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Conservation of an endangered medicinal plant *Taxus baccata* through air layering; A method of rapid multiplication

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ABSTRACT

Taxus baccata is categorized as a highly medicinal but endangered species, due to production of several economically important metabolites. *Taxus baccata,* a high altitude medicinal plants attract attention recently because its bark and leaves contain an alkaloid, which is the primary origin of Taxol, a vigorous anti-cancer drug. The formation of adventitious roots during air layering can be induced by various root promoting treatments and hormones which increase the root length and shoot development. Two plant growth hormones Indole-3-Butyric Acid and Napthelic Acetic Acid (IBA and NAA) with different concentration have been used for air layering on *Taxus baccata.* Each treatment was applied to 20 layers in replicates of 4. All the layers were carried out under uniform cultural practice during the entire period of examination. The experiments were conducted at village Ravigram, Joshimath, Chamoli (Uttarakhand) India. The stem layers pre-treatment with IBA 1000 ppm was observed most suitable condition for plant multiplication and regeneration in comparison to NAA. Effect of layering time and different concentration of growth regulators shows variation in formation of roots and callus. So 80.833% survivability was achieved with 1000 ppm of IBA and all other best results were obtained with this concentration followed by 750 ppm NAA.

Key words: *Taxus baccata, Air layering, IBA, NAA, Conservation*

Introduction

The Himalayan region of Uttarakhand is very rich in biodiversity of varieties of medicinal and aromatic plants Kaushik and Dhiman, (2000). *Taxus baccata* is categorized as a highly medicinal but endangered plant, due to production of several economically important metabolites. 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 and multiplication in Himalaya. Due to an increase in consumption and over exploitation of plants from nature, populations of many of them are now depleting regularly (Kumari *et al.,* 2012). It has been heavily exploited to produce the anticancer drug Taxol (Saqib *et al.,* 2006). Taxol has a unique quality of preventing the growth of cancerous cells and exciting potential as an anti-cancer drug. Therefore it is now being used in the treatment of breast cancer, ovarian cancer and non-small cell lung carcinomas (Rowinsky, 1997). Seed of Taxus posses a long dormancy phage ranges from 1.5 to 2 year (Stenfield, 1992) and does not germinate easily and

establish naturally.It ranges from 1800m to 3300masl in Uttarakhand Himalayas (Shakarishvili, 2009). The Himalayan yew is locally known as "Thuner", has greater medicinal properties and economic values existing with other flowering plants in the region. Leaves or needles are also applied externally on forehead in form of paste to provide relief from headache (Gaur, 1999).

Air layering is a way of plant multiplication, in which development and enlargement of adventitious roots are induced in aril stems while still attached to the parent plant. The formation of adventitious roots during air layering can be induced by various root promoting treatments and hormones which increase the root length and shoot development. Success of air layering depends on early root initiation and formation of sufficient fibrous roots. Growing substratum is prime components on which seedling development, establishment and survivability of branches and cuttings are depended Suitable growing medium with proper nutrient content dressing can promote seedling growth, establishment and survival percentage (Sileshi *et al.,* 2007. Season play an important role for successful layering in woody plant species because callus formation and rooting on layers are increased by photoperiod, optimum temperature and sufficient moisture content present in plant habitat (Bose *et al.,* 1986). Auxin is a root promoting hormone used in wide population of plant species (Henrique *et al.,* 2006) and IBA is widely used as a non toxic auxin for root development. Availability of constant moisture content and plant growth regulators are essential conditions for successful air layering (Nautiyal, 2002; Butola and Badola, 2007).

Locations and Climatic conditions of Experimental site

The present investigation is focused on the regeneration potential of *Taxus baccata* through various means of propagations used for clonal multiplication of plant. The experiments were conducted in experimental field at village Ravigram, Joshimath block, District Chamoli (Uttarakhand) India situated 30.57°North and 79.57°East at an elevation of 6150 feet (1890 masl), temperature ranges between 26 °C-27 °C in Summer and minimum temperature 11 °C - 2 °C in winter.

