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## Effect of Wounding and Plant Growth Regulators (IBA and NAA) on root proliferation of *Taxus wallichiana* shoot cuttings

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#### Abstract

Meghalaya, a hilly state of Northeast India is blessed with a few natural stocks of Taxus wallichiana, a highly valued medicinal tree. However its habitat destruction due to several reasons posed a serious threat to its existence in the region. Therefore, ex-situ conservation measures are urgently needed to prevent this valuable resource from perishing. In order to optimize the propagation protocol of Taxus wallichiana by shoot cuttings in subtropical climate of Northeast India, the present study was carried out to investigate the effect of shoot type (softwood, semi-hardwood and hardwood) wounding (light and severe), plant growth regulators (IBA and NAA) and their interactive effect on adventitious rooting. Semi-hardwood (29.7%) and hardwood cuttings (26.1%) had higher survival percentage than softwood (5.6%). Overall better rooting response was exhibited by semi-hardwood cutting having 43.9% rooted cuttings initiated root development earlier than light wounded (76.9 days vs. 87.0 days). In addition, severe wounding also increased root production (10.5) in shoot cuttings exhibited overall better rooting response. In contrast, application of NAA proved phyto-toxic to plant as all of them died due to basal necrosis. For, softwood and semi hardwood higher rooting success was achieved with 6.15 mM IBA.

Keywords: Taxus wallichiana, Meghalaya, ex-situ conservation, shoot type, wound type, plant growth regulators.

### Introduction

Taxus wallichiana Zucc. (synonym Taxus baccata ssp wallichiana (Zuccarini). Pilger) also known as Himalayan Yew belongs to family Taxaceae is a long lived and coniferous tree species. It is distributed throughout the temperate and subalpine region of Himalaya at altitudes of 1800 to 3300 m a.s.l. from Pakistan to Southwest China<sup>1</sup>. In Indian Himalayan Region (IHR), it is found at elevation of 2,300 to 3,400 m a.s.l from Ladakh eastward to Khasi and Jaintia Hills, Naga Hills and Manipur<sup>2</sup>. This species is primarily valued for 'paclitaxel' a mitotic inhibitor, extracted from its leaves and barks, used in the treatment of patients with lung, breast and ovarian cancer<sup>3</sup>. After the discovery of 'paclitaxel', its market demand increased manifolds which led to the intense and reckless exploitation of Taxus species all over the world. In IHR, over exploitation coupled with habitat destruction had reduced the natural population of this valuable tree species to a greater extent<sup>4</sup>. Moreover its conservation crisis is aggravated by the species poor natural regeneration process, slow growth rate and long seed dormancy period of 1.5-2 years<sup>5</sup>. Since Taxus regeneration through seed is very difficult and requires complex treatment like *in vitro* culture and precocious germination<sup>6,7,8</sup>, vegetative propagation might seem to be only practical solution to improve and manage its natural stock as well as regeneration.

Taxus species is reported as plant with relative potential of regeneration by adventitious rooting of cuttings<sup>9,10</sup>. However, Taxus baccata unlike other Taxus species is difficult to root and often requires longer time<sup>11,12</sup>. But efficient rooting treatment by selection of proper planting material, use of rooting inducing wounding and modifying chemical, the surrounding environment can result in rapid root initiation, high percentage of rooting and higher quality of root system<sup>13</sup>. Although adventitious rooting of Taxus wallichiana stem cutting using plant growth regulator is reported by other workers<sup>10,14-16</sup>, but most of the research works so far has been restricted to the temperate region (Central Himalayan Region) of the country. Despite being a subtropical region, Meghalaya is also home to a few good population of *Taxus wallichiana*, where it is considered as a rare plant <sup>17</sup>. In order to give high conservation priority, the species is now listed under the endangered category<sup>18</sup>. However no standard propagation package by use of cuttings is available for subtropical climate. Therefore, the present study was carried out to evaluate the effect of wounding and plant growth regulators on feasibility of adventitious rooting of different kind of shoot cuttings of Taxus wallichiana.

#### **Material and Methods**

**Study area:** The experiment was conducted in the experimental garden of the Department of Environmental

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Studies, North-Eastern Hill University, located at Shillong in Meghalaya, India (91°53' E; 25°36' N and at an altitude of 1426 m asl). The predominant vegetation of the area is subtropical. The mean annual temperature ranges between a minimum of 7°C to a maximum of 28°C. The average annual rainfall of the region is 2200mm, where 80-90% of annual rainfall is received during monsoon season (July to September) and lowest (<5%) during winter season (January to March). Dry weather condition prevails during spring (April to June) whereas autumn is relatively moist (October to December).

