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RESEARCH ARTICLE

CLONAL VARIATION IN ADVENTITIOUS ROOTING OF *TAXUS BACCATA* L. STEM CUTTINGS

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ABSTRACT

A study was conducted to examine the clonal variation, effect of cutting type and IBA treatment on adventitious rooting of *Taxus baccata*. Six clones were selected from different provenances in East Khasi Hills districts of Meghalaya, India. From each clones two types of cuttings (lignified and non lignified) were made and given four IBA treatments (0, 1000, 2000 and 5000 ppm). Results revealed that clonal variation was significant for all the cutting parameters. Among the six clones studied; C2 (Botanical Survey of India, Shillong, Meghalaya, India) have given the highest rooting response (45%).The influence of IBA treatment was significant only for rooting percentage and root length, where 1000 and 2000 ppm was most effective in stimulating the rooting. However effect of type of stem was insignificant for all the parameters except root number where lignified (2.16) cuttings exhibited higher value than the non lignified ones (1.54).

INTRODUCTION

Taxus baccata L. (Himalayan Yew) of the family Taxaceae is a long lived evergreen and dioeciously conifer, about 6-12 meters height with horizontally branched and dense canopy bearing spirally arranged leaves. It is naturally found in temperate and subalpine environment of Himalaya from Pakistan to Southwest China at altitudes between 1800 to 3300 m amsl (**Lanker *et al*., 2010**). In India, *Taxus* is distributed all along the Himalayas from Ladakh eastward to Khasi and Jaintia Hills, Naga Hills and Manipur between 2,300 and 3,400 m amsl. (**Bhatnagar and Moitra, 1996**). Unlike other common coniferous species, population of *Taxus baccata* is not continuous and usually occurred in patches. Its habitat are mainly characterized by moist, mixed coniferous tree forests or cool broad-leaved forests. Due to shade demanding nature, *Taxus baccata* is usually found in association with large tree species such as *Betula utilise*, *Abies pindrow*, *Acer cesium*, *Pinus wallichiana*, *Quercus semecarpifolia and Rhododendron arboreum* (**Rikhari *et al*., 1998**). *Taxus baccata* is considered as a multipurpose useful tree for its timber, ornamental and various ethno-botanical values. But the species is globally known for the medicinal properties of 'taxol' an anticancer drug obtained from its leaves and bark (**Wani *et al*., 1971 and Busing, 1995**). Unsustainable harvesting, grazing and wide scale deforestation in Indian Himalayan Region (IHR) has posed a serious threat to prosperity of this valuable Himalayan tree (**Pant and Samant, 2008**). 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. Moreover, species poor natural regeneration process, its slow growth rate and long seed dormancy period of 1.5-2 years (**Stenfield, 1992**) contributes significantly to its conservation crisis. Clonal (vegetative) propagation could, therefore be one of the practical options to augmenting its natural regeneration. *Taxus* species is reported as plant with relative potential of regeneration by adventitious rooting of cuttings (**Schneck, 1996**). Unlike other *Taxus* species, *Taxus baccata* is difficult to root and requires longer time (**Fordham and Spraker, 1977**). Rooting of *Taxus baccata* using stem cutting is well documented (**Mitter and Sharma, 1993; Nandi *et al*., 1996; Khali and Sharma, 2001**), but majority of works reported so far were from Central Himalayan region. In addition, vegetative propagation technique of *Taxus baccata* L. for subtropical climate of Eastern Himalayan region is yet to be standardized. Hence, the present experiment was conducted to evaluate the rooting potential of two types of cuttings obtained from different clones of *Taxus baccata* with application of IBA.

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MATERIALS AND METHODS

Experimental site

The experiment was carried out in the experimental garden of Department of Environmental Studies, North-Eastern Hill

University, located at Shillong in Meghalaya, India (91°53' E; 25°36' N and 1413 m amsl). The experimental site falls in subtropical zone having average annual rainfall 2359 mm. The study period was between June to October, 2012.

