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REGULAR ARTICLE

EFFECT OF PHYSIOLOGICAL AGE OF STEM AND IBA TREATMENT ON ROOTING OF BRANCH CUTTINGS OF *TAXUS BACCATA* L.

SAUMITRO DAS[1*], L. K. JHA[2]

[1]Karimganj Forest Division (Territorial), Karimganj 788712, Assam, India

[2]Department of Environmental Studies, North-Eastern Hill University, Shillong 793022, Meghalaya, India

ABSTRACT

The natural population of *Taxus baccata* L. (Himalayan Yew) throughout the Indian Himalayan Region is greatly reduced due to its extensive and reckless exploitation for "Taxol" an anticancer drug. The effects of overexploitation are exacerbated by the species poor regeneration process, slow growth rate and prolonged seed dormancy. Therefore vegetative propagation by branch cuttings seems to be only practical solution for its large scale multiplication. A study was conducted on six candidate trees (CTs) to examine the effect genotype, physiological age of stem, IBA treatment on rooting of *Taxus baccata* cuttings. Results revealed that rooting behaviour of cuttings was significantly affected by all the factors under study. Among the six CTs studied, CT 2 (from BSI, Shillong) had given the highest rooting response (46.28%). The juvenile cuttings have the higher rooting capacity; however the callusing was more prominent in mature cutting. The influence of IBA treatment was also significant for rooting where 1000 was most effective for stimulating rooting juvenile cuttings and 2000 ppm in mature cuttings.

Keywords: Branch cuttings, Indole-3-butyric acid, Taxol, *Taxus baccata*, Vegetative propagation.

INTRODUCTION

Taxus baccata L. (Himalayan Yew) is an important high altitude medicinal plant species that found in subtropical and temperate region of Himalaya at an altitude between 1800 and 3300 m amsl [1]. It is a long lived conifer species belonging to family Taxaceae and has immense ethnobotanical importance in Indian Himalayan Region [2]. Traditionally, the extract derived from the bark and leaves is used to cure various ailments like bronchitis, asthma, poisonous insect bites and also acts as an aphrodiasic [3]. However the species is globally known for 'Taxol' a mitotic spindle inhibitor extracted from its leaves and barks. This compound is used in the treatment of patients with lung, breast and ovarian cancer [4]. The extraction of taxol is economically unfeasible and biological unsustainable as only 0.01 to 0.03% of the dry phloem weight is taxol, against the requirement of a minimum of 2 gms of purified taxol for full regimen of anti-cancer treatment [5]. Despite soaring prices, the demand for this drug is growing at an annual rate of 20%. The global demand of taxol is 800-1000 kg annual [6]. In India, its annual demand was estimated at 500 tonnes [7].

Due to the huge demand of taxol, *Taxus baccata* is being exploited by the collectors, traders and industrialists recklessly beyond its regeneration capacity rendering it to the status of endangered species. The species which was earlier found abundant in the Himalayan region are now restricted to few places that have suitable microclimate. Meghalaya, India was once home to very good population of *Taxus baccata*, but due to rapid destruction of its natural habitat, the species has now become rare and found only in sacred groves and nursery of government owned forest departments.

Due to growing concern on sustainable use and survival of *Taxus* sp., domestication programmes have been initiated in many places in India where *Taxus* grows naturally. The challenge, however, is that it is very difficult to regenerate naturally through seeds as it requires complex treatment like *in vitro* culture and precocious germination [8, 9]. So, major focus has been paid on vegetative propagation by branch cuttings to augment its natural regeneration *Taxus* species is reported as plant with relative potential of regeneration by adventitious rooting of cuttings [10]. Unlike other *Taxus* species, *Taxus baccata* is difficult to root and requires longer time [11]. The workers from states in Central Himalayan Region viz. Jammu and Kashmir, Himachal Pradesh and Uttarakhand have developed and standardised the cutting propagation protocol for their respective region [12, 13, 14 and 15] The protocols that have been developed in Central Himalayan regions are area specific; and so are suitable there only. The vegetative propagation technique of *Taxus baccata* for subtropical region of Meghalaya particularly yet to be standardised.