Collection and preparation of shoots: The branches of Taxus wallichiana were collected from four phenotypically superior tree growing in the nursery of Botanical Survey of India (East Khasi Hill), during the second week of July (2013). The branch bearing at least 10 internodes or more were excised at the point of its origin from the main shoot. The final cuttings of 15-20 cm long with 3-4 nodes and maximum of 1 cm diameter were cut from the excised branches with the help of sharp shear. The needles at the basal 2 cm of the shoot were stripped off. Finally the cuttings were grouped according to the kind of wood. The first three internodes from the apical part of shoot with new flush of leaves were classified as soft wood (current or 1 year old shoot); next middle three internodes as semi-hardwood (2 or 3 year old shoot) and basal three internodes with dark brown colour as hardwood (> 3 year old). When broken by applying pressure, semi-hard and hardwood cuttings produced a distinct cracking sound due to their mature and firm stem tissue. Due to delicate nature, softwood cuttings held at a point of breaking. Flaccid softwood were avoided.

**Experimental design:** Factorial arrangement of treatments on the basis of randomised complete block design was used as experimental design: 3 blocks (replications) and 20 cuttings per treatment per block. 10 out of 20 cuttings per treatment were used for estimation of days taken for root initiation. The main treatments were as (i) three types of shoot: softwood, semi-hardwood and hardwood; (ii) two types of wounding: light and severe; (iii) two plant growth regulators: IBA and NAA. The concentration tested for IBA was 6.15 and 24.6 mM; and for NAA concentration was 0.25 and 1 mM. In addition to these, control solution and combined solution of 6.15 mM IBA and 0.25 mM NAA was also tested. According to Khali *et al.* (2003) and Kaul (2008) higher concentration of NAA was not effective in rooting of *Taxus wallichiana*<sup>10, 16</sup>.

**Wounding and exogenous auxin treatment:** Light wounding consisted of one simple cut at the base while preparing cuttings from branch and no additional cut or incision was made on the cutting.. Severe wounding consisted of 4 to 5 incisions of 2-3 cm on the basal shoot to a depth of 5-10 mm, reaching secondary xylem with the help of sharp razor. Following wounding, the cuttings were given plant growth regulator (PGR)

treatment. PGRs (IBA /NAA) solution were prepared by dissolving required amount of chemical in a very small volume (5-10 ml for 1000 ml of solution) of 0.1N NaOH and finally, the desired concentration was obtained by adding distilled water. Quick dip method of IBA application<sup>19</sup> was used throughout the experiment. All cuttings were treated with fungicide 0.05% carbendezim solution and immediately planted in perforated poly-pot (18 cm deep and 6.5 cm diameter) containing equal proportion of soil, sand and farmyard manure (1:1:1). Finally all cuttings were kept under bamboo made poly-house and watered regularly as and when depending on the weather conditions and moisture status of rooting medium.

Observation recording and Statistical analysis: Observation for days required for root initiation was recorded at an interval of 1 week by taking single cutting per treatment from 45<sup>th</sup> day to 130<sup>th</sup> days. All the cuttings were harvested after 6 months of planting and data was recorded on number of cuttings survived (survival), number of cuttings rooted (rooting percentage), and average number of roots formed per cutting and average root length per treatment. Following the data of recorded parameters were subjected to analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) 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 data of survival and rooting percentage were transformed by arc sine transformation, whereas data of root number was transformed by square root transformation before statistical operation<sup>20</sup>.

**Survival percentage:** All three main factors *i.e.* shoot type (p = 0.000), wound (p = 0.019) and PGRs treatment (p = 0.000) had significant influence on survivability of cuttings (Table-1). Mean separation by HSD revealed that the semi-hardwood cuttings (29.7%) followed by hardwood cuttings had higher surviving capacity (26.1%) (Table-3). Light wounded cuttings (22%) had better survival rate than severe wounded (18.9%) (table-3). Among PGRs treatment, greater survival success rate was achieved with IBA, having maximum value with 6.15 mM (55%) followed by 24.6 mM (42.3%) and control (20.6%) (Table-2). NAA treatment resulted in worst survival performance, where all cutting died within one month of planting. Combined treatment of 0.25 mM NAA and 6.15 mM IBA also had lower success rate having survival percentage of just 5% (table-2).