Collection and preparation of cuttings

Six different provenances of *Taxus baccata* in East Khasi Hills district of Meghalaya (Table 1) were selected. The branches were collected from a phenotypically superior tree growing in each provenance and classified them as clone C1, C2, C3, C4, C5 and C6 (Table 1). The cuttings were then brought to the Department laboratory in moist polythene bag and used within 12 hours after collection. The final cuttings of 15 to 20 cm in length and 0.5 to 1 cm diameter were made from given branches. A maximum of 3-4 nodes were retained in each cutting and the needles at the basal 2 cm of the stem were removed. Finally the cuttings were categorized into two groups according to the type of stem. The cuttings with diameter less than 0.5 cm and green or light brown color were classified as 'non-lignified'. 'Lignified cuttings' were taken as stems with diameter more than 0.5 cm and dark brown or grey in color.

Preparation of IBA solution and planting

A stock solution of 10000 ppm of indole-3-butyric acid (IBA) was prepared by dissolving 10 g of hormone in 1000 ml of distilled water. Due to insoluble in water, the hormone was dissolved first in very small amount of 1N NaOH and later the volume was made up to 1000 ml by adding distilled water. From the stock solution, 1000, 2000 and 5000 ppm IBA solution were prepared by dilution in distilled water. For control treatment, only distilled water i.e., 0 ppm IBA concentration was used. Quick dip method of IBA application (**Hartmann *et al*., 2009**) was used throughout the experiment. The cuttings were soaked at basal 2 cm with prepared IBA solutions for few seconds and allowed to dry for 15 minutes. After that, every cutting was given fungicide treatment by dipping their basal portion in 0.05% carbendezium solution. Following chemical treatment, cuttings were planted in slightly slant angle at a depth of 4-6 cm perforated poly-pot (18 cm deep and 6.5 cm diameter) containing sterilized rooting medium (equal proportions of soil, sand and FYM). Finally all potted cuttings were brought under poly-house and allotted according to the experimental design. The cuttings were watered regularly as when depending on weather condition and moisture status of rooting medium.

Experimental design

The experiment was laid out in a completely randomized block design with a factorial arrangement of treatments. Every treatment was replicated three times and each replicate had 5 cuttings. A total of 720 cuttings were used throughout the experiment (n = 720; 3 replications x 5 cuttings x 6 clones x 2 types of cuttings x 4 IBA treatments).

Observation recording and data analysis

The cuttings were assessed for following parameters after six months of planting: number of cuttings survived (survival percentage), number of cuttings rooted (rooting percentage), and mean number of roots formed per cutting and mean root Length per treatment). 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 sort ((x + 0.5)/100), and data of root number was transformed into square root (x + 0.5) (**Hoshmand, 1994**).

Table 1. Geographical location of clones

Name of Clone	Place	Latitude	Longitude	Altitude (m amsl)
C1	North Eastern Hill University (NEHU). Mawkynroh Umsing	25°36'44.56"N	91°53'51.86"E	1413.9
C2	Botanical Survey of India, Shillong	25°34'44.93"N	91°53'54.10"E	1445.4
C3	Forest beat House, 4½ miles, Upper Shillong	25°32'49.77"N	91°51'00.69"E	1760.5
C4	Village Forest, Mawphlang	25°27'11.79"N	91°45'16.12"E	1828.2
C5	Botanical garden, Umiam, Shillong	25°40'37.48"N	91°54'11.56"E	987.9
C6	Forest beat house, Raid Laban, Shillong	25°33'27.17"N	91°52'17.04"E	1566.7

Table 2. Analysis of variance for the effect of clone, type of cuttings and treatment of IBA on rooting parameters of *Taxus baccata*

Source of Variation	Df	Mean Sum of Square			
		Survival percentage	Rooting percentage	Mean number of roots per cuttings	Mean length of root per cuttings
Clone	5	0.00	0.00	0.00	0.00
Cutting type	1	0.51	0.91	0.03	0.05
IBA	3	0.07	0.01	0.11	0.00
Clone x Cutting type	5	0.00	0.00	0.00	0.00
Clone x IBA	15	0.03	0.04	0.00	0.02
Cutting type x IBA	3	0.07	0.03	0.29	0.78
Clone x Cutting type x IBA	15	0.66	0.64	0.98	0.26

*Values in bold are significant at $p < 0.05$ level

RESULTS

Survivability

The data analysis revealed that there was significant ($p < 0.05$) clonal variation for survivability of cuttings (Table 2 and 3). The cuttings obtained from clone C2 (BSI, Shillong) exhibited

Table 3. Mean values of survival percentage of *Taxus baccata* stem cuttings in response to clone, cutting type and various concentration of IBA