Propagation by cuttings is the most commonly used

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*Corresponding Author

Saumitro Das

Karimganj Forest Division (Territorial), Karimganj 788712, Assam, India

Email: suman.envt@gmail.com

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method to propagate numerous woody species as it give faster growth rate, greater stock stand uniformity, better site matching and true-to type planting material. Successful propagation of trees by rooting of cuttings depends on many factors such as physiological age of cutting, the propagation environment and the concentration of applied auxin [16, 17 and 18]. Among auxin, generally Indole-3-butyric acid are reported to have better rooting because of its long retention time at the site of the application and is effective in promoting rooting of a large number of tree species [19]. However, the effectiveness of auxin is determined by sensitivity of tissue of cuttings. This sensitivity of tissue is somewhat associated with genetic characteristics of stock plants [20]. So it is necessary to select out best individual tree having better rootabilty prior to propagation program.

In view of this, a rooting trial was conducted to evaluate the rooting potential of two types of cuttings obtained from different candidate trees of *Taxus baccata* L. with application of IBA.

MATERIALS AND METHODS

Experimental site

The rooting trial was conducted in experimental garden of Department of Environmental Studies, North-Eastern Hill University, located at Shillong in Meghalaya, India (91 °53' E; 25 °36' N and 1426 m m amsl). Six candidate trees (CTs) of age group of 10-15 y growing at six different location in East Khasi Hills were identified and tagged as CT 1, CT 2, CT 3, CT 4, CT 5 and CT 6. (The geographic coordinates of location of CTs are given in table 1) The experiment was conducted in a completely randomized block design and a total of 720 cuttings were used (n = 720; 3 replications x 5 cuttings x 6 CTs x 2 types of cuttings x 4 IBA concentration including control). The study period was between April to October 2012.

Collection and preparation of cuttings

The collection of branches was made during last week of April. The branches were procured from each CT and brought to the departmental laboratory in moist polythene bag to avoid desiccation. The branches so collected were made into cuttings of 15-20 cm in length and 0.3 to 1 cm in diameter. The needles at the basal portion of 2 cm were stripped off. Finally cuttings were categorised into two groups according to the physiological nature of stem. The cutting with diameter is between 0.3-0.5 cm and green or light brown colour was classified as 'Juvenile cutting'. 'Mature cutting' was taken as cutting with more than 0.5 cm but less than 1.0 cm and dark brown or grey in colour.

IBA treatment and planting

Four IBA concentrations was tested *i.e.,* control (0 ppm), 1000 ppm, 2000 ppm and 5000 ppm. For control treatment, only distilled water was used. The cuttings were soaked at basal 2 cm with prepared IBA solutions for few seconds and allowed to dry for 15 min. The treated cuttings were planted in perforated polypot (18 cm deep and 6.5 cm diameter) filled with rooting medium containing equal proportions of soil, sand and FYM. Finally the planted polypots were placed in polyhouse and water regularly according to weather condition and moisture status of rooting medium.

Observation recording and data analysis

The cuttings were assessed for following parameters after six months of planting: number of cuttings rooted (rooting percentage), a number of cutting callused (Callusing percentage), mean number of roots formed per cutting and mean root length. Analysis of variance (ANOVA) was carried out for all the parameters and Least Significant Difference (LSD) test at 5% probability was used to compare significantly different means using GLM procedure in the SPSS (Statistical Package for Social Sciences version 16). To ensure normality and variance homogeneity, the survival and rooting percentage data were converted into arc sine sqrt ((x+0.5)/100), and data of root number was transformed into square root (x+0.5) [21].

RESULTS

The data on all the rooting parameters evaluated are furnished in table 2 to table 5.

Perusal of the data in table 2 and 3, rooting and callusing of *Taxus baccata* was significantly influenced by CT and IBA concentration. Among six CTs, highest rooting (46.28%) and callusing percentage (62.50%) was observed in the cuttings made from CT 2. Owing to highest rooting percentage, CT 2 exhibited maximum number of roots per cutting (3.33 no.) and longest root (2.43 cm) (fig. 1(c) and 1(d)). Cuttings from CT 1 and CT 3 showed moderate rooting response where their rooting percentage was recorded as 22.23 and 26.06 % respectively. The poor rooting response was observed in cuttings from CT 4, CT 5 and CT 6 where their mean value of rooting and callusing percentage was less than 10.84%. Data analysis (table 2) reveals that physiological age of cuttings on rooting percentage was statistically insignificant. However unlike rooting, rate of callusing of cutting was significantly affected by age of cutting where mature cutting exhibited more callusing than juvenile ones (30.56% vs 25.28%) (table 3). Different IBA concentration also resulted in differential rate of rooting (table 2 and fig. 1(e) and 1 (f)). The highest rooting for IBA treatment was achieved with concentration of 2000 ppm and 1000 ppm with overall mean percentage was 18.89% and 18.15% respectively.