Among interaction, shoot type x IBA concentration had significant (p = 0.000) effect. Semi hardwood cuttings treated with 6.15 mM IBA had maximum survival capacity (85%) (Table-4). The other best interactions were hardwood x 24.6 mM IBA (61.7%), hardwood x 6.15 mM IBA (58.3%) and semi-hardwood x 24.6 mM IBA (56.7%) (table-4)

Table-1
ANOVA mean sum of the square and p-value for the survival percentage and number of days taken for root initiation of
Taxus wallichiana shoot cuttings

Source of variation	Su	rvived cuttings	s (%)	No. of days taken for root initiation $^{\Omega}$			
Source of variation	df	MS <i>p</i> -value		df	MS	<i>p</i> -value	
Replicate Block	2	29.4	0.946 <sup>ns</sup>	2	285.16	0.737 <sup>ns</sup>	
Shoot type	2	3827.6	0.000*	2	13562.73	0.000*	
Wound	1	259.6	0.019*	1	475.95	0.001*	
PGRs treatment	5	7336.2	0.000*	2	1272.90	0.000*	
Shoot type x Wound	2	115.7	0.081 <sup>ns</sup>	2	25.75	0.045*	
Shoot type x PGRs treatment	10	632.3	0.000*	4	244.32	0.000*	
Wound x PGRs treatment	5	32.5	0.612 <sup>ns</sup>	2	5.39	0.846 <sup>ns</sup>	
Shoot type x Wound x PGRs treatment	10	64.1	0.192 <sup>ns</sup>	2	5.61	0.840 <sup>ns</sup>	
Error	70	45.8		24	31.98		
Total	107			39			

<sup> $\Omega$ </sup> Only control and two IBA treatment (6.15 & 24.6 mM) are consider for number of days taken for root initiation.\* indicates significant and not significant at  $p \le 0.05$ .

 Table-2

 ANOVA mean sum of the square and *p*-value for rooting percentage, number of roots per cutting and mean root length of *Taxus wallichiana* shoot cuttings

		Means square					
Source of variation	df	Rooted cuttings (%)		Root number/cutting		Mean root length (cm)	
		MS	<i>p</i> -value	MS	<i>p</i> -value	MS	<i>p</i> -value
Replicate Block	2	14.9	0.960 <sup>ns</sup>	0.2	0.888 <sup>ns</sup>	2.5	0.702 <sup>ns</sup>
Shoot Type	2	4840.1	0.000*	29.3	0.000*	81.4	0.000*
Wound	1	141.0	0.062 <sup>ns</sup>	1.1	0.050*	0.00	0.986 <sup>ns</sup>
IBA concentration	2	2365.4	0.000*	5.7	0.000*	30.5	0.000*
Shoot type x Wound	2	703.8	0.000*	5.9	0.000*	13.1	0.004*
Shoot type x IBA concentration	4	234.0	0.000*	0.4	0.197 <sup>ns</sup>	7.4	0.014*
Wound x IBA concentration	2	51.5	0.270 <sup>ns</sup>	0.95	0.038*	0.9	0.636 <sup>ns</sup>
Wound x IBA concentration x Cutting	1	19.1	0.287 <sup>ns</sup>	0.1	0.691 <sup>ns</sup>	12	0.685 <sup>ns</sup>
age	7	77.7	0.207	0.1	0.071	1.2	0.005
Error	34	39.2		0.3		2.00	
Total	53						

\* indicates significant and not significant at  $p \le 0.05$ .

**Root initiation:** Like survival percentage, effect of all the main factors was found to be highly significant ( $p \le 0.001$ ) for number of days required for root initiation (Table-1). Softwood cuttings (62.7 days) and semi-hardwood (69.2 days) rooted earlier than hardwood (121.6 days) (Table-3). Severe wounding (76.9 days) and PGR treatment (83.1days for 6.15 mM IBA; 84.6 days for 24.6 mM IBA) took less number of days for cuttings to root as compared to light wounded (87 days) and control (103.5 days) respectively.

According to ANOVA, significant interactive effect on root initiation was observed between shoot type x wound (p = 0.045) and shoot type x PGR treatment (p = 0.000) (Table-1). Mean separation by HSD showed that the rapid root initiation was reported from wounded cutting of both softwood (56 days) and semi-hardwood cuttings (55.5 days) (Table-4). In case of shoot

type x PGR treatment, root initiation was quicker in 24.6 mM IBA treated cuttings of both softwood (56 days) and semi hardwood (56.4 days) (Table-4). However it was not statistically significant from 6.15 mM IBA treated cutting of softwood (58.4 days) and semi-hardwood cuttings (61.1 days); and control softwood cuttings (63 days) (table-4).