Clone	Cutting type	0 ppm	1000 ppm	2000 ppm	5000 ppm	Mean of clone x cutting type
C1	NL	33.33 (35.01)	46.67 (43.08)	46.67 (43.08)	13.33 (21.69)	35.00 (35.71)
	L	20.00 (26.24)	26.67 (30.78)	20.00 (26.56)	6.67 (17.47)	18.33 (25.26)
Mean of clone x IBA		26.67 (30.62)	36.67 (36.93)	33.33 (34.82)	10.00 (19.58)	26.67▼ (30.49)
C2	NL	60.00 (51.15)	80.00 (63.76)	66.67 (54.99)	53.33 (46.92)	65.00 (54.21)
	L	46.67 (43.08)	53.33 (46.92)	73.33 (59.22)	66.67 (55.69)	60.00 (51.23)
Mean of clone x IBA		53.33 (47.11)	66.67 (55.34)	70.00 (57.11))	60.00 (51.31)	62.50▼ (52.72)
C3	NL	6.67 (17.47)	26.67 (30.78)	13.33 (22.01)	20.00 (26.24)	16.67 (24.13)
	L	26.67 (30.78)	46.67 (43.08)	73.33 (59.54)	73.33 (59.92)	55.00 (48.33)
Mean of clone x IBA		16.67 (24.13)	36.67 (36.93)	43.33 (40.78)	46.67 (43.08)	35.83▼ (36.23)
C4	NL	6.67 (17.47)	6.67 (17.47)	0.00 (12.92)	0.00 (12.92)	3.33 (15.19)
	L	13.33 (22.01)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	3.33 (15.19)
Mean of clone x IBA		10.00 (19.74)	3.33 15.19)	0.00 (12.92)	0.00 (12.92)	3.33▼ (15.19)
C5	NL	6.67 (17.47)	6.67 (17.47)	6.67 (17.47)	6.67 (17.47)	6.67 (17.47)
	L	13.33 (22.01)	0.00 (12.92)	6.67 (17.47)	0.00 (12.92)	5.00 (16.33)
Mean of clone x IBA		10.00 (19.74)	3.33 15.19)	6.67 (17.47)	3.33 (15.19)	5.83▼ (16.90)
C6	NL	6.67 (17.47)	20.00 (26.24)	13.33 (22.01)	0.00 (12.92)	10.00 (19.66)
	L	6.67 (17.47)	6.67 (17.47)	6.67 (17.47)	0.00 (12.92)	5.00 (16.33)
Mean of clone x IBA		6.67 (17.47)	13.33 (21.85)	10.00 (19.74)	0.00 (12.92)	7.50▼ (17.99)
Mean of cutting type x IBA	NL	20.00 (26.00)	31.11 (33.13)	24.44 (28.75)	15.56 (23.03)	22.78 (27.73)
	L	21.11 (26.93)	22.22 (27.35)	30.00 (32.20)	24.44 (28.64)	24.44 (28.78)
Mean of IBA		20.56 (26.47)	26.67 (30.24)	27.22 (30.47)	20.00 (25.83)	
LSD @ p < 0.05		Clone = 5.46; Cutting type = NS; IBA = NS; Clone x cutting type = 7.73; Clone x IBA = 10.93; cutting type x IBA = NS; Clone x cutting type x IBA = NS				

Figures in parenthesis are mean of arc sine transformed values of replicate, 'NL' non lignified,' L' lignified, ▼ Mean of clone, italic number represents three factors interaction mean of clone, cutting type and IBA

Over all highest survival percentage with mean value of 62.50%. The cuttings from clone C1 (NEHU, Mawkynroh Umsing) and C3 (4½ Miles, Upper Shillong) also had moderate survival success having mean values of 26.67% and 35.83% respectively. Worst survivability was observed in cuttings from remaining 3 clones where least mean values 3.33% was recorded in C4 (Mawphlang). However, neither the type of cutting nor IBA treatment had significant effect on survivability. Instead, two way interactive effects of clone x cutting type and clone x IBA was found significant. The interaction between clone and cuttings implies that the effect of cutting type on survivability was non uniform across the clones. The non lignified cuttings (35%) showed significantly higher survival capacity than the lignified ones (18.33%) in C1 whereas lignified cuttings (55%) had higher survivability in C3. No significant difference noticed between two types of stem cuttings in rest of the clones (C2, C4, C5 and C6). Both lignified (60%) and non lignified cuttings (65%) from C2 had overall higher survival success followed by lignified cuttings From C3 (55%). In the interaction between clone and IBA treatment, it was observed that variation due various IBA level was least in C2 where survival percentage oscillated between 53.33 to 70%. Moreover, mean survival percentage in each concentration level including 0 ppm in C2 (53.33) was higher than any of the IBA treatment from rest of the clones. The higher success rate due to effect of IBA treatment in C1, C2 and C3 was exhibited by 1000 ppm (36.67%), 2000 ppm (70%) and 5000 ppm (46.67%) respectively.

