



Original Research Article

Propagation and conservation of Himalayan Yew (*Taxus baccata* L.) through air layering: A Simple Method of Clonal PropagationKamal Kishor^{1*}, Brij M Upreti, YPS Pangtey¹, Ashish Tewari² and Lalit M Tewari¹¹Department of Botany D.S.B. Campus, Kumaun University, Nainital, Uttarakhand, India²Department of Forestry and Environmental Sciences, Kumaun University, Nainital, Uttarakhand, India**Received:** April 02, 2015; **Accepted:** April 27, 2015.

Abstract: Himalayan Yew (*Taxus baccata* L. subsp. *wallichiana* (Zucc.) Pilg.) is a valuable medicinal plant of Himalayan region. Yew is used by Himalayan people by various ways like medicinal, timber and making drinks etc. Poor seed germination, slow growth rate and excessive harvesting of *T. baccata* from the forests, all along the Indian Himalayan Region are the main reason for regeneration failure of this species. In the present study role of some auxins, indol-3-butyric acid (IBA), indole-3-acetic acid (IAA) and α -naphthalene acetic acid (NAA) have been examined for their stimulatory effects on adventitious root formation in air layered shoots of this species. Various concentrations of auxins were applied by using soil paste and with cotton plug on girdled shoots of healthy plants in high altitude forests of Munsyari. The higher concentrations (1000 μ M) of IBA effectively resulted in rooting in air layered shoots, compared to no rooting in control. Clonal plants raised through this method were successfully established *in situ* (Native Habitat) as well as *ex situ* (Mist Chamber and garden) conditions.

Key Words: *Taxus baccata* L., Conservation, Air layering, Auxin, Rooting, Himalaya.

Introduction

Taxus baccata L. subsp. *wallichiana* (Zucc.) Pilg. is an evergreen non-resinous gymnosperm tree attaining heights up to 10-15 m, often with multiple trunks and spreading, rounded or pyramidal canopy. *T. baccata* belongs to family Taxaceae of order Taxales, commonly known as "Lwait" or "Thuner" in Kumaun Himalaya. This genus is widely but sparsely distributed along temperate belt between different altitudes. This tree is found in temperate forests in the Pacific, North-West of North America, East Asia, North Africa and Europe. In the British Isles, *T. baccata* grows from sea level to 425 m asl in England and Wales and to 470 m asl on Purple Mountain, Kerry (Alt. range Br. Pl.) (Thomas and Polwart, 2003). In the north of its range, including Britain, *T. baccata* grows only at fairly low altitudes whereas in the south it is primarily a montane tree growing only on higher mountains in the Mediterranean region, although in the Caucasus Mountains it grows from sea-level to 2050 m asl (Thomas and Polwart, 2003; Bugala, 1978). In Indian Himalayan Region (IHR), *T. baccata* is distributed in temperate zone between 1800 and 3300 m asl (Anonymous, 1976). In central Himalayan region, *Taxus* grows in association with some high altitude tree species like *Abies pindrow* (D. Don) Royle, *Quercus semecarpifolia* Sm. and *Betula utilis* and *Acer caesium* Wall. ex Brandis (Purohit et al., 2001).

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In recent years *T. Baccata* has received much attention during the recent past because its leaves and bark are the prime source of "Taxol", a potent anti-cancer drug (Wani et al., 1971, Purohit et al., 2001). In the Unani system of medicine, the extract from the bark and leaves of *T. baccata* is the source of a drug "Zarnab" used for the treatment of various disorders (Anonymous, 1976). *T. baccata* also have great ethnobotanical significance as people of Himalaya use *T. baccata* bark paste in treatment of bone fracture and externally on the forehead for relief from headache (Gaur, 1999). The extract from the bark and leaves is also used for the treatment of various diseases like bronchitis, asthma, poisonous insect bites and also as an aphrodisiac (Beckstrom-Sternberg and Duke, 1993). The famous traditional winter drink "Jya" is also prepared from the bark extract of *T. baccata* by "Shauka" Tribal community in Munsyari region of Kumaun Himalaya.

