management of *T. baccata* species. For many species, propagation from seeds is the most common and the cheapest method used in nurseries (Macdonald, 2006). However, a major constraint to the sexual propagation of *T. baccata* species is the poor germination due to deep seed dormancy (Vance and Rudolf, 2008). In this case, vegetative propagation techniques could be alternatively used in order to achieve the aim of conservation and restoration of the species. According to Husen and Pal (2006), vegetative propagation is widely used to multiply valuable trees growing in natural population to exploit the genetic variability.

However, there are several factors that affect the rooting of cuttings (Vakouftsis, 2006). Adventitious root formation of stem cuttings is affected by age of the donor plants as well as by the season of collection. The effect of age of donor plant on rooting ability of stem cuttings has been reported in many species (Bhardwaj and Mishra, 2005; Husen and Pal, 2006; Amri et al., 2010). According to Hartmann et al. (2014), rooting ability of cuttings decreases with increasing age of the donor plants. Several explanations for the decreasing rooting ability of cuttings taken from mature plants have been given. These include accumulation of rooting inhibiters, decrease in the endogenous content of auxin and/or root promoters, and decreased sensitivity of tissues to auxins with physiological aging of the stock (Haffner et al., 1991; Husen and Pal, 2006). In an attempt to increase rooting ability of woody species, auxins are applied to the basal part of the stem cuttings where adventitious root initiation and development will take place. Studies have shown that the use of auxins is essential for obtaining the maximum number of rooted cuttings in a short period of time (Metaxas et al., 2004; Bayraktar et al., 2018; Kostas et al., 2021; Tsaktsira et al., 2021). The indole-3-butyric acid (IBA) is the most commonly used auxin for rooting in commercial propagation; other auxins often used are indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) (De Klerk et al., 1999; Hartmann et al., 2014). Furthermore, vegetative propagation with cuttings under a mist system has become the most accepted method worldwide (Hartmann et al., 2014). The mist reduces transpiration and leaf temperature, increases the relative humidity and keeps the cuttings and leaves in turgor during the process of root induction (Hartmann et al., 2014).

There are studies that have shown that vegetative propagation of *T. baccata* is possible (Metaxas et al., 2004; Bayraktar et al., 2018; Das and Jha, 2018a, 2018b). However, in most of the research in rooting of *Taxus* stem cuttings, the plant material used was not derived from individuals growing in their natural habitat. The aim of the present study is to investigate the effect of donor tree age, collection time, and the K-IBA (indole-3-butyric acid potassium salt) application on rooting of *T. baccata* stem cuttings collected from plants growing in their natural habitat.

Materials and methods

Propagated material

The propagation material (branches) was collected from a number of individuals growing in their natural habitat (40º26′17′′N, 23º31′30′′E, 730 m asl) in the Cholomontas mountains in the northern part of Greece. The propagation material was collected in two different times of the year 2013: in spring (20 March) and in autumn (23 November). In each collection time, the branches were taken separately from healthy individuals of adult age and from individuals of juvenile age. Adults were classified as the individuals in which the diameter at breast height was ranged from 43 to 60 cm, whereas the height was ranged from 6.5 to 9.0 m. Unfortunately, the determination of their age was not possible due to the presence of rot inside the trunk. Juveniles were characterised as the individuals with height from 1.0 to 1.5 m and diameter at the base (ground level) from 1.5 to 2.0 cm. The age of juveniles was determined from a random sample of six individuals and was ranged from 16 to 23 years old. In both age groups, the propagated material was taken from the lower part of the crown.

Stem cuttings treatment

The experiments were conducted in the greenhouse of the Laboratory of Silviculture, School of Forestry and Natural Environment, Aristotle University of Thessaloniki, Greece.