**Rooting percentage:** The percent rooted cuttings of *Taxus* wallichiana was significantly (p = 0.000) influenced by shoot type and IBA concentration (Table-2). Mean separation by HSD showed that semi-hardwood cuttings (43.9%) had maximum percentage followed by hardwood cuttings (19.4%) (Table-3). Among the different IBA concentration, 6.15 mM had significantly higher rooting success (39.9%) (Table-3). The lowest value for rooting percentage was exhibited by softwood (5.6%) and control cuttings (10.00%) (table-3).

	percentage, number of root per cutting and mean root length of <i>raxus watternana</i> shoot cuttings								
Variables	df	Survived cuttings (%)	Root initiation (days)	Rooted cuttings (%)	Root number/cut- ting	Mean root length			
Shoot type	Softwood (SW)	5.6 c	62.7a	5.6 c	2.6 c	1.8 c			
	Semi-hardwood (SHW)	29.7 a	69.2 b	43.9 a	15.9 a	6.0 a			
	Hardwood (HW)	26.1 b	121.6 c	19.4 b	8.8 b	3.6 b			
Wound	Light	22.0 a	87.0 b	20.0 a	7.7 a	3.5 a			
	Severe	18.9 b	76.9 a	25.9 a	10.5 a	3.9a			
IBA concentrati on	Control	20.6 c	103.5 b	10.0 c	6.2 c	2.2 b			
	IBA 6.15 mM	55.0 a	83.1 a	39.9 a	11.9 a	4.7 a			
	IBA 24.6 mM	42.3 b	84.6 a	19.4 b	9.26 b	4.3 a			
	NAA 0.25 mM	0.0 d	-	-	-	-			
	NAA 1.0 mM	0.0 d	-	-	-	-			
	6.15 mM IBA + 0.25 mM NAA	5.0 d	-	-	-	-			

# Table-3 Influence of shoot type, wound and IBA treatment on survival percentage, number of days taken for root initiation, rooting percentage, number of root per cutting and mean root length of *Taxus wallichiana* shoot cuttings

Mean values within each variable from same column followed .by same alphabet are not significantly different at p = 0.05 level (HSD test).

**Rooting percentage:** The percent rooted cuttings of *Taxus wallichiana* was significantly (p = 0.000) influenced by shoot type and IBA concentration (Table-2). Mean separation by HSD showed that semi-hardwood cuttings (43.9%) had maximum percentage followed by hardwood cuttings (19.4%) (Table-3). Among the different IBA concentration, 6.15 mM had significantly higher rooting success (39.9%) (Table-3). The lowest value for rooting percentage was exhibited by softwood (5.6%) and control cuttings (10.00%) (table-3).

Interactive effect of shoot type x wound as well as shoot type x IBA concentration were found to be highly significant (p = 0.000) for rooting percentage (Table-2). Maximum rooting percentage (51.1%) was achieved with semi hardwood cuttings having severe wound (Table-4). Light wounded semi hardwood cuttings (36.6%) also had moderate rooting success which was statistically at par with severely wounded hardwood cuttings (25.6%) (Table-4). Among the shoot type x IBA concentration interaction, semi-hardwood cuttings treated with 6.15 mM IBA had significantly higher rooting success (75%) (table-4).

**Root number and root length:** In the present study, rooting percentage had good correlation with mean number of root produced ( $R^2 = 0.8$ ) and root length per cutting ( $R^2 = 0.7$ ). It implies that the factors which significantly affected the rooting percentage also had significant effect on root number and root length. However, according to ANOVA (table-2) the few differences encountered in case of root number. The interactive effect was found insignificant in case of shoot type x IBA concentration (p = 0.197), whereas it was significant for wound x IBA (p = 0.038).

Semi-hardwood and 6.15 mM IBA had higher root number and longest root (Table-3). However the length of root of 24.6 mM IBAtreated cuttings (4.3 cm) was not significantly different from those of 6.15 mM (4.7 cm). Mean separation by HSD also revealed

that higher root number for interaction between wound x IBA concentration was achieved with severe wounding x 6.15 mM (13.0). For interaction between shoot type x wound, greater number of roots (19.1) and longest root length (6.5 cm) was exhibited by severe wounded semi-hardwood cuttings. In case of interaction between shoot type x IBA concentration maximum root number was recorded in semi-hardwood cuttings treated with 6.15 mM (19.2), whereas longest root length was resulted from semi-hardwood cutting treated with 24.6 mM IBA (7.3 cm).