Materials and Methods

Two plant growth hormones Indole-3-Butyric Acid and Napthelic Acetic Acid (IBA and NAA) with different concentration have been used for air layering on *Taxus baccata.* Each treatment was applied to 20 layers in 4 replications. All the stem layers were carried out under uniform cultural practice during the entire time period of examination. For preparation of one litter of stock solutions of 1500 ppm of IBA and NAA, 1500 mg of both growth regulators was weighed and then dissolved in small amount of alcohol and added few drops of ammonium hydroxide to dissolve and to stop precipitation. This is diluted with distilled water to make up one litter. At the time of cutting treatment, the working solutions of various concentrations were prepared from stock solution. For preparation of rooting media, soil+ Farmyard manure + vermicompost are mixed in the ratio of (2:1:1) and sphagnum moss is used as a base media for experiment. The sphagnum moss is dipped in the solutions of growth regulators till half an hour before conducting experiment. Excess moisture was not removed from the moss and then the outer side of the moss placed over soil mixture. The plants of same age were selected and the healthy stems were taken. One inch bark is removed off, two cuts were placed around the bark and were peeled off in between the cuts, after removing the bark a handful rooting media was applied around the peeled stem portion and polythene sheet was wrapped around the medium and both the ends were tightly closed with strings.

Air layered shoots of *Taxus baccata.*

In the present investigation, air layering was carried out in the different month of season; early spring in Feb, late spring in April and the rainy season of July

to take advantage of (i) active growth and (ii) high humidity and favourable condition for root development with sphagnum moss as a rooting media with application of different concentration of IBA and NAA which has already been reported to favour rooting of cuttings (Loach, 1988). The rooting percentage varies in response to season; the optimum temperature between (20-25ºC) and high humidity (75–95%) in rainy season are favourable for maximum growth. Sphagnum moss used as rooting media for air layering absorbs moisture while temperature helps in root initiation.

Results and Discussion

Root development from air layered shoots of *Taxus baccata*

Air layering is an alternative method of multiplication where the roots formation is very slow from stem cuttings. Our observations are supportive with the findings of (Nautiyal, 2002) in Ficus spp. Due to high rain and humidity rooting percentage of survived cuttings varies in response to season because rainy season with rooting hormone provides a favourable environment for inducing roots. The optimum concentration of auxins required for rooting of stem cuttings have already been mentioned by (Blazich, FA. 1988). Further, it depends upon the conditions of stock plant species irrespective whether it is herb, shrub or tree.

The survival of air layers requires high moisture holding rooting media and root promoting growth substances and well matured shoots. Sphagnum moss is one of the best rooting media used for air layering of shoots for highest survival. The main

Table 1. Effect of layering time and different concentration of (IBA and NAA) on average number of roots, root length, root diameter, average no of rooted stem layers and survival percentage of air layered shoots of *Taxus baccata*.

Layering time	Average number of roots	Root length (cm)	Root diameter (mm)	Avg. no of rooted stem layers	Survival (%)
Feb 15(T_1)	9.938	4.454	1.092	8.500	42.500
April 15(T_2)	5.321	4.425	0.944	9.568	47.568
June 15(T_3)	8.616	4.339	1.048	10.545	52.727
CD (5%)	1.084	N/A	0.119	0.760	3.809
S.Em.±	0.386	0.131	0.042	0.270	1.355
Concentration					
IBA 250	7.175	3.416	0.983	7.750	38.750
IBA500	9.625	5.227	1.273	12.083	59.417
IBA750	9.968	6.789	1.322	13.333	66.667
IBA1000	13.053	8.403	1.550	16.167	80.833
IBA1250	8.258	5.670	1.299	8.583	42.917
NAA 250	6.250	2.789	0.794	6.667	33.33
NAA 500	5.500	3.435	1.021	9.667	48.333
NAA750	12.432	6.148	1.293	14.333	71.667
NAA 1000	10.925	3.460	1.023	12.167	60.833
NAA 1250	4.363	3.133	0.754	4.167	20.833
Control	0.000	0.00	0.000	00.00	0.00
CD (5%)	2.076	0.703	0.228	1.455	7.293
S.Em.±	0.738	0.250	0.081	0.571	2.594

aim of this experiment was to trace out best concentration of growth hormone among IBA and NAA for survival and root development of air layers. Present investigation was aimed to demonstrate effective method of clonal propagation for regeneration of *Taxus baccata* through air layering of matured shoots.