The collected branches were placed in plastic bags, were transferred to the Laboratory of Silviculture and were stored in a refrigerator (3–5 ºC). The next day, the collected branches were cut to a length of about 12–15 cm (Figure 1b). In both collection times, only terminal cuttings were used (Figure 1a). The diameter at the base of cuttings was at least 3 mm. Then, the needles were removed from the basal half of each cutting and the basal

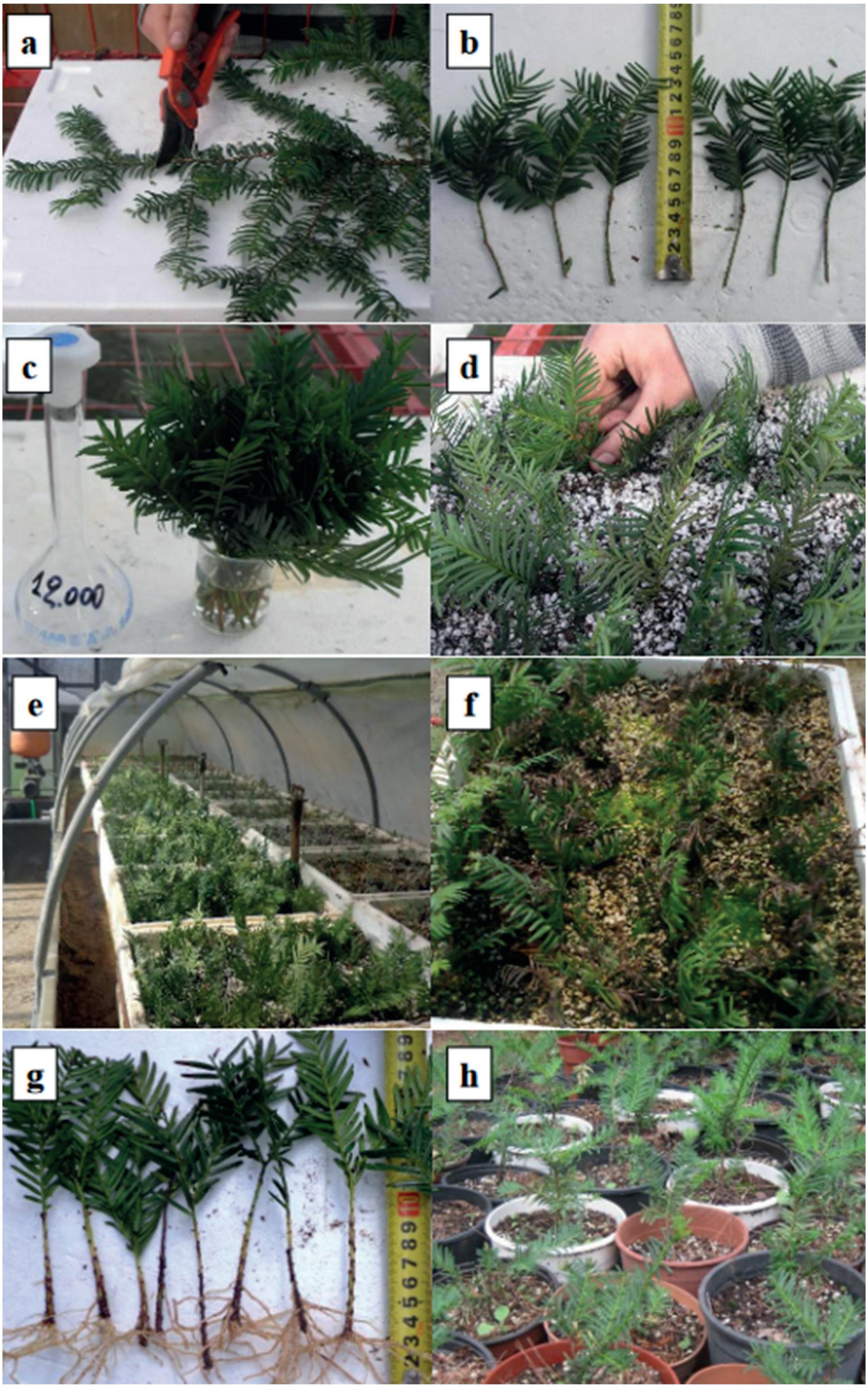

Fig. 1. Preparation of shoot cuttings from branches of *T. baccata* (a), *T. baccata* shoot cuttings (b), application of 12 g L^{-1} K-IBA root regulator for ten seconds (c), planting of shoot cuttings in boxes with substrate perlite and peat (d), boxes with shoot cuttings in mist rooting system (e), *T. baccata* shoot cuttings in the end of the experiment (f), rooted *T. baccata* shoot cuttings (g) and plantlets of *T. baccata* in pots four months after rooting (h).