Taxus species have an extremely slow regeneration process including rooting of cuttings which often takes as long as 6-8 months. It is thus necessary to keep alive the shoot cuttings of Taxus wallichiana until the onset of rooting process. The survivability of cuttings can be determined by the nature of the shoots. In present study, overall, semi-hardwood (2<sup>nd</sup>-3<sup>rd</sup> years) cuttings had higher survival rate and thus higher rooting ability. Our result is partially agreement with the finding of Kaul (2008) where highest rooting response was exhibited by 2<sup>nd</sup> year cutting In the present experiment worst survival and rooting response was exhibited by softwood (less than 1 year old shoot) cuttings. Morpho-histologically semi-hardwood cuttings are green but not as soft or flexible as the softwood, and tough but not as hard or recalcitrant as the hardwood. Besides, higher tissue sensitivity and greater meristematic activity of semi-hardwood has made it superior than other two kinds of shoot in terms of rooting ability. Lower rooting ability of hardwood cutting could be attributed to it lower level of endogenous root promoter, increased lignifications and accumulation of inhibitory substances such as phenolics<sup>21,19</sup>. Despite having greater rooting potential, softwood cuttings displayed high mortality, which could be due to their leafy and delicate nature causing excessive water loss through transpiration and thereby resulting in wilting. In addition, it has been observed that only few softwood survived even after rooting due to loss of its plant inertia<sup>19</sup>. Therefore their propagation requires high cost maintenance such as specialised poly-house, mist and fog system<sup>19</sup>.

The hardwood cuttings, on the other hand, had better survival and rooting success than softwood due to their hard lignified outer covering which protect them from rapid desiccation and pest or fungal decay.

In present study, though there was no significant difference between severe and light wounded cuttings of Taxus wallichiana for rooting percentage, the former showed earlier rooting than the latter. Basal wounding of cutting had proved helpful for root production in many species such as juniper, arborvitae, rhododendron, maple, magnolia and holly<sup>19,22</sup>. Wounding stimulates rooting in woody species by damaging the sheath of materials which exist between bark tissue and wood tissue. This sheath has capability of inhibiting root development<sup>22</sup>. In certain conifer species such as Thuja orientalis L. and false cypress (Chamaecyparis lawsoniana), basal wounding of cuttings reported to have increased the number of root production by 80-90%<sup>23</sup> Furthermore, wounding when combined with IBA treatment had significantly increased the root number of Taxus wallichiana shoot cuttings According to Hartmann et al. auxin applied at the base of cuttings absorbed predominantly via cut (wounded) surface of cuttings and not through the epidermis or periderm<sup>18</sup>. However present experiment also indicates the higher mortality rate of severe wounded cuttings than light one, especially in case of softwood cutting. Additional incision in softwood caused extensive tissue

damage which leads to rotting of its basal part. So, it is suggested that wounding should be done particularly in semi-hardwood or hardwood cuttings. The injury to deep tissue should be avoided by restricting the incision to the 5-10mm from the surface.

Only IBA treated cuttings exhibited better rooting performance in terms of both root quantity and quality. All NAA treated cuttings showed basal necrosis and the leaf abscission cutting died without rooting within one month of planting. Even control cuttings showed better survival and rooting success than NAA in the present study. Our findings are in contrast to that of Kaul (2008) who found that lower concentration of NAA increased rooting in stem cutting of Taxus wallichiana<sup>16</sup>. It is also necessary to consider tissue sensitivity in relation to plant growth regulators<sup>24</sup>. Softwood and semi-hardwood cuttings have sufficient level of endogenous auxin due to their younger tissue and greater meristemetic activities and therefore low dose of IBA is enough to trigger rooting process. On the other hand, due to poor tissue sensitivity, hardwood cutting take longer time to root. Optimally high concentration (24.6mM) could improve the rooting process of hardwood. Application of higher IBA concentration in case of younger shoot (softwood and semihardwood) seems to be detrimental rather than beneficial because the entire auxin concentration is raised to supra optimal level.

