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Regeneration of *Taxus baccata* through vegetative propagation using different concentrations of IBA and NAA

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Abstract
Taxus baccata is a highly important medicinal plant commonly known as 'thuner" in Uttarakhand. The present research was accomplished to investigate the effect of different concentration of IBA and NAA on varying diameter in stem cuttings of *Taxus baccata* under different conditions for multiplication. The experimental work was performed at Ravigram Joshimath Chamoli under two environmental conditions in net house and in open nursery. Stems were cut into an equal length of 6 inches and divided into two varying size of diameter 0-4mm and 4-8mm from the field at the same day of treatment. Cuttings were pretreated for 24 hours in different concentration of IBA and NAA (100, 300, and 500ppm). After pretreatment of 24 hours these were transferred to the polythene bags containing Soil + vermicompost + Farm-yard manure at a ratio of 2:1:1. Proper irrigation was provided as per requirement of planted cuttings in rooting media. The highest survival percentage (58.25%) was obtained from IBA 500ppm followed by NAA100ppm. The Maximum fresh weight (27.237gm) was resulted from IBA 500ppm and dry weight (2.719gm) obtained from NAA100ppm. The study revealed that IBA 500PPM is the most optimum hormone for rooting percentage, fresh weight and dry weight of rooted stem cuttings of *Taxus baccata.*

Keywords: *Taxus baccata*, farm-yard manure, IBA, NAA, vegetative propagation

Introduction
Taxus baccata locally known as 'thuner' is an important medicinal plant having fair demands in national and at international market for their parts as a raw material. *Taxus baccata* also known as Yew is an endangered forest tree species with low regeneration capacity. It is also reported from the Himalayan countries of Nepal and Bhutan (Sahni, 1990) [21]. Taxus is a slow growing small to medium sized evergreen tree containing seeds like red 'berries'(covered by arils). *Taxus baccata* having often with a rounded or pyramidal canopy, bark reddish brown in colour with scaly thin leaves, long branches, whorled green twigs and irregularly alternate. This is non-resinous gymnosperms attaining height up to 20-28m, and grows primarily in the understory of moist, forested habitats in temperate climates. It is an evergreen tree found with an altitude ranging between 1500m to 3200m. Seeds of *Taxus baccata* prove difficult to germinate under normal condition.

It has been heavily exploited for its bark and leaves for obtaining 'taxol' an anti-cancerous alkaloid (Gaur, 1999; Saqib *et al*. 2006) [9, 22]. Taxol originally extracted from the bark of the slow growing pacific yew, *Taxus brevifolia.* It is also used for treating bronchitis, asthma, epilepsy, snake bites, scorpion stings, internal injuries, lung diseases, and diabetes, and also as an aphrodisiac (Beckstrom *et al*. 1993; Dhyani *et al.* 2014) [4, 8] alzhiemers disease polycystics kidney disease In India pharmaceutical companies got success in isolation of taxol from *Taxus baccata* leaves (Purohit *et al.* 2001) [20]. Taxol has a unique property of preventing the growth of cancerous cells and therefore it is now being used in the treatment of ovarian malignant tumors and breast cancer (Kovacs *et al.* 2007) [15]. *Taxus baccata* is categorized as a highly medicinal but endangered plant, due to production of several economically important metabolites (Wink *et al.* 2005) [26]. As the economic and medicinal status of *Taxus baccata* is very high, it could be highly beneficial to human beings if conservational strategies are initiated through its cultivation in the Himalaya. It has been stated that many medicinal plants which have a slow growth rate, population density are not wider and they are restricted to a small area geographical portions, those species are easily extinct form nature (Nautiyal *et al*. 2002) [18].

Vegetative propagation is one of the potential and useful methods to overcome with the problem of propagation, multiplication and In-situ or Ex-situ conservation of various useful plant species. Vegetative propagation is a convenient method to those seeds which possess any

kind of problems for their multiplication and faces difficulties to regenerate through seeds. There are different kinds of plant growth hormones are available to improve root and shoot growth of stem cuttings planted for the purpose of multiplication (Nadeem *et al*. 2000; Butola and Badola, 2007) [16, 5].