portion of each cutting, about 2 cm in length, was dipped into different concentrations (0, 3, 6 or 12 g L^{-1}) of K-IBA for 10 seconds (Figure 1c). The cuttings were planted in plastic boxes (with holes at their base for drainage) filled with a ratio 1:2 (v/v) mixture of peat (Klassmann TS1) and perlite (Figure 1d). Subsequently, the plastic boxes with the planted cuttings were transferred on the bench under an intermittent mist system for rooting (Figure 1e). The frequency and the duration of mist applying were adjusted according to environmental conditions. Furthermore, the temperature at the base of the bench was set to 20 °C. In each collection time and for each age group and treatment with K-IBA, 60 terminal cuttings were used (four replications of 15 cuttings).

At the end of a 3-month period, the cuttings were carefully exported from the substrate and the number of rooted cuttings was counted and their percentage (%) was calculated for each treatment (Figure 1f, g). A cutting was considered rooted if it had at least one root ≥ 0.5 cm. After rooting evaluation, the rooted cuttings were transplanted in pots (Figure 1h).

Statistical analysis

In each age group, the experimental design was a completely randomised factorial design. The factors were the collection time and the concentration of K-IBA (2 × 4 factorial design). The data were analysed using the ANOVA method in the frame of the GLM (General Linear Model) (Gomez and Gomez, 1984). The rooting percentage data was transformed to arc-sine square root values, before analysis (Snedecor and Cochran, 1980). The transformed data were checked for normality and homogeneity of variances and then analysed by ANOVA, while the comparisons of the means were made using the Bonferroni test (Toothaker, 1993). In the comparisons between the two age groups for each treatment with K-IBA in the two collection times, the T test was used (Klockars and Sax, 1986). All statistical analyses were carried out using SPSS 21.0 (SPSS, Inc., USA).

Results

In the cuttings taken from adult individuals, the application of K-IBA only affected the rooting percentage (Table 1). In detail, the cuttings treated with K-IBA, regardless the concentration, exhibited significantly higher rooting percentages (24.2–30.8%) than those of control (5.0%). Furthermore, no significant difference in rooting percentages among the cuttings treated with the K-IBA solutions was observed (Figure 2).

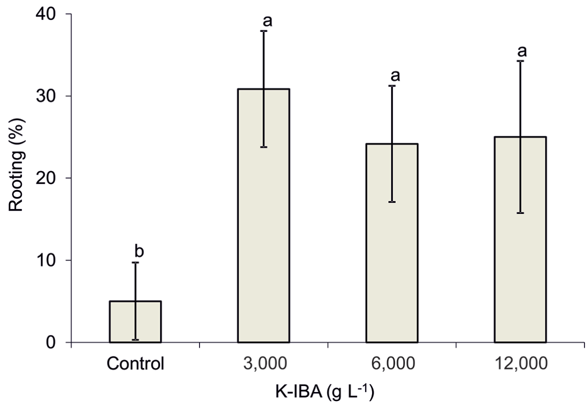

Fig. 2. Effect of different concentrations of K-IBA on rooting of *T. baccata* stem cuttings taken from adult plants, regardless the collection time. Columns accompanied with the same letter do not differ significantly. The comparisons were made using Bonferroni test.

In the cuttings taken from juvenile individuals, the main effects (collection time and K-IBA application) as well as their interaction were statistically significant (Table 2). In the presence of a significant interaction, it was considered that the interpretation of main effects was of less importance.