Rooting response

Interclonal variation was also found significant ($p < 0.05$) for all the rooting parameters (Table 2). Maximum rooting response was observed in the cutting obtained from clone C2 (BSI, Shillong) (45%) followed by C3 (4 ½ miles, Upper, Shillong) (30.00%) and C1 (NEHU, Mawkynroh Umsing) (17.50%) (Table 4). The effects of variables on adventitious rooting of stem cuttings are explained as follow:

Table 4. Mean values rooting percentage of *Taxus baccata* stem cuttings in response to clone, cutting type and various Concentration of IBA

Clone	Cutting type	0 ppm	1000 ppm	2000 ppm	5000 ppm	Mean of clone x cutting type
C1	NL	20.00 (26.24)	40.00 (38.85)	26.67 (30.78)	6.67 (17.47)	23.33 (28.34)
	L	6.67 (17.47)	13.33 (22.01)	20.00 (26.56)	6.67 (17.47)	11.67 (20.88)
Mean of clone x IBA		13.33 (21.85)	26.67 (30.43)	23.33 (28.67)	6.67 (17.47)	17.50▼ (24.61)
C2	NL	46.67 (43.08)	73.33 (59.54)	53.33 (46.92)	33.33 (34.31)	51.67 (45.96)
	L	26.67 (30.78)	33.33 (35.01)	53.33 (46.92)	40.00 (38.85)	38.33 (37.89)
Mean of clone x IBA		36.67 (36.93)	53.33 (47.27)	53.33 (46.92)	36.67 (36.58)	45.00▼ (41.93)
C3	NL	6.67 (17.47)	26.67 (30.78)	6.67 (17.47)	26.67 (30.78)	16.67 (24.13)
	L	20.00 (26.56)	46.67 (43.08)	53.33 (46.92)	53.33 (46.92)	43.33 (40.87)
Mean of clone x IBA		13.33 (22.01)	36.67 (36.93)	30.00 (32.20)	40.00 (38.85)	30.00▼ (32.50)
C4	NL	6.67 (17.47)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	1.67 (14.06)
	L	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)
Mean of clone x IBA		3.33 (15.19)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	0.83▼ (13.49)
C5	NL	6.67 17.47)	0.00 (12.92)	0.00 (12.92)	0.00 (12.92)	1.67 (14.06)
	L	6.67 (17.47)	0.00 (12.92)	6.67 (17.47)	0.00 (12.92)	3.33 (15.19)
Mean of clone x IBA		6.67 (17.47)	0.00 (12.92)	3.33 (15.19)	0.00 (12.92)	2.50▼ (14.63)
C6	NL	0.00 (12.92)	13.33 (21.69)	6.67 (17.47)	0.00 (12.92)	5.00 (16.25)
	L	0.00 (12.92)	6.67 (17.47)	0.00 (12.92)	0.00 (12.92)	1.67 (14.06)
Mean of clone x IBA		0.00 (12.92)	10.00 (19.58	3.33 (15.19)	0.00 (12.92)	3.33▼ (15.15)
Mean of cutting type x IBA	NL	14.44 (22.44)	25.56 (29.45)	15.56 (23.08)	11.11 (20.22)	16.67 (23.80)
	L	10.00 (19.69)	16.67 (23.90)	22.22 (27.29)	16.67 (23.67)	16.39 (23.63)
Mean of IBA		12.22 (21.06)	21.11 (26.68)	18.89 (25.18)	13.89 (21.94)	
LSD @ p < 0.05		Clone = 4.71; Cutting type = NS; IBA = 3.85; Clone x cutting type = 6.67; Clone x IBA = 9.42; Cutting type x IBA = 5.44; Clone x cutting type x IBA = NS				