The rooting behaviour of juvenile and mature cutting varied among CTs as interactive effect of CT and physiological age of cutting found to be significant for rooting percentage, root number and root length (table 2, 4 and 5). The juvenile cuttings from CT 2 exhibited overall highest rooting success (51.67%) followed by the mature cuttings from CT 3 (43.33%) and CT 2 (38.33%). The root proliferation was prominent in CT 3 (fig. 1(b) and table 4 and 5), where mature cuttings exhibited maximum value for mean root number (5.26 no. s) and mean root length (2.89 cm). The study also showed that juvenile and mature cuttings responded differently to different concentrations of IBA. Juvenile cutting showed maximum rooting at concentration of 1000 ppm where mean rooting success was recorded as 25.56 % whereas rooting of mature cutting was highest in 2000 ppm with 22.22 % mean rooting.

Rooting and callusing of two types of cutting varied with candidate tree and IBA treatment. The best rooting performance were seen in CT 2 and CT 3 as responded better to IBA treatment than rest the CTs. Juvenile cuttings from CT 2 treated with 1000 IBA had induced overall highest rooting success (73.33%). From same tree mature cuttings yielded maximum rooting percentage of 53.33% at 2000 ppm IBA. The mature cuttings showed best result in CT 3 where IBA treatment at 1000 and 2000 ppm resulted in 53.33% rooting success. The rate of callusing was highest in CT 2 (62.50%), where 1000 ppm IBA treated juvenile cuttings scored highest callusing percentage (80.00%) followed by 2000 ppm IBA treated mature cuttings (73.33%). Mature cuttings from CT 3 (fig. 1(b)) also exhibited better callusing rate (73.33%) at higher concentration of IBA *i.e.*2000 and 5000 ppm.

DISCUSSION

CT 2 (Botanical Survey of India, Shillong) represents the best genotype for cutting propagation of *Taxus baccata*. For rooting of mature cuttings, CT 3 is best candidate tree. Variation in rooting ability of among individual of *Taxus baccata* as revealed from the present investigation may be controlled by genetic makeup of the donor plant or may be caused by plant environmental factors. It is well known that different species varies in rooting ability, but differences in rooting potential of plant between provenances and individual trees (clones) within species were also reported in many species such as *Triplochiton scleroxylon* [22], *Calliandra calothyrus* [23], *Dalbergia sisoo* [24] and *Tectona grandis* [25]. According to Ahuja and Libby, genotypes-environmental interaction causes specific changes in the biology and chemistry of the clone, that affects the rooting of clone [26]. In addition to this, microclimate where the clones grow influences the accumulation of carbohydrates within stem tissue also varies from year to year which cause variation in rooting [27 and 28].

Rooting and callusing of *Taxus baccata* branch cutting was also affected by physiological age of stem. Though, mature cuttings showed low root production than juvenile cuttings, it exhibited more callusing than latter. Similar result was also reported in *Dalbergia melanoxylon* by Amri *et al.* (2010) where juvenile cuttings recorded higher rooting percentage and mature cuttings showed higher rate of callus formation [29]. Poor rooting performance of physiological old cuttings can be attributed to accumulation of inhibitory substances, decreased rate of photosynthesis, low endogenous auxin content and altered carbohydrate metabolism [30 and 31]. In present, experiment callus formation somewhat caused delay in root initiation resulting in low rooting percentage in mature cuttings. Das and Jha (2014) found that hardwood cuttings of *Taxus wallichiana* took considerably longer time than softwood to root [32].