Average number of roots

As the data obtained from present investigations revealed that different layering time and different concentrations of IBA and NAA showed significant effect on average number of roots The maximum number of roots were found in February (9.93) followed by June (8.61). Among the different concentrations of IBA and NAA, IBA showed best results for maximum number of roots (13.03) found under IBA 1000ppm which was followed by NAA 750ppm (12.43) in comparison to the control conditions where there was no growth. The lower concentrations of growth regulators have not proved helpful to develop the substantial number of roots.

The results obtained from interaction between layering time and different concentrations of IBA and NAA found significant on number of roots. The maximum number of roots were recorded (16.20) form T_1C_4 followed by T_1C_8 (15.93) during layering time of February month. In mid April the maximum number (9.00) of roots were found from T_2C_4 followed by T_2C_5 (6.92). In month of June, the maximum (15.27) roots were found from T_3C_8 followed by T_3C_4 (13.95) and the minimum number of roots were obtained from T_1C_0, T_2C_0 and as compared to control set (T_3C_0) where no growth has been observed. Data revealed that the most suitable time for layering is February and June. The IBA 1000 ppm and NAA 750 ppm have been best concentrations for performing air layering to obtain maximum number of roots of *Taxus baccata*. The lower concentrations of growth regulators might be not helpful to develop the maximum number of roots because for root development proper nutrient and plant growth regulators are needed for callus initiation.

Suitable concentration of growth substances is required for proper root induction and its development in Taxus species. The different concentrations of IBA and NAA found significant in the present experiment towards the maximization of root which are similar to the findings of (Kaul, 2008). In an another study on air layering conducted by (Purohit *et al.,* 2011), IBA 1000 ppm was found most effective in case of C. tamala. The interaction effect between layering time and different concentration of IBA and NAA was found significant on number of roots. This result indicated that IBA and NAA had synergistic effect on the number of primary roots developed per rooted layer.

The maximum root length (4.45) was found in February followed by April month (4.42) on the month of performing layering. The maximum root length (8.40) cm were found from IBA 1000 ppm followed by IBA750 ppm (6.78) as compared to the root length which was not visible under control set (0.00). The length of roots is affected by layering time; the early spring and rainy seasons are best time for air layering of stems due to favorable environmental conditions. The interaction between layering time and different concentration of IBA and NAA found significant on average root length. The maximum root length (9.15) was recorded form T_{1C4} followed by T_1C_5(7.61) in layering time of February month. In mid April the maximum roots (7.615) were found under T_2C_3 followed by T_2C_4 (7.15). In June the maximum root length (8.905) were found from T_3C_4 followed by T_3C_8 (6.70). There was no development in the root length from control set of all

Table 2. Analysis of variance for effect of layering time and different concentration of (IBA and NAA) on average number of roots on air layered shoots.

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	3	7.782			
Layering time (T)	2	497.612	248.806	38.048	0.00000*
Growth regulators(C)	10	1,770.088	177.009	27.069	0.00000*
Interaction layering time X growth regulator	20	337.534	16.877	2.581	0.00111*
Error	96	627.770	6.539		
Total	131	3,240.786			

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

the three months. The increased in survival percentage might be due to the better water holding capacity of sphagnum moss for longer time.

Similar observations have been mentioned in a study where Sphagnum moss has been reported to have better absorption of water and minerals from the growing media as well as created more favourable environment for root and shoot growth resulting in higher survival percentage of guava air layers (Naik *et al.,* 2017). Geoffery and Sani (2017) performed an investigation in order to find out the best rooting hormone for air layering of in *Synsepalum dulcificum* and found that mean root numbers in per rooted cutting increases with higher concentrations of IBA treatment. Krajnc *et al.,* (2013) observed that IBA play an important role in controlling the growth and development of adventitious roots which is similar to present findings. The increased number of primary and secondary roots might have helped to gain maximum absorption of nutrient and moisture for roots development (Venkatesan *et al.,* 2010).