Figures in parenthesis are mean of arc sine transformed values of replicate, 'NL' non lignified,' L' lignified, ▼ Mean of clone, italic numbers represents three factors interaction mean of clone, cutting type an IBA

Rooting percentage (Table 4): In addition to clonal effects, IBA treatment also exhibited significant influence on rooting percentage where IBA concentration 1000 ppm (21.11%) resulted in maximum rooting followed by 2000 ppm (18.89%). Statistically, the rooting success due to 1000 ppm was not significantly different from that of 2000 ppm. Like survivability, the rooting capacity of two different kind of cuttings did not bear any significant different in present experiment. However, all two way interactions i.e. clone x cutting type, clone x IBA and cutting type x IBA showed significant effects on rooting percentage. Interactive effects of Clone x cutting type showed that non lignified cuttings from C2 exhibited overall highest rooting percentage (51.67%) followed by lignified cuttings from clone C3 (43.33%) and C2 (38.33%). In case of clone x IBA treatment, cuttings from clone C2 treated with 1000 and 2000 ppm IBA had induced overall highest rooting success (53.33%). Surprisingly, 5000 ppm IBA treatment showed similar rooting percentage (36.67%) to that of control treatment in same clone. In clone C1 (26.67%) and C3 (40.00%) highest rooting percentage was due to the treatment of 1000 and 5000 ppm IBA respectively. In case of interaction between cutting type and IBA treatment, overall maximum rooting (25.56%) was exhibited by combined effect of non lignified cuttings and 1000 ppm IBA treatment followed by lignified cuttings and 2000 ppm IBA (22.22%).

Root number (Table 5): Clonal variation was also seen in mean root number. Owing to highest rooting percentage, clone C2 exhibited maximum number of roots per cutting. Unlike other rooting parameters, cutting type showed significant influence on mean root number where lignified cuttings (2.16) borne more number of roots than non lignified ones (1.54). In present experiment, IBA treatment did not show any variation in root number. Furthermore, only one interaction i.e. clone x cutting type was found significant for rooting number. Maximum number of roots (5.26) was exhibited by lignified

Table 5. Mean values root number of *Taxus baccata* stem cuttings in response to clone, cutting type and various concentration of IBA

Clone	Cutting type	0 ppm	1000 ppm	2000 ppm	5000 ppm	Mean of clone x cutting type
C1	NL	2.00 (1.48)	4.56 (2.24)	3.33 (1.95)	2.00 (1.32)	2.97 (1.75)
	L	0.67 (1.00)	2.33 (1.57)	4.33 (2.20)	1.67 (1.25)	2.25 (1.50)
Mean of clone x IBA		1.33 (1.24)	3.44 (1.90)	3.83 (2.08)	1.83 (1.29)	2.61▼ (1.63)
C2	NL	3.61 (2.03)	4.13 (2.15)	4.17 (2.16)	2.89 (1.70)	3.70 (2.01)
	L	4.33 (2.19)	4.00 (2.12)	5.72 (2.49)	5.42 (2.42)	4.87 (2.30)
Mean of clone x IBA		3.97 (2.11)	4.07 (2.14)	4.94 (2.32)	4.15 (2.06)	4.28▼ 2.16)
C3	NL	0.67 (1.00)	2.33 (1.66)	1.33 (1.18)	3.17 (1.90)	1.88 (1.43)
	L	3.00 (1.86)	4.94 (2.33)	5.06 (2.35)	8.03 (2.88)	5.26 (2.35)
Mean of clone x IBA		1.83 (1.43)	3.64 (1.99)	3.19 (1.76)	5.60 (2.39)	3.57▼ (1.89)
C4	NL	0.33 (0.88)	0.00 0.71)	0.00 (0.71)	0.00 (0.71)	0.08 (0.75)
	L	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
Mean of clone x IBA		0.17 (0.79)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	0.04▼ (0.73)
C5	NL	0.67 (1.00)	0.00 (0.71)	0.00 (0.71)	0.00 0.71)	0.17 (0.78)
	L	1.00 (1.10)	0.00 (0.71)	0.67 (1.00)	0.00 (0.71)	0.42 (0.88)
Mean of clone x IBA		0.83 (1.05)	0.00 (0.71)	0.33 (0.85)	0.00 (0.71)	0.29▼ (0.83)
C6	NL	0.00 (0.71)	1.17 (1.14)	0.67 (1.00)	0.00 (0.71)	0.46 (0.89)
	L	0.00 (0.71)	0.67 (1.00)	0.00 (0.71)	0.00 (0.71)	0.17 (0.78)
Mean of clone x IBA		0.00 (0.71)	0.92 (1.07)	0.33 (0.85)	0.00 (0.71)	0.31▼ (0.83)
Mean of cutting type x IBA	NL	1.21 (1.18)	2.03 (1.43)	1.58 (1.28)	1.34 (1.17)	1.54 (1.27)
	L	1.50 (1.26)	1.99 (1.40)	2.63 (1.57)	2.52 (1.45)	2.16 (1.42)
Mean of IBA		1.36 (1.22)	2.01 (1.42)	2.11 (1.43)	1.93 (1.31)	
LSD @ p < 0.05		Clone = 0.24; Cutting type = 0.14; IBA = NS; Clone x Cutting type = 1.06; Clone x IBA = NS; cutting type x IBA = NS; Clone x cutting type x IBA = NS				