Application of IBA improved rooting performance in *Taxus baccta* cuttings. But the relative response towards a particular growth regulator varied with place, clone, and age of cuttings and donor plant [30]. The interaction between IBA and CT in the present experiment indicates that not all CT responded in the same way to the IBA treatment. Similar results were reported in *Dianthus caryophyllus* by Purnachandra *et al.* (2017) where genotype of plants resulted in differential IBA effect on rooting [33].

Increasing IBA concentration resulted in increased rooting in cuttings but within a certain range as it was observed in CT 1 and CT 2 where the maximum rooting was recorded in 1000-2000 ppm IBA. In addition, interaction between IBA and type of stem also suggests that consideration of the tissue sensitivity in relation to plant growth regulators. Juvenile cuttings being younger than mature cutting require optimally low concentration of IBA (1000 ppm) for root initiation whereas rooting performance of mature cuttings can be improved by optimally higher concentration of IBA as seen in CT 3 where greater rooting percentage was obtained with 2000 and 5000 ppm.

CONCLUSION

The present study reveals that rooting capacity of *Taxus baccata* branch cutting was strongly influenced by genotypes. So it is necessary to select appropriate individual (CT) with good rooting potential propagation programme. Candidate Tree 2 can be served as donor plant for cutting in that part of the state. In addition, physiological age of stem and IBA concentration in relation to particular individual should be also taken into account. The lower concentration of IBA at 1000 ppm for juvenile cutting and higher concentration at 2000 ppm for mature cutting could be best combination for vegetative propagation of *Taxus baccata*. It is hoped that findings of present study will be helpful for developing propagation protocol of *Taxus baccata* L. especially for subtropical region of Meghalaya.

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Table 1: Geographical coordinates of region candidate trees were selected

Candidate tree (CT)	Place	Latitude	Longitude
CT 1	North Eastern Hill University (NEHU). Mawkynroh Umsing	25 °36'43.55"N	91 °53'51.77"E
CT 2	Botanical Survey of India, Shillong	25 °34'45.50"N	91 °53'55.37"E
CT 3	Forest Beat Office, 4½ miles, Upper Shillong	25 °32'49.57"N	91 °51'0.59"E
CT 4	Village Forest, Mawphlang	25 °27'11.77"N	91 °45'15.96"E
CT 5	Botanical garden, Umiam, Shillong	25 °40'41.23"N	91 °54'26.48"E
CT 6	Forest Beat Office, Raid Laban, Shillong	25 °33'30.24"N	91 °52'26.76"E

Table 2: Effect of physiological age of stem and IBA treatment on rooting of cuttings from six candidate trees of *Taxus baccata*

Physiol. Age	IBA conc. (ppm)	Candidate tree (CT) CT 1	CT 2	CT 3	CT 4	CT 5	CT 6	Mean
Juvenile	*Control*	20.00 (26.24)	46.67 (43.08)	6.67 (17.47)	6.67 (17.47)	6.67 (17.47)	0.00 (12.92)	14.44 (22.44)
	1000	40.00 (38.85)	73.33 (59.54)	26.67 (30.78)	0.00 (12.92)	0.00 (12.92)	13.33 (21.69)	25.56 (29.45)
	2000	26.67 (30.78)	53.33 (46.92)	6.67 (17.47)	0.00 (12.92)	0.00 (12.92)	6.67 (17.47)	15.56 (23.08)
	5000	6.67 (17.47)	33.33 (34.31)	26.67 (30.78)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	11.11 (20.22)
	Mean	23.33 (28.38)	51.67 (45.96)	16.67 (24.12)	1.67 (14.06)	1.67 (14.06)	5.00 (16.25)	16.67 (23.80)
Mature	*Control*	6.67 (17.47)	26.67 (30.78)	20.00 (26.56)	0.00 (12.92)	6.67 (17.47)	0.00 (12.92)	10.00 (19.69)
	1000	13.33 (22.01)	33.33 (35.01)	46.67 (43.08)	0.00 (12.92)	0.00 (12.92)	6.67 (17.47)	16.67 (23.90)
	2000	20.00 (26.56)	53.33 (46.92)	53.33 (46.92)	0.00 (12.92)	6.67 (17.47)	0.00 (12.92)	22.22 (27.29)
	5000	6.67 (17.47)	40.00 (38.85)	53.33 (46.92)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	16.67 (23.67)
	Mean	11.67 (20.88)	38.33 (37.89)	43.33 (40.87)	0.00 (12.92)	3.33 (15.20)	1.67 (14.06)	16.39 (23.64)
Mean of CT		22.23 (24.61)	46.28 (41.93)	26.06 (32.50)	5.76 (13.49)	6.62 (14.63)	8.26 (15.15)	
Mean of IBA conc.			*Control* 12.22 (21.7)	*1000* 21.12 (26.68)	*2000* 18.89 (25.19)	*5000* 13.89 (21.95)		
LSD @ 5%	CT = 4.71, Physiological age = NS, IBA conc. = 3.85, CT x Physiol. Age = 0.56, CT x IBA conc.= 0.79, Physiol. Age x IBA conc. = 0.45, CT x Physiol. Age x IBA conc.= NS.							