The main reasons for the decline of Yew populations globally are widespread deforestation, selective felling of Yew and grazing (Bugala, 1978; Titinssor, 1980; Jahn, 1991). Poor seed germination, slow growth rate and excessive harvesting of *T. baccata* from the forests all along the Indian Himalayan Region for the extraction of "Taxol" (Shukla et al., 1994; Rikhari et al.,

1998) is the main reason for reduction of this species from forest areas. Degradation of old populations and low regeneration in its natural habitat are the main problems in the conservation of *T. baccata*. The natural regeneration of this species through seeds is adversely affected by low seed viability due to prolonged dormancy period and poor establishment of seedlings in the natural conditions. Propagation through vegetative means by using stem cuttings and tissue culture methods has been also practiced for multiplication of this species in past (Nandi et al., 1996, 1997). The success of establishment of cuttings by this method is 15%-55% and most of plants generally fail to survive during hardening and field trials. Therefore, the present study was undertaken to develop simple and efficient propagation method for *T. baccata* through air layering of lateral shoots.

Materials and Methods

The air layering experiment was carried out over two years (July 2012-October 2014) in natural habitat of *T. baccata* in high altitude forests of Munsyari region of Kumaun Himalaya. The mature healthy plants were selected in Kalamuni (Ele: 2720m asl, N 30°02'01.0" E0 80°12'39.5") and Thamrikund (Ele: 2910m asl, N 30°01'56.0" E0 80°14'57.0") forest for air layering in Munsyari region. The air layering experiments were carried out during rainy season. The layered shoots were treated with various concentrations test chemicals (500, 1000, 2000 and 5000 μ M) of indol-3-butyric acid (IBA), Indole-3-acetic acid (IAA) and α -naphthalene acetic acid (NAA) (All from HIMEDIA, Mumbai, India) and systematic fungicide Bavistin (BASF India Ltd., Mumbai, India). Total 10 shoots of 5-8 mm diameter (250-320 cm, CBH trees) were used for each treatment and untreated was used for control. The careful removal of bark (1.5-2cm width) with sharp knife at the node of shoot was followed by application of various test chemicals mixed with soil paste or directly by using test solution dipped cotton plug around the layer. The layer is then covered by moss pad and wrapped with perforated polythene sheet, tied with thread at both the end and observations were taken at 20-25days intervals. Afterwards well rooted shoot were cut 5 cm below the girdle with help of sharp secateurs. Out of these 50% rooted cuttings were directly planted in their natural habitat and 50% were transported in laboratory in

wet cotton sheets. These rooted cuttings were transferred in pots containing mixture of soil and farmyard manure and kept inside mist chamber. Following this, after 25 days these plants were transplanted in open air at D.S.B. Campus Botanical Garden and further observation were carried out.

Results and Discussion

The callus formation and rooting in air layered shoots were recorded upto 120 days after application of different concentrations of hormonal treatments with soil paste and cotton strip. Maximum rooting (90%) was induced by IBA at 1000 μ M concentrations when test solution applied as soil paste with an average rooting of 18.21 ± 5.52 roots per layered shoot and an average of 10.58 ± 2.68 cm and 2.02 ± 0.48 mm, root length and diameter respectively (Table 1 & 2) compared to no rooting in control. While, when test solutions were applied by dipped cotton plug, maximum rooting (80%) were induced by 500 μ M and 1000 μ M concentrations equally with different average number of roots, length and diameter (Table 2). The effect of different concentrations of IBA, IAA and NAA on rooting, root length and diameter are presented in table 1 and 2.



Figure 1: (A): Air layered shoot in habitat (B-C) Cuttings of rooted shoots (D) Transferred rooted air layered shoots in pots under mist chamber (E) One year old plant raised through air layering growing in Botanical Garden D.S.B. Campus Nainital (F) One year old plant raised through air layering growing in Natural habitat (Munsyari forest).

Table 1: Effect of Auxin Concentrations on *T. baccata* rooting (percentage) in air layered shoots

S No.	Concentrations (μM)	Solution Mixed Soil Paste			Solution Dipped Cotton Plug			Control
		IBA	IAA	NAA	IBA	IAA	NAA	
1	250(μM)	50%	30%	20%	50%	40%	20%	0%
2	500(μM)	80%	70%	40%	80%	70%	40%	0%
3	1000(μM)	90%	80%	70%	80%	70%	60%	0%
4	2000(μM)	20%	40%	70%	40%	70%	70%	0%
5	5000(μM)	Toxic	0%	50%	0%	0%	50%	0%

0% indicates no rooting at all and Toxic indicates no rooting and killing shoots above layer

Table 2: Effect of Auxin Concentrations on *T. baccata* rooting after 120 day of treatment