Figures in parenthesis are mean of square root transformed values of replicate , 'NL' non lignified,' L' lignified, ▼ Mean of clone, italic number represents three factors interaction mean of clone, cutting type and IBA

Table 6. Mean values root length of *Taxus baccata* stem cuttings in response to clone, cutting type and various concentration of IBA

Clone	Cutting type	0 ppm	1000 ppm	2000 ppm	5000 ppm	Mean of clone x cutting type
C1	NL	0.85	2.76	2.64	1.00	1.81
	L	0.77	1.27	2.00	0.43	1.12
Mean of clone x IBA		0.81	2.02	2.32	0.72	1.47▼
C2	NL	2.27	2.87	2.25	1.79	2.30
	L	1.53	2.63	3.02	3.04	2.56
Mean of clone x IBA		1.90	2.75	2.64	2.41	2.43▼
C3	NL	0.47	1.32	0.67	2.05	1.13
	L	2.30	3.53	3.06	2.66	2.89
Mean of clone x IBA		1.38	2.42	1.87	2.36	2.01▼
C4	NL	0.17	0.00	0.00	0.00	0.04
	L	0.00	0.00	0.00	0.00	0.00
Mean of clone x IBA		0.08	0.00	0.00	0.00	0.02▼
C5	NL	0.23	0.00	0.00	0.00	0.06
	L	0.48	0.00	0.62	0.00	0.28
Mean of clone x IBA		0.36	0.00	0.31	0.00	0.17▼
C6	NL	0.00	0.64	0.68	0.00	0.33
	L	0.00	0.67	0.00	0.00	0.17
Mean of clone x IBA		0.00	0.65	0.34	0.00	0.25▼
Mean of cutting type x IBA	NL	0.66	1.27	1.04	0.81	0.94
	L	0.85	1.35	1.45	1.02	1.17
Mean of IBA		0.76	1.31	1.25	0.91	
LSD @ p < 0.05		Clone = 0.39; Cutting type = NS; IBA = 0.32; Clone x cutting type = 0.56; Clone x IBA =0.79; Cutting type x IBA = NS; Clone x cutting type x IBA = NS				

'NL' non lignified,' L' lignified, ▼ Mean of clone, italic numbers represent three factors interaction mean of clone, cutting type and IBA

Figure 1. Clonal propagation of *Taxus baccata*: 1(a) A mature tree of *Taxus baccata* growing in natural condition; 1(b) Treated planting materials in poly-house; 1(c) Rooting of IBA treated lignified cuttings of *Taxus baccata* 1(d) Rooting of IBA treated non lignified cuttings of *Taxus baccata*

cuttings from C3 followed by lignified (4.87%) and non lignified cuttings (3.70) from C2.