Fig. in parenthesis are mean of arc sine transformed values of replicate.

Table 3: Effect of physiological age of stem and IBA treatment on callus formation of cuttings from six candidate trees of *Taxus baccata*

Physiol. Age	IBA conc. (ppm)	Candidate tree (CT) CT 1	CT 2	CT 3	CT 4	CT 5	CT 6	Mean
Juvenile	*Control*	33.33 (35.01)	60.00 (51.15)	6.67 (17.47)	6.67 (17.47)	6.67 (17.47)	6.67 (17.47)	20.00 (26.01)
	1000	46.67 (43.08)	80.00 (63.76)	26.67 (30.78)	6.67 (17.47)	6.67 (17.47)	20.00 (26.24)	31.11 (33.13)
	2000	46.67 (43.08)	66.67 (54.99)	46.67 (43.08)	0.00 (12.92)	6.67 (17.47)	13.33 (22.01)	30.00 (32.26)
	5000	13.33 (21.69)	53.33 (46.92)	46.67 (43.08)	0.00 (12.92)	6.67 (17.47)	0.00 (12.92)	20.00 (25.83)
	Mean	35.00 (35.72)	65.00 (54.21)	31.67 (33.60)	3.33 (15.20)	6.67 (17.47	10.00 (19.66)	25.28 (29.31)
Mature	*Control*	20.00 (26.24)	46.67 (43.08)	26.67 (30.78)	26.67 (30.46)	33.33 (35.01)	20.00 (26.56)	28.89 (32.02)
	1000	26.67 (30.78)	53.33 (46.92)	46.67 (43.08)	20.00 (26.56)	0.00 (12.92)	20.00 (26.24)	27.78 (31.08)
	2000	53.33 (46.92)	73.33 (59.22)	73.33 (59.54)	6.67 (17.47)	6.67 (17.47)	6.67 (17.47)	36.67 (36.35)
	5000	33.33 (34.63)	66.67 (55.69)	73.33 (59.92)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	28.89 (31.50)
	Mean	33.33 (34.64)	60.00 (51.23)	55.00 (48.33)	13.33 (21.85)	10.00 (19.58)	11.67 (20.80)	30.56 (32.74)
Mean of CT		34.17 (35.18)	62.50 (52.72)	43.34 (40.97)	8.33 (18.52)	8.34 (18.53)	10.84 (20.23)	
Mean of IBA conc.			*Control* 24.45 (29.01)	*1000* 29.45 (32.11)	*2000* 33.34 (34.30)	*5000* 24.45 (28.67)		
LSD @ 5%	CT = 6.02, Physiological age = 3.47, IBA conc. = NS CT x Physiol. Age = NS, CT x IBA conc. = 1.00, Physiol. Age x IBA conc. = NS, CT x Physiol. Age x IBA conc.= NS.							

fig. in parenthesis are mean of arc sine transformed values of replicate.