If any plant parts are treated with growth regulators then they developed the ability of root and shoot induction for vegetative propagation. And vegetative regeneration is a process by which new seedlings arise without seed production or sexual reproduction. Stem cuttings and tissue culture methods have been also practiced for the multiplication of *Taxus baccata* in the past for vegetative regeneration (Nandi *et al.* 1996) [17].

Production of true-to-type plants can only be achieved through vegetatively propagated plant parts in short time duration. This method is helpful for production of superior plants or large scale commercial plantation with quick productive gains (Pandey *et al.* 2011) [19]. The external use of plant hormone on stem cuttings can be helpful for conservation of medicinally valuable and commercially prioritized plants. Vegetative regeneration though stem cuttings could be a feasible way to achieve conservation and restoration goals as well as to meet the demand of plants for toxol or paclitaxel. To overcome with this problem of seed dormancy stem cutting is a quick and reliable method to develop a new plant population in a short duration; with the help of plant growth hormones (IBA and NAA) rooting time was shorter in comparison with control conditions or without any hormonal treatment.

Material and Method

The present study was conducted in the nursery condition of village Ravigram Joshimath Chamoli Uttarakhand India. The cuttings were planted in the second week of the July month in the rainy season.

Preparation of rooting media: Soil+ FYM and vermicompost are mixed in the ratio of 2:1:1. The mixture was filled in black polythene bags (1/2 kg capacity) tightly leaving two inch space at top. The plants selected for preparing cuttings were taken from moderately vigourous and healthy shoots having mature 4-5nodes. The length of the cuttings varied between 6-8mm. Cuttings were taken early in the morning when leaves and shoots of stock plants were turgid. The lower cut on stem was made just below the node and the upper slanting cut was made about 1.5cm above the node without any injury to the buds and leaves.

Preparation of rooting mixture: For preparing stock solutions of 1000ppm concentration of NAA and IBA, 1gm was weighted and then dissolved in a small quantity of ethyl alcohol and few drops of ammonium hydroxide are added to dissolve the growth regulators and to stop precipitation. This is diluted with distilled water to make up one liter. The freshly prepared solution is used for experiment. The working solution of plant growth regulators of various concentrations i.e. (100ppm, 300ppm, 500ppm) was prepared from the stock solution.

Collection, preparation and planting of stem cuttings: shoot cuttings were collected from the lower and mid crown of mature plants of *Taxus baccata*. The cuttings were collected from 4 to 5 randomly selected healthy plants of same age from their natural habitat. The cuttings were taken and then immediately rapped in wet jute bags to retain moisture and carried to the experimental site of Ravigram Joshimath Chamoli for further experimental work, then the cuttings were cut into equal length of 6 inch and then the diameter were measured and divide them into two categories 1-4mm and 4.1 to 8mm.Then the cuttings were pretreated for 24 hours, cuttings were dipped in solutions of different concentration of IBA and NAA. The low concentration is used for treatment because for a long duration the hormone is equally absorbed by all the cuttings and the phenolic compounds were leached out, this is beneficial to plants because phenolic compounds creates hindrance in root development.

The pretreated cuttings without any mechanical or physiological injury to the buds or stems were inserted in the polythene bags having the mixture of the rooting media for root induction and development. 1/3 basal portion of the shoots cuttings were placed into the rooting media. Each polythene bag was consisted of one cutting. The data recorded were subjected to statistical analysis by using Factorial Randomized block Design (FRBD). Four replicates and 25 cuttings per treatment per replicate were used for multiplication studies of *Taxus baccata*.

Result and Discussion

In present study, overall 0-4mm diameter cuttings with low concentration of NAA and High concentration of IBA cuttings planted in the open nursery shows the highest survival percentage, higher rooting ability with fresh and dry weight. The rooting responses of *Taxus baccata* cuttings with two types of cuttings used for experiment, different concentration of growth regulators (NAA and IBA) and growing conditions of the open nursery and the net house shows a significant effect on the survival percentage of cuttings, fresh weight and dry weight of roots. The rooting efficiency observed in *Taxus baccata* showed satisfactory results in open nursery condition with stem diameter varying from 0-4mm.