In Table 3 the interaction is analysed. Specifically, in November collection, the cuttings treated with 12

Table 1. Significance of factors and their interaction on rooting percentage of *T. bacatta* stem cuttings taken from adult plants, estimated by ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Collection time	33.54	1	33.54	0.99	0.330
K-IBA	2,656.29	3	885.43	26.07	0.000
Collection time × K-IBA	253.35	3	84.45	2.49	0.085

Table 2. Significance of factors and their interaction on rooting percentage of *T.bacatta* stem cuttings taken from juvenile plants, estimated by ANOVA

Source	Sum of Squares	df	Mean Square	F	Sig.
Collection time	1,802.25	1	1,802.25	59.23	0.000
K-IBA	3,685.63	3	1,228.54	40.37	0.000
Collection time × K-IBA	1,681.56	3	560.52	18.42	0.000

Table 3. Effect of different concentrations of K-IBA on rooting percentage of *T. baccata* stem cuttings taken from juvenile plants in combination with the cutting time

Collection	Rooting percentage (% ± S.D.)			
Time	Control	3 g L^{-1}	6 g L^{-1}	12 g L^{-1}
March	41.67 b[1] A[2] ± 6.38	61.67 a B ± 10.00	55.00 ab B ± 8.39	58.33 ab B ± 10.00
November	35.00 c A ± 8.39	78.33 b A ± 8.39	81.67 b A ± 6.38	98.33 a A ± 3.33

[1]In a row, percentages are statistically different at $p < 0.05$, when they don't share a common letter (small letters).
[2]In a column, percentages are statistically different at $p < 0.05$, when they don't share a common letter (capital letters).
The comparisons were made using Bonferroni test.

Table 4. Comparisons of the rooting percentages of *T. baccata* stem cuttings between the two age groups for each concentration of K-IBA application in each collection time

Donor Tree	Rooting percentage (% ± S.D.)			
Age	Control	3 g L^{-1}	6 g L^{-1}	12 g L^{-1}
		March		
Adult	6.67 b[1] ± 5.44	31.67 b ± 6.38	20.00 b ± 5.44	18.33 b ± 5.44
Juvenile	41.67 a ± 6.38	61.67 a ± 5.44	55.00 a ± 8.39	58.33 a ± 10.00
		November		
Adult	3.33 b ± 3.85	30.00 b ± 8.61	28.33 b ± 6.38	31.67 b ± 6.38
Juvenile	35.00 a ± 8.39	78.33 a ± 8.39	81.67 a ± 6.38	98.33 a ± 3.33

[1]In a column, percentages are statistically different at $p < 0.05$, when they don't share a common letter.
The comparisons were made using T test.

g L^{-1} of K-IBA exhibited the highest rooting percentage (98.3%), whereas the untreated cuttings (control) exhibited the lowest rooting percentages (35.0%). No significant difference between the treatments with 3 and 6 g L^{-1} of K-IBA was observed. In March collection, the cuttings treated with 3 g L^{-1} of K-IBA exhibited higher rooting percentages (61.7%) than those of control (41.7%). In cuttings taken from juvenile individuals, the K-IBA application, regardless the concentration, significantly improved the rooting of cuttings collected in November compared with those collected in March. However, no significant difference in rooting percentages of untreated cuttings (control) between the collection times was observed.

Furthermore, in each treatment with K-IBA for both collection times, the cuttings taken from juvenile individuals, exhibited significantly higher rooting percentages than those taken from adult individuals (Table 4).

Discussion

According to the results of the present study, it was determined that the K-IBA treatment had a positive effect on rooting of *T. baccata* stem cuttings. Regardless of the age of donor tree and collection time, higher rooting percentages were observed in the cuttings treated with K-IBA compared with untreated cuttings. Possibly, the exogenous auxin K-IBA resulted in elevation of endogenous auxin causing the beginning of rooting initiation phase and subsequently the rooting expression phase. According to Metaxas et al. (2004), the application of K-IBA and IBA in semi-hardwood cuttings of a *T. baccata* genotype with high rooting ability resulted in high percentages of rooting (100 and 92.2 %, respectively), while no rooting occurred without auxins treatment. Furthermore, in the former study the auxins K-IBA and IBA were the most efficient in rooting of *T. baccata* stem cuttings compared with other auxins (IAA, NAA, Paclobutrazol). Similarly, Singh (2006), Bayraktar et al. (2018), and Das and Jha (2018b) found the highest rooting percentage in stem cuttings of *T. baccata* treated with IBA. In other species of *Taxus*, the similar effect of auxin treatment on adventitious root formation has been observed (Eccher, 1988; Kaul, 2008).

