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AN INVESTIGATION ON USING OF GROWTH REGULATORS IN PROPAGATION OF *TAXUS BACCATA* BY CUTTINGS

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Abstract

The feasibility of enhancing the propagation of *Taxus baccata* by stem cuttings has been studied using the application of exogenous growth regulators IBA and/or GA3 at different concentrations (4000, 6000, 8000 and 10000 ppm). IBA at 10000 ppm induced the highest germination percentage along with better root system characteristics. Although, GA3+IBA at 10000 ppm each was inferior IBA alone, it has obtained promising results.

Keywords: *Taxus baccata*, cutting, rooting, propagation, IBA, GA3

INTRODUCTION

Apart from its ornamental uses especially in hedges and topiary, and the uses of its strong and flexible wood in making fine musical instruments, cabinets, and utensils (Ambasta, 1986), *Taxus* species has gain additional economic importance since it was discovered to harbor Taxol®. This diterpene extracted from *Taxus* bark, needles, twigs, and roots, has been identified as one of the most promising anti-cancer drugs (Heinstein and Chang, 1994). The propagation of English yew, *Taxus baccata,* has drawn a lot of attention in the past several years as a response to the collection of needles for the extraction of 10-Deacetylbaccatin III, which is a homolog of Taxol®, and is considered the most appropriate precursor for synthesizing Taxol® derivatives (Kikuchi and Yatagai, 2003).

T. baccata could be propagated by seed, cutting, grafting as well as *in vitro* culture means. Since seed germination in *T. baccata*, even after application of different techniques of breaking dormancy, is very poor, vegetative propagation through stem cuttings may perhaps be the only viable option to improve and manage its natural stock as well as the regeneration process (Singh, 2006).

Skimming the previous available literature, It is obvious that rooting hormone treatment is important or even essential for successfully rooting of *T. baccata* cutting (Mitchell, 1997), without which no rooting could be induced (Metaxas *et al.*, 2004). Although, IBA has been reported as the most successful rooting treatment, a wide range of variation regarding its rooting effect has been noticed varying from 29 up to 100% using the same level (8000-10000 ppm) (Nelson, 1959; Bell, 1975; Eccher, 1989; Mitchell, 1997). This reason of these variations lies in the high level of inter- and intraspecific variations in *Taxus* populations (Von Kornya, 1976; Mitchell 1997), even among individuals (Schneck, 1996)

Thus, the present study aimed to determine a proper rooting hormonal application by which a high rooting percentage of stem cuttings as well as better seedling growth of *T. baccata* could be attained. Since previous investigations concentrated on comparing different auxins, our study managed to assay the possibility of using GA3 comparing with IBA or their combination at different levels.

MATERIALS AND METHODS

Terminal cuttings taken from 1-2-year-old twigs were collected from a single mature *Taxus baccata* tree at the Central Park, Plovdiv during the end of January to the beginning of February for three successive seasons of 2008, 2009 and 2010. Uniform cuttings (7-10 cm long) were prepared and needles were striped of the basal 4 cm portion. Subsequently, cuttings were dipped at the basal end (2-3 cm basal portion) for (15-20 min) in different concentrations (4000, 6000, 8000 and 10000 ppm) of IBA and/or GA3. Cuttings dipped in distilled water were served as a control treatment. Cuttings were immediately struck at 3-4 cm depth into frames containing a 1:1 peat: perlite (1:1) mixture. Treatments were arranged in a randomized complete block design with four replicates. Each experimental plot comprised 20 cuttings. The cultures were kept under greenhouse conditions, and the relative humidity being maintained at about 70% using a fogging system (misting). Previcur® Fungicide (2%) was applied weekly. Cuttings were periodically checked and data were recorded on the beginning of callus and root initiation. Six months after cuttings were struck, rooting percentage, number of roots, mean root length, root fresh and dry matter, root volume and number of lateral shoots per cutting were taken. The same procedures were followed as above for the three seasons. The results were subjected to analysis of variance (ANOVA) and differences among means were tested by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

It could be deduced from the data shown in Table 1. that, using IBA and/or GA3 at any level significantly enhanced rooting of *Taxus* cuttings comparing to untreated ones (control). Generally, using IBA alone resulted in significantly higher rooting percentage than GA3 or their combinations. This effect was more obvious as the treatment concentration was increased, where the highest concentration (10000 ppm) of IBA induced the highest rooting percentage (56.92). This percentage is more than two-fold the rooting percentage achieved using GA3 at the same level, and about 4-fold that of untreated cuttings (control). Hence, our results strongly emphasize that fact, previously mentioned by (Mitchell, 1997), that rooting hormone treatment is important or even may be essential for successfully rooting of *T. baccata* cutting. In spite of such a low rooting percentage in untreated cuttings (control) (14.92%), it is considered quite height comparing with that obtained by Metaxas *et al.* (2004) (zero %).