Average root diameter

The average root diameter shows a significant effect with layering time and different concentrations of growth regulators of IBA and NAA. The maximum root diameter (1.09 mm) was found from February followed by June (1.04 mm). Among the growth regulators used, the maximum root diameter was observed under IBA 1000 ppm (1.55 mm) followed by (1.32mm) in IBA750 ppm concentration. The minimum root diameter (0.75 mm) was observed under NAA 1250 ppm. The interaction between layering time and different concentration of IBA and NAA has been observed non-significant on average root diameter. The maximum root diameter (1.77 mm) were recorded form C_4T_1 followed by C_3T_1 (1.49 mm) under the layering time of February. In mid April, the maximum root diameter (1.50mm) were obtained from T_2C_8 followed T_2C_4 and T_2C_5 (1.29 mm). In the month of June the maximum root diameter (1.58 mm) were found in T_3C_4 followed by T_3C_2 (1.35mm). No root length (0.00) obtained from T_1C_0, T_2C_0 and T_3C_0. Paul and Ch Aditi, (2009) performed an experiment on air-layering of water apple (*Syzygium javanica* L.) and reported NAA (1000 ppm) and IBA (1000 ppm) statistically more significant among all other concentrations of IBA and NAA those are similar to our findings. IBA and NAA at concentration of 1000 ppm level are more effective for enhance rooting on air-layering of *Taxus baccata*. The rainy season is best for air layering, continuous moisture for root formation and optimum

Table 3. Analysis of variance for effect of layering time and different concentration of (IBA and NAA) on mean root length of air layered shoots.

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	3	3.114			
Layering time (T)	2	0.318	0.159	0.212	0.80923ns
Growth regulators(C)	10	641.107	64.111	85.440	0.00000*
Interaction layering time X growth regulator	20	78.759	3.938	5.248	0.00000*
Error	96	72.034	0.750		
Total	131	795.332			

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

Table 4. Analysis of variance for effect of layering time and concentrations of IBA and NAA on average root diameter of air layered shoots.

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	3	0.036			
Layering time (T)	2	0.508	0.254	3.228	0.04400*
Growth regulators(C)	10	21.012	2.101	26.710	0.00000*
Interaction T X C	20	1.576	0.079	1.002	0.46754ns
Error	96	7.552	0.079		
Total	131	30.684			

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

temperature are suitable factors in *Taxus baccata*. Present observation is identical to the findings recorded by Kadami and Dabral, (1954) and Nautiyal (2002) in Spondias piñata. Callus formations found similar under all treatments in all months. Root formation and all other attributes are highest in July month of rainy season as compared to treatments with other months.

The maximum number of rooted stem layers were recorded under June (10.54) followed by April (9.56). Among the concentrations of growth regulators, the maximum numbers of rooted stem layers (16.16) were found in IBA1000 ppm concentration followed by NAA 750 ppm (14.33). No root has been observed under control set (0.00). This method of vegetative propagation is generally successful, because water stress is minimized and carbohydrate and mineral nutrient levels are high. The effect of interaction between layering time and different concentrations of IBA and NAA shows significant impact on numbers of rooted stem layers in each replicate. In the February month the maximum numbers of rooted stem layers (15.25) were found from T_1C_4 followed by NAA 750 ppm (13.25). In April the maximum rooted stem layers were found under IBA concentration of 1000 ppm (15.00) followed by 750 ppm NAA (14.250). Similarly, in July the maximum number of rooted stem layers was found in IBA 1000 ppm (18.25) followed by IBA 750 ppm (15.75). Several factors contribute towards success in the propagation of tree plants. Some of them are stem treatment, water and nutrient supply, environmental condition, age of stock, application of root promoting substances and rooting media etc.