Table 4: Effect of physiological age of stem and IBA treatment on mean root number produced by cuttings from six candidate trees of *Taxus baccata*

Physiol. Age	IBA conc. (ppm)	Candidate tree (CT)						Mean
		CT 1	CT 2	CT 3	CT 4	CT 5	CT 6	
Juvenile	*Control*	2.00 (1.48)	3.61 (2.03)	0.67 (1.00)	0.33 (0.88)	0.67 (1.00)	0.00 (0.71)	1.21 (1.13)
	1000	4.56 (2.24)	4.13 (2.15)	2.33 (1.66)	0.00 (0.71)	0.00 (0.71)	1.17 (1.14)	2.03 (1.44)
	2000	3.33 (1.95)	4.17 (2.16)	1.33 (1.18)	0.00 (0.71)	0.00 (0.71)	0.67 (1.00)	1.58 (1.52)
	5000	2.00 (1.32)	2.89 (1.70)	3.17 (1.90)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	1.34 (1.29)
	Mean	2.97 (2.26)	3.70 (2.01)	1.88 (1.44))	0.08 (0.75)	0.17 (0.70))	0.46 (0.89	1.54 (1.34)
Mature	*Control*	0.67 (1.00)	4.33 (2.19)	3.00 (1.86)	0.00 (0.71)	1.00 (1.10)	0.00 (0.71)	1.50 (0.92)
	1000	2.33 (1.57)	4.00 (2.12)	4.94 (2.33)	0.00 (0.71)	0.00 (0.71)	0.67 (1.00)	1.99 (1.05)
	2000	4.33 (2.20)	5.72 (2.49)	5.06 (2.35)	0.00 (0.71)	0.67 (1.00)	0.00 (0.71)	2.63 (1.11)
	5000	1.67 (1.25)	5.42 (2.42)	8.03 (2.88)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	2.52 (1.00)
	Mean	2.16 (1.51)	3.53 (2.31)	3.16 (2.36)	0.00 (0.71)	0.52 (0.8)	0.50 (0.70)	1.70 (1.02)
Mean of CT		2.57 (1.88)	3.62 (2.16)	2.52 (1.90)	0.04 (0.73)	0.35 (0.75)	0.17 (0.48)	
Mean of IBA conc.			*Control* 1.36	*1000* 1.79	*2000* 2.11	*5000* 1.93		
LSD @ 5%	CT = 0.75, Physiological age = 0.43, IBA conc. = NS CT x Physiol. Age = 0.09, CT x IBA conc. = NS, Physiol. Age x IBA conc. = NS, CT x Physiol. Age x IBA conc.= NS.							

fig. in parenthesis are mean of square root transformed values of replicate.

Table 5: Effect of physiological age of stem and IBA treatment on mean root length (cm) of cuttings from six candidate trees of *Taxus baccata*

Physiol. Age	IBA conc. (ppm)	Candidate tree (CT)						Mean
		CT 1	CT 2	CT 3	CT 4	CT 5	CT 6	
Juvenile	*Control*	0.85	2.27	0.47	0.17	0.23	0.00	0.66
	1000	2.76	2.87	1.32	0.00	0.00	0.64	1.27
	2000	2.64	2.25	0.67	0.00	0.00	0.68	1.04
	5000	1.00	1.79	2.05	0.00	0.00	0.00	0.81
	Mean	1.81	2.30	1.13	0.04	0.06	0.33	0.94
Mature	*Control*	0.77	1.53	2.30	0.00	0.48	0.00	0.85
	1000	1.27	2.63	3.53	0.00	0.00	0.67	1.35
	2000	2.00	3.02	3.06	0.00	0.62	0.00	1.45
	5000	0.43	3.04	2.66	0.00	0.00	0.00	1.02
	Mean	1.12	2.56	2.89	0.00	0.28	0.17	1.17
Mean of CT		1.47	2.43	2.01	0.02	0.17	0.25	
Mean of IBA conc.			*Control* 2.01	*1000* 1.54	*2000* 1.51	*5000* 1.38		
LSD @ 5%	CT = 0.39, Physiological age = NS, IBA conc. = 0.32 CT x Physiol. Age = 0.05, CT x IBA conc. = 0.07, Physiol. Age x IBA conc. = 0.04, CT x Physiol. Age x IBA conc.= NS.							

Fig. 1: Propagation of *Taxus baccata* L. by branch cutting (a) A candidate tree of *Taxus baccata*; (b) Callused formation in mature cutting from CT 3; (c) Vigorous rooting of juvenile cuttings obtained from CT 2; (d) Vigorous rooting of mature cuttings obtained from CT 2. (e) Rooting of IBA treated juvenile cuttings of *Taxus baccata*; (f) Rooting of IBA treated mature cuttings of *Taxus baccata*

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