The period of the year in which cuttings are taken, can play an important role in rooting. According to Hartmann et al. (2014), for many species there is an optimal period of the year for rooting. Therefore, in knowing the optimal period, the propagators can maximise the rooting process of cuttings. In the present study, the collection time influenced the rooting of cuttings taken only from juvenile individuals. According to the results, the control cuttings of *T. baccata* rooted with more or less difficulty. However, taking cuttings from juvenile individuals during late autumn and applying K-IBA, higher rooting percentages were obtained than cuttings taken in March. Hartmann et al. (2014) have demonstrated that the rooting of *Taxus* cuttings is lowest during the season of active vegetative growth and highest during the dormant period. Furthermore, Lanphear and Meahl (1961) observed significantly lower number of cuttings rooted of *Taxus cuspidate* 'Nana' taken during late winter.

The stem cuttings of *T. baccata* taken from juvenile individuals rooted in higher percentages than cuttings taken from mature individuals. This result indicates that the age of donor tree is crucial for rooting. The cuttings collected from juvenile individuals in late autumn and treated with 12 g L^{-1} of K-IBA exhibited the highest rooting percentage (98.33%). In evaluating the effect of genotype on rooting, Metaxas et al. (2004) found that the rooting of terminal semi-hardwood cuttings of *T. baccata* taken from 5-year old individuals and treated with 10 g L^{-1} K-IBA ranged from 50 to 100%. However, the donor plants of the seven genotypes used in their study were grown in the greenhouse under controlled environmental conditions and the cuttings were planted in perlite and placed for rooting in a fog system. In Bayraktar et al.'s (2018) study, the cuttings of *T. baccata*, which were taken in March from a 28-year old male individual growing in campus of Karadeniz Technical University and were treated with 5 g L^{-1} of IBA and then planted in perlite in greenhouse (air temperature at 20 ± 2 °C, rooting table temperature at 25 ±2 °C), exhibited the highest rooting percentage (80%). It is well-known that cuttings taken from juvenile plants have higher ability to root than those collected from mature plants (Macdonald, 2006). Talbert et al. (1993) concluded that rooting ability of conifer cuttings taken from mature plants has limited success. This effect of donor tree age on rooting of cuttings has been demonstrated in many other species (Berhe and Negash, 1998; Bhardwaj and Mishra, 2005; Amri et al., 2010). Macdonald (2006) found that many plants reach an optimum stage for rooting effectively, after which the percentage success rate drops and the vigor of plants declines. An example for this was the rooting capacity of ×*Cupressocyparis leylandii* cuttings taken from 5-year old, 20-year old and 50-year old trees, which was 94%, 34% and 5% respectively (Macdonald, 2006).

The present study was the result of a preliminary investigation of rooting of *T. baccata* stem cuttings, which were collected from a natural population of the species. For the improvement of rooting ability of cuttings from adult individuals, the effect of more factors, such as the auxin type and the concentration, the substrate composition (ratio of mixture of perlite:peat), the rooting system (fog, aeroponic system) on cutting rooting needs to be investigated. Furthermore, as an alternative to propagation by cuttings, in vitro techniques (Al-Mayahi et al, 2020; Konopkova et al, 2020; Al-Mayahi and Ali, 2021) could be used for an optimal multiplication of *T. baccata* adult individuals.

Conclusions

The results of the present study can constitute a basic step for both in situ and ex situ conservation efforts or for sustainable exploitation strategies of this valuable phytogenetic resource. According to preliminary results of the present study, the stem cuttings of *T. baccata* rooted in low percentage without K-IBA treatment. Regardless of the collection time and age of donor tree, the treatment of cuttings with K-IBA improved significantly the rooting percentage. The collection time affected the rooting only in stem cuttings taken from juvenile individuals of *T. baccata*. Particularly, cuttings collected in late November exhibited higher rooting percentage than those collected in March. In both collection times, the age of donor trees affected the rooting. Regardless of the K-IBA application, the cuttings taken from juvenile individuals exhibited significantly higher rooting percentages than those taken from adult individuals. For the vegetative propagation of *T. baccata*, collection of stem cuttings from juvenile individuals in late November and treatment with 12 g L^{-1} of K-IBA is recommended.

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