Plate 1: Nursery condition

Plate 2: The Net house condition

Table 1: Effect of type of cuttings, different concentration of growth regulators (NAA and IBA) and growing conditions of an open nursery and the net house on the survival percentage of cuttings

Type of cutting	survival percentage	Fresh weight (gm)of roots	Dry weight (gm) of roots
0-4mm	49.143	20.300	2.535
4.1-8mm	28.071	4.405	0.486
C.D (5%)	2.823	1.410	0.173
S.Em.±	1.003	0.501	0.062
Growth regulators			
100PPM, NAA	51.500	19.422	2.719
300PPM, NAA	30.00	15.640	2.142
500PPM, NAA	11.750	4.238	0.478
100ppm, IBA	39.500	10.634	1.190
300ppm, IBA	46.75	13.216	1.500
500ppm, IBA	58.2500	21.273	2.260
Control	32.500	2.045	0.285
C.D (5%)	5.282	2.637	0.324
S.Em.±	1.877	0.937	0.511
Growing conditions			
Open nursery	43.00	15.552	1.988
Net house	34.214	9.153	1.034
C.D (5%)	2.823	1.410	0.173
S.Em.±	1.003	0.501	0.062

Table 2: Anova of the survival percentage of shoot cuttings

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	p-value
Replication	3	31.286			
Type of cutting(T)	1	12,432.143	12,432.143	220.606	0.00000*
Growth regulators(C)	6	23,229.714	3,871.619	68.701	0.00000*
Interaction (TXC)	6	7,770.857	1,295.143	22.982	0.00000*
Growing condition(G)	1	2,161.286	2,161.286	38.352	0.00000*
Interaction TXG	1	1.286	1.286	0.023	0.88032ns
Interaction C X G	6	933.714	155.619	2.761	0.01711*
Type of cutting X Growth regulators X Growing condition	6	417.714	69.619	1.235	0.29704ns
Error	81	4,564.714	56.354		
Total	111	51,542.714			

*indicates significant and ns-non significant $p \leq 0.05$.

The effect of types of cuttings shows a significant effect on the survival percentage of stem cuttings, the maximum survival percentage (49.143%) was obtained from the 0-4mm diameter of the stem cutting followed by 4.1-8mm (28.071%). Among the different concentration of growth regulators used for propagation of stem cuttings, the maximum survival percentage (58.25%) were obtained from IBA 500ppm followed by NAA 100ppm (51.50%) and the minimum survival were obtained by (11.75%) 500pp NAA. Among the nursery conditions, the maximum survival percentage were obtained from open nursery conditions (43.00%) followed by the net house (34.25%). Simple net house conditions without any temperature and moisture control can increase temperature inside the net house due to that excess temperature can damage the plants and leaves. Excess water supply and high temperature is also deteriorating the growth of shoot cuttings.

The effect of various factors shows a significant effect like type if cuttings, different concentrations of growth regulators and growing conditions etc. but interaction between types of cuttings and growing conditions shows a non-significant effect on the survival percentage and also the interaction between all the three factors also shows non- significant effect. The chemical concentrations play an important role in root-shoot development and effective treatment of the stem cuttings show higher rooting percentage and a higher quality root system with with developed roots (De Klerk *et al.* 1997) [7].

Table 3: ANOVA for average fresh weight of stem cuttings of *Taxus baccata*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	p-value
Replication	3	14.424			
Type of cutting(T)	1	7,074.563	7,074.563	503.500	0.00000*
Growth regulators(C)	6	5,058.173	843.029	59.999	0.00000*
Interaction (TXC)	6	3,470.536	578.423	41.167	0.00000*
Growing condition(G)	1	1,146.770	1,146.770	81.616	0.00000*
Interaction TXG	1	1,011.827	1,011.827	72.012	0.00000*
Interaction C X G	6	1,030.218	171.703	12.220	0.00000*
Type of cutting X Growth regulators X Growing condition	6	1,105.784	184.297	13.117	0.00000*
Error	81	1,138.112	14.051		
Total	111	21,050.407			

*indicates significant and ns- non significant $p \leq 0.05$.