Root length (Table 6): As stated earlier that clonal variation was significant for all rooting parameters, so for the root length. Longest mean root length (2.43 cm) was exhibited by clone C2 followed by C3 (2.01 cm). IBA treatment also had significant influence on mean root length where longest root length was induced by 1000 ppm (1.31 cm) followed by 2000 ppm (1.25 cm). Type of cutting also showed nearly significant effect (p = 0.053). Among interaction, clone x cutting type and clone x IBA were found significant. Overall, lignified cuttings from clone C3 exhibited longest root length (2.89 cm). It was followed by lignified (2.56 cm) and non lignified cuttings (2.30 cm) from clone C2. The interactive effect of clone x IBA showed that the cuttings from clone C2 response better to IBA treatment. In whole experiment, highest root length (2.75 cm) was from clone C2 cutting receiving 1000 ppm IBA treatment followed by 2000 (2.64 cm) and 5000 (2.41 cm). In clone C1 (2.32 cm) and C3 highest (2.42) root length was induced by 2000 ppm and 1000 ppm IBA respectively.

DISCUSSION

The results of the present investigation clearly demonstrate that the variations do exist among clones in same region (East Khasi Hill) in terms of survival and rooting ability of the *Taxus baccata* cuttings Clone C3 (Botanical Survey of India, Shillong) represents best genotype for adventitious rooting. Interclonal variation in rooting of cuttings is a common phenomenon and had been reported in many other tree species such as *Triplochiton scleroxylon* (**Leakey *et al.*, 1982**), *Calliandra calothyrus* (**Dick *et al.*, 1996**), *Dalbergia sisoo* (**Husen, 2004**) and *Tectona grandis* (**Husen, 2013**).

However, the reason behind interclonal variation is still largely unknown. According to **Zsuffa (1976b)** variable rooting response among clones could be due to either internal or environmental factors or interaction of the two (**Zsuffa, 1976b**). Internal factors affecting rooting might be genetic, morphological or physiological characteristics of stock plant. The main environmental factors which affect rooting are light, temperature and moistures, Genotypes-environmental interaction cause specific changes in the biology and chemistry of the clone, which affect the rooting of clone. (**Ahuja and Libby, 1993**). Effect of stem type was insignificant for all the rooting parameters except root number. However, it was revealed from the study that significant interaction between clone and cutting type existed which implies that the effect of type of stem was not uniform among clones. Distinct differences in rooting capacity were observed in clone C1 and C3, where non-lignified cuttings had higher rooting capacity in

clone C1 and lignified ones in clone C3. Due to large diameter, the lignified cuttings had greater number of root primordial (**Zalenshy, 2003**) and so was its root number. In clonal propagation, the adventitious rooting also depends on C/N ratio (**Hartmann *et al*., 2009**), where cuttings with more carbohydrates reserves in tissues induce higher rooting response. Carbon being less mobile nutrient than nitrogen gets accumulated in older tissue which increases the C/N ratio in that part of the plant (**Taiz and Zeiger, 2006**). So it can be said that woody lignified cuttings had more carbohydrate reserves than non lignified ones and this could be responsible for its greater degree of rooting.

Application of IBA significantly increased rooting percentage. It is now well known that IBA is best rooting hormones for *Taxus* species and it was confirmed by many workers (**Mitter and Sharma, 1993; Nandi *et al*., 1996; Khali and Sharma; 2001, Kaul 2008**). But the relative response toward particular growth regulator varied with place, clone, and age of cuttings and donor plant. The interaction between IBA and clone in present experiment indicates that not all clones responded in the same way to the IBA treatment. Increasing IBA concentration resulted in increasing rooting in cutting but within at certain range as it was observed in clone C1 and C2 where maximum rooting was recorded in 1000-2000 ppm IBA. In addition, interaction between IBA and type of stem also suggests considering tissue sensitivity in relation to plant growth regulators. Non lignified cuttings being younger than lignified ones require optimally low concentration of IBA (1000-2000 ppm) for root initiation than latter. It was clearly depicted in clone C3 (Table 4) where maximum of lignified cuttings rooted and the rooting percentage increased steadily as concentration of IBA increase.

Conclusion

The results of present experiment demonstrate that survival and rooting of *Taxus baccata* stem cuttings was strongly influenced by genotypes. So it is necessary to select genotypes with good rooting potential. In addition, appropriate cutting type and IBA concentration in relation to particular clones should be also taken into account. It is suggested that IBA concentration 1000-2000 ppm could be optimum for root initiation in cutting, and due to rigid nature and high degree of rooting; lignified cutting should be given preference. We hoped that our findings will be helpful for developing propagation protocol of *Taxus baccata* L. especially for subtropical climate of Eastern Himalayan Region.

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