Table 1. Effect of IBA and/or GA3 application at different concentrations (4000, 6000, 8000 and 10000 ppm) on rooting %, and callus and root initiation time of *T. baccata* cuttings.

Treatment ppm	Rooting %	Callus initiation day	Root initiation day
Control	14.92 [f]	29.250 [a]	42.917 [ns]
IBA			
4000	23.92 [e]	24.917 [cd]	42.833
6000	32.50 [cd]	22.917 [f]	37.750
8000	46.00 [b]	20.500 [g]	38.667
10000	56.92 [a]	18.083 [h]	35.750
GA3			
4000	24.75 [e]	27.917 [b]	43.250
6000	28.75 [de]	27.417 [b]	42.250
8000	28.76 [de]	27.667 [b]	42.833
10000	26.25 [e]	27.583 [b]	43.250
IBA+GA3			
4000	26.42 [e]	25.750 [c]	42.500
6000	33.67 [cd]	24.167 [de]	40.417
8000	35.42 [c]	24.083 [de]	38.580
10000	44.75 [b]	23.667 [ef]	36.417

Mean separation by DMRT , $P \leq 0.05$

ns≡ not significant at 5% level of significance.

A wide range of rooting percentage have been reported in *T. baccata* cuttings using either 8000 or 1000 ppm IBA by many other investigators; 29-72.9% (Verma *et al.,* 2007),60-80% (Nelson, 1959; Bell, 1975; Singh 2006) up to 100% (Eccher, 1989; Sabo, 1976; Fordham, 1966). Such Variations could be attributed to two factors. Firstly, variations in ortets (parents) age, as increasing ortets age is known to reduce rooting success in *Taxus* (Goo *et al.*, 1990). Secondly, even using the same rooting hormone with the same type of cutting taken from donor plants in the same age could surprisingly result in contradicting outcomes due to the high level of inter- and intraspecific variations in *Taxus* populations (Von Kornya, 1976; Mitchell, 1997), even among individuals (Schneck, 1996).

When comparing callus and root initiation period, it is evident that although there were considerable differences among treatments in terms of callus initiation, root initiation weren't hastened by early callus initiation in IBA-treated cuttings (Fig 1). Therefore, it's quite evident that IBA application seem to increase rooting percentage though it doesn't hasten the process, which has been previously revealed by Eccher (1988). Even though, treatments characterized by early callus initiation attained higher rooting percentage and vice versa, it is not clear whether there is any reliable relationship between both of them. Gibberellin (GA3), on the other hand, has been found to be inferior to IBA treatment (Khali and Sharma, 2003; Singh, 2006).