Among these factors concentration of growth regulators and time of operation contribute much towards successful propagation. Davis (1988) concluded that photosynthesis promotes adventitious root formation in most types of leafy cuttings. Several air-layers were found to form only callus at the upper part of the cut in other operating months of layering except rainy seasons. Failure to form root are due to lacking several factors that may lead to unfavorable condition for rooting. Factors such as time for treatment, size of branch, type and amount of rooting hormone used and the moisture content in rooting media may be varied that will affect rooting potential of the plant. The maximum number of primary and secondary roots might be due the accumulation of internal nutrient content and their downward movement in layered stems. Maximum length of longest roots, suggests that higher concentration of IBA stimulated faster growth of roots resulting in maximum length as reported by Tyagi and Patel, (2004).

Various operational timing on layering and dif-

Table 5. Analysis of variance for effect of layering time and different concentration of IBA and NAA on the average number of rooted air layered shoots.

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	3	8.265			
Layering time (T)	2	92.106	46.053	14.332	0.00000*
Growth regulators (C)	10	2,723.061	272.306	84.741	0.00000*
Interaction T X C	20	248.894	12.445	3.873	0.00000*
Error	96	308.485	3.213		
Total	131	3,380.811			

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

Table 6. Analysis of variance for effect of layering time and different concentration of (IBA and NAA) on the survival percentage of rooted air layered shoots.

Source of Variation	DF	Sum of Squares	Mean Squares	F-Calculated	Significance
Replication	3	245.356			
Layering time (T)	2	2,301.197	1,150.598	14.250	0.00000*
Growth regulators (C)	10	67,781.970	6,778.197	83.947	0.00000*
Interaction T X C	20	6,287.803	314.390	3.894	0.00000*
Error	96	7,751.394	80.744		
Total	131	84,367.720			

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

ferent chemical concentrations of IBA and NAA show significant effect on survival percentage of rooted stem layers. The layering time and growth regulators both affect the survivability of *Taxus baccata*. The highest survival percentage (52.72%) was obtained with layering time in the month of June followed by April (42.56%). The highest survivability of rooted stem layers were obtained from IBA 1000 ppm (80.83%) followed by NAA 750 ppm (71.66%). No results were obtained from control conditions (0.00). The effect of interaction between layering time and different chemical concentrations shows a significant effect on survivability of rooted stem layers. The highest survivability percentage (76.25%) was found under IBA 1000 ppm followed by NAA 750 ppm (66.25%) in the month of February. In month of April the highest survival percentage is (75.00). The highest survivability was found under operational period of June with IBA1000 ppm (91.25%) followed by IBA750 ppm with (78.75%) percent. No growth and survivability (0.00) have been observed under control conditions. The rate of survivability shows that the June month is the best time of air layering of *Taxus baccata* stems. Better rooting response of air layers was observed with IBA1000 ppm during the month of July.

IBA has greater chemical stability and low mobility in plant which results in prolonged action giving better chance of success as reported by (Audus 1963). These results have verified the findings of Chauhan and Dua (1982) and Puri and Nagpal (1988) in Dalbergia sissoo. Many workers conducted the studies to prove the effectiveness and superiority of IBA on air layering in several plants species (Chatterjiee *et al.*, 1989; Shukla and Bist, 1994). Layering is usually done during rainy season because rooting process, callus formation and root development nutrient gets accumulated in the plants and encourages quick healing and better callusing. For better development proper concentration of carbohydrates is needed in the branches for air layering. This might be due to the fact that at higher concentration of IBA, the quantity of auxin reaching the cambial region may be adequate for initiating root primordia, so the highest performance was seen at higher concentrations of IBA indicating the possibility of better success with employing higher concentrations of IBA.

The results revealed that application of plant growth regulators IBA 1000 ppm concentration was found to be best in terms of most of the vegetative growth parameters and multiplication rate. This concentration is recommended for establishment of air layers of *Taxus baccata.* This promoted rooting capacity with various root characters of *Taxus baccata.* The stem layers pre-treatment with IBA 1000 ppm was observed most suitable condition for plant multiplication and regeneration in comparison to NAA. It is hoped that findings of present study will be helpful in developing propagation protocol of *Taxus baccata* through air layering.

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