In 1974 (Ghosh and Basu) reported that the effect and the root regeneration or initiation capacities of different growth regulators are different for regeneration and elongation of roots for different plants species is different. The effect of types of cuttings shows a significant effect on fresh weight, the maximum fresh weight (20.300) was obtained from the 0-4mm diameter of stem the cutting followed by 4.1-8mm (4.405) gm. Among the different concentration of growth regulators used for propagation of stem cuttings, the maximum weight (21.273) was exhibited by 500ppm IBA followed by (19.422) 100ppm NAA and the minimum weight (2.045) was obtained by control C_0. The effect of growing conditions shows a significant effect on average fresh weight of roots, the maximum weight obtained from open nursery conditions (15.552) followed by the net house (9.153). All the factors play a significant role on fresh weight of roots because the growth hormones, growing conditions and types of cuttings absorb the nutrients and hormones according to the size of cutting diameter and the cuttings affected by both conditions whether the conditions are favourable or adverse conditions.

Table 4: ANOVA for average dry weight of stem cuttings of *Taxus baccata*

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	p-value
Replication	3	0.160			
Type of cutting(T)	1	117.625	117.625	553.152	0.00000*
Growth regulators(C)	6	81.470	13.578	63.855	0.00000*
Interaction (TXC)	6	66.997	11.166	52.511	0.00000*
Growing condition(G)	1	25.480	25.480	119.822	0.00000*
Interaction TXG	1	23.699	23.699	111.449	0.00000*
Interaction C X G	6	29.421	4.903	23.059	0.00000*
Interaction T X C X G	6	30.343	5.057	23.782	0.00000*
Error	81	17.224	0.213		
Total	111	392.419			

*indicates significant and ns- non significant $p \leq 0.05$.

The effect of types of cuttings shows a significant effect on dry weight of roots, the maximum dry weight (2.535) were obtained from 0-4mm diameter of stem cutting followed by 4.1-8mm (0.486)gm. Among the different concentration of growth regulators used for propagation of stem cuttings, the maximum weight (2.719) were obtained from 100ppm, NAA followed by 500ppm IBA (2.260) and the minimum weight obtained by (0.285) control C_0.The effect of growing conditions shows a significant effect on an average fresh weight of roots, the maximum weight obtained from open nursery conditions (1.988gm) followed by the net house (1.034gm).

The pretreatment of growth hormones like IBA and NAA may have a direct or indirect effect to stimulate the cuttings for root development by enhancing sugar movement and translocation, to the edge of the cuttings and consequently stimulate rooting (Haissing, 1974) [12]. The effect of growth hormones and planting media used for plant propagation exhibits a significant effect on growth and survival of stem cutting used for plant growth. By using growth hormones the rate of plant survival is increased as compared to control. It has been reported that after pretreatment of IBA for root development in stem cuttings of *Taxus baccta* IBA have been reported as a most effective root inducting hormone (Nandi *et al*. 1996) [17]. Due to that IBA treated cuttings exhibited better rooting performances both in terms of quantity and quality. findings are correlated with Kaul (2008) [14] how found that Cuttings treated with high concentration of NAA shows plant necrosis but lower concentration were helpful to increase rooting percentage in shoot cuttings of Taxus *Taxus wallichiana*. As a result of treatment of cuttings with higher concentration, large numbers of cuttings are died in within a short duration.

The present study exhibited that IBA at 500ppm was the most effectual growth hormone for root initiation and higher rooting percentage in stem cuttings of *Taxus baccata* and similar results were obtained by (Aslam *et al.* 2007; S.P. Singh, 2006) [1, 24] in several number of other yew cultivars. The IBA treatment is important for root regeneration and development and it is helpful for successful propagation and conservation of plant population. Rooting rates have been found to be greater in cuttings from younger and lateral shoots (Goo *et al*. 1990) [11]. It has also been suggested by some other researchers that optimum concentration of Auxins is favorable, while excess/high concentrations of Auxins are toxic to cuttings as well as inhibit root initiation and regeneration (Chauhan and Reddy, 1974; Avanzato *et al.* 1998) [6, 2].

A. Cutting treated with NAA 100ppm

B. Cutting treated with IBA 500ppm

C. Cutting without hormonal treatment

D. Shoots regeneration

Conclusion

The results concluded that the induction of adventitious rooting of *Taxus baccata* stem cuttings can be boost up by hormonal treatment. It indicates that treatment of IBA 500ppm and treatment of NAA 100ppm with diameter 0-4mm in open nursery condition shows significant results for survival percentage, fresh weight and dry weight of rooted cuttings as compared to control. Application of this method is an easy way to achieve a large number of rooted cuttings within a short time period for multiplication and to meet the market demands of species for their products.

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