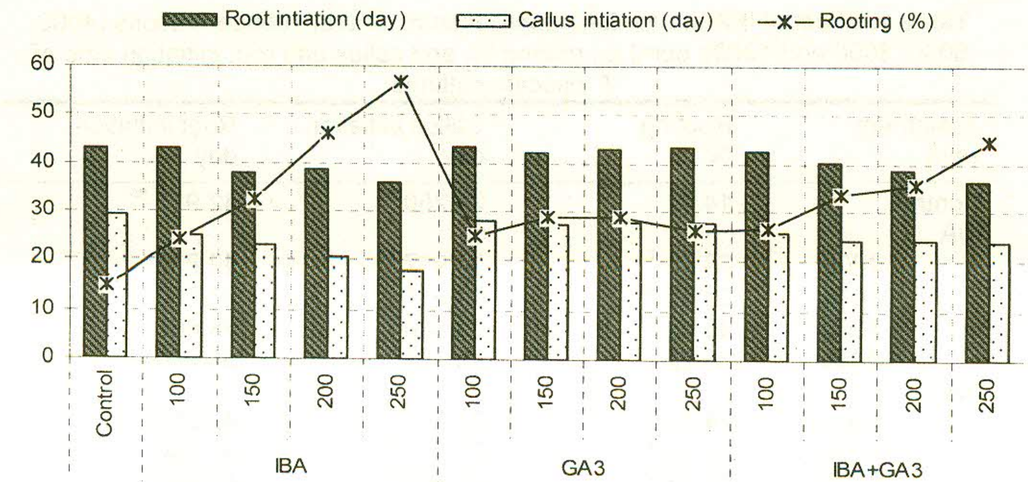

Figure 1. Effect of IBA and/or GA3 application at different concentrations (4000, 6000, 8000 and 10000 ppm) on rooting % (line graph), and callus and root initiation time (column graph) of *T. baccata* cuttings.

Table 2. Effect of IBA and/or GA3 application at different concentrations (4000, 6000, 8000 and 10000 ppm) on rooting %, and callus and root initiation time of *T. baccata* cuttings. (FM≡ fresh matter, DM≡ dry matter, LG≡ length)

Treatment ppm	No. roots	Mean root LG cm	Root FM g	Root DM* %	Root volume cm^3	No. lateral shoots
Control	1.575 ^{f}	3.500 ^{f}	1.133 ^{f}	51.343 ^{e}	0.675 ^{f}	1.710 ^{ns}
IBA						
4000	1.975 ^{e}	3.800 ^{f}	1.450 ^{e}	53.320 ^{e}	0.992 ^{e}	1.196
6000	2.183 ^{de}	4.342 ^{e}	1.708 ^{d}	58.560 ^{d}	1.333 ^{cd}	1.227
8000	2.677 ^{c}	5.450 ^{c}	2.267 ^{b}	65.853 ^{ab}	1.608 ^{b}	1.260
10000	3.242 ^{a}	6.633 ^{a}	2.650 ^{a}	67.003 ^{ab}	1.858 ^{a}	1.320
GA3						
4000	2.100 ^{de}	5.908 ^{b}	1.658 ^{d}	59.170 ^{d}	1.183 ^{de}	1.277
6000	2.067 ^{de}	5.850 ^{b}	1.775 ^{d}	59.027 ^{d}	1.108 ^{de}	1.343
8000	2.075 ^{de}	5.917 ^{b}	1.792 ^{d}	60.500 ^{cd}	1.258 ^{d}	1.327
10000	2.050 ^{e}	5.983 ^{b}	1.792 ^{d}	60.270 ^{bc}	1.217 ^{de}	1.337
IBA+GA3						
4000	2.292 ^{d}	3.808 ^{f}	1.450 ^{e}	53.367 ^{e}	1.000 ^{e}	1.360
6000	2.558 ^{c}	4.150 ^{e}	1.650 ^{d}	58.427 ^{d}	1.233 ^{de}	1.327
8000	2.733 ^{c}	4.892 ^{d}	2.075 ^{c}	64.370 ^{bc}	1.558 ^{bc}	1.317
10000	2.942 ^{b}	5.700 ^{bc}	2.308 ^{b}	68.787 ^{a}	1.750 ^{ab}	1.353

* Dry matter (DM) was measured as percentage of fresh matter (FM)
Mean separation by DMRT , $P \leq 0.05$
ns≡ not significant at 5% level of significance

The promotive effect of the highest level used of IBA (10000 ppm) extended to include inducing the best root system (root length, volume, fresh and dry weight) (Table 2.), followed by the combination of IBA+GA3 at the highest level. Meanwhile, GA3 at any level resulted in significantly longer roots comparing with the other treatments excluding IBA at 10000 ppm.

Despite the high differences noticed in root characters, number of shoots insignificantly varied among treatments. However, we suspect that cuttings with better root system are more likely to survive and to gain better vegetative growth in the future. In this concern, Singh (2006) assured that a higher root to shoot is advisable for achieving a higher survival rate after field transplantation.

CONCLUSIONS

Amongst all the combinations between IBA and GA3 investigated in the present experiment, IBA at 10000 proved to be the most effective treatment inducing the highest rooting percentage and attaining the best rooting characteristic in cuttings of *T. baccata*. Our results further revealed that although GA3 alone or in combination with IBA resulted in relatively lower values, it has obtained promising results.

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