 Table-4

 Influence of two way interaction of shoot x wounding, shoot x IBA concentration and wounding x IBA concentration on different rooting parameters of Taxus wallichiang shoot cuttings

unterent rooting parameters of Taxus waachaana shoot cuttings									
			Survived	Root	Rooted	Root	Root		
Interactive Variables			cuttings	initiatio	cuttings	number/c	length		
			(%)	n (days)	(%)	utting	(cm)		
	SW	light	17.8 b	60.1 ab	10.0 c	4.4 c	2.8 c		
		severe	4.4 c	56.0 a	1.1 d	0.9 d	0.8 d		
Sheet type y Weynd	SHW	light	55.6 a	72.8 b	36.6 b	12.8 b	5.5 ab		
Shoot type x wound		severe	58.9 a	55.5 a	51.1 a	19.1 a	6.5 a		
	HW	light	52.2 a	72.8 d	13.3 c	6.0 c	3.1 c		
		severe	46.7 a	100.6 c	25.6 b	11.6 b	4.0 bc		
Shoot type x IBA concentration	SW	Control	3.3 e	63 ab	1.7 e	0.5 e	0.6 e		
		6.15 mM	21.6 cd	58.0 ab	13.3 cd	5.4 c	5.6 abc		
		24.6 mM	8.3 de	56.0 a	1.7 e	2.0 de	0.9 e		
	SHW	Control	30.0 c	74.9 b	20.0 bc	13.0 ab	4.2 c		
		6.15 mM	85.0 a	61.1 ab	75.0 a	19.2 a	6.6 ab		
		24.6 mM	56.7 b	56.4 a	36.7 b	15.6 ab	7.3 a		
	HW	0 mM	28.3 c	116.1 c	8.3 de	5.1 cd	2.1 e		
		6.15 mM	58.3 b	110.4 c	20.0 bc	11.2 b	3.8 cd		
		24.6 mM	61.7 b	106.9 c	30.0 b	10.2 b	4.8 bc		
Wound x IBA concentration	light	Control	21.1 cd	96.9 a	6.7 d	3.9 c	2.1 c		
		6.15 mM	58.9 a	82.4 a	37.8 a	10.8 a	4.9 a		
		24.6 mM	45.6 ab	86.2 a	15.6 bc	8.4 b	4.3 ab		
	severe	Control	20.0 d	83.3 a	13.3 c	8.5 ab	2.5 bc		
		6.15 mM	51.1 a	75.3 a	41.1 a	13.0 a	4.5ab		
		24.6 mM	38.9 bc	73.3 a	23.3 b	10.1 ab	4.3 ab		

Mean values within each interactive variable from same column followed by same alphabet are not significantly different at p = 0.05 level (HSD test).



Figure-1

Rooting of *Taxus wallichiana* shoot cuttings: (a) Rooting of IBA treated softwood cuttings; (b) Rooting of IBA treated semi-hardwood cuttings (c) Rooting of IBA treated hardwood cuttings; (d) Rooting of light and severe wounded cuttings

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Besides physical and chemical treatment, time of collection and season should be also considered for successful propagation of Taxus wallichiana cuttings. As suggested by Kaul (2008), in temperate region, spring season was ideal for cutting propagation of Taxus wallichiana on the account of its favourable temperature condition with day temperature of 21- $29^{\circ}$ C combined with night temperature  $14-20^{\circ}$ C<sup>16</sup>. In the sub tropical climate of Meghalaya with an elevation ranging from (1400-1500 m.a.s.l), the required temperature condition was observed only during monsoon season where average day temperature and night temperature is 28.2°C and 17.8°C respectively. Spring season cuttings had higher potential of rooting and they showed good rooting response when the relative humidity and surrounding air temperature were maintained. Simple poly-house conditions without temperature management raised temperature inside the poly-house resulting in burning of the leaves. So spring season cuttings required special care such as building of a poly-house under shady area or covering it with green agro-net (50-70 % shade) to bring down the temperature.

Moreover regular spraying of plants (6-7 times) is necessary for maintaining the relative humidity for survival cutting inside poly-house during spring season. Post monsoon (October to February) cuttings are not suitable for propagation as most of the buds are in dormant stage and the activity of the cambial cell of the plant was drastically reduced.

## Conclusion

The result of this study demonstrates that the adventitious rooting of *Taxus wallichiana* shoot cuttings can be improved by taking into consideration of types of shoot and wounding along with the plant growth regulators. Semi hardwood and hardwood wood cuttings which are less perishable and easier to handle should be employed in cutting propagation. It is also suggested that the rooting of shoot cuttings is boosted by IBA treatment and careful wounding (4-5 cuts) of the shoots at the basal portion. The method is easy and inexpensive way to obtain a well rooted cutting within a short span of time and could be helpful in *ex-situ* conservation of *Taxus wallichiana* Zucc..

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