S. No.	Treatments	Solution Mixed Soil Paste			Solution Dipped Cotton Plug		
		Avg. no. of Roots (Mean \pm SE)	Avg. Root Length (cm) (Mean \pm SE)	Avg. Root Diameter(mm) (Mean \pm SE)	Avg. no. of Roots (Mean \pm SE)	Avg. Root Length (cm) (Mean \pm SE)	Avg. Root Diameter (mm) (Mean \pm SE)
1	IBA(250 μM)	13.58 \pm 3.83	4.25 \pm 1.98	1.02 \pm 0.12	6.15 \pm 2.36	3.66 \pm 0.88	1.05 \pm 0.15
2	IBA(500 μM)	16.25 \pm 2.54	7.31 \pm 0.52	1.26 \pm 0.19	8.61 \pm 2.68	5.82 \pm 0.75	1.16 \pm 0.12
3	IBA(1000 μM)	18.21 \pm 5.52	10.58 \pm 2.68	2.02 \pm 0.48	10.21 \pm 4.18	8.56 \pm 1.56	1.62 \pm 0.12
4	IBA(2000 μM)	11.00 \pm 1.02	7.42 \pm 0.85	1.23 \pm 0.22	5.20 \pm 2.63	4.42 \pm 1.28	1.03 \pm 0.00
5	IBA(5000 μM)	0.00	0.00	0.00	0.00	0.00	0.00
6	IAA(250 μM)	11.26 \pm 1.58	4.58 \pm 1.98	1.38 \pm 0.12	4.96 \pm 1.66	2.88 \pm 1.42	1.04 \pm 0.18
7	IAA(500 μM)	12.00 \pm 1.09	4.86 \pm 1.53	1.42 \pm 0.12	6.25 \pm 2.13	2.86 \pm 0.98	1.12 \pm 0.25
8	IAA(1000 μM)	16.35 \pm 3.55	6.91 \pm 2.06	1.50 \pm 0.23	8.32 \pm 1.96	4.11 \pm 1.11	1.21 \pm 0.22
9	IAA(2000 μM)	11.48 \pm 0.84	3.52 \pm 1.10	1.33 \pm 0.38	5.45 \pm 2.22	3.52 \pm 1.12	1.20 \pm 0.00
10	IAA(5000 μM)	0.00	0.00	0.00	0.00	0.00	0.00
11	NAA(250 μM)	9.16 \pm 0.94	2.86 \pm 0.75	0.96 \pm 0.11	3.26 \pm 1.11	2.19 \pm 0.22	0.90 \pm 0.00
12	NAA(500 μM)	10.26 \pm 0.76	3.75 \pm 0.58	1.05 \pm 0.15	5.82 \pm 1.98	3.56 \pm 1.58	0.96 \pm 0.00
13	NAA(1000 μM)	14.58 \pm 1.25	5.62 \pm 0.63	1.27 \pm 0.18	5.18 \pm 1.25	5.15 \pm 1.56	1.12 \pm 0.15
14	NAA(2000 μM)	12.86 \pm 1.68	5.13 \pm 1.13	1.13 \pm 0.18	2.94 \pm 1.25	6.13 \pm 0.98	1.17 \pm 0.13
15	NAA(5000 μM)	11.94 \pm 1.33	3.15 \pm 1.15	1.08 \pm 0.12	2.55 \pm 0.58	3.25 \pm 0.86	0.98 \pm 0.15
16	Control	0.00	0.00	0.00	0.00	0.00	0.00

SE = Standard error of mean. All values are an average of 10shoots.

0.00 indicates no rooting at all.

The air layering is an effective method for clonal propagation of highly medicinal and ethnobotanically important Himalayan tree species *T. baccata* by treatment with various concentrations of auxins (IBA, IAA and NAA). While various concentrations of auxins (IBA, IAA and NAA) were examined upon girdle with soil paste and cotton plug. 1000 μM solution is found most effective when applied as soil paste as well as dipped cotton plug. It was also observed that higher concentrations of IBA ($\geq 5000\mu\text{M}$) are toxic and kills layered shoots, while IAA at this concentration do not behave such manner but does not stimulate root induction. The auxin (IBA) has also been reported effective in recent past inducing rooting in layered shoots of higher altitude trees like *Quercus serrata* Murray, *Myrica esculenta* Buch.-Ham. ex D. Don, *Quercus glauca* Thunb. *Cinnamomum tamala* (Buch.-Ham.) Nees & Eb erm. (Srivastava et al., 2000; Purohit et al., 2004, 2005, 2011). However successful layering has also been reported in plants growing in lower altitudes (Nagpal et al., 1985; Gupta et al., 1998) by auxin treatments.

Conclusion

This study reveals that auxins play a vital role in rooting of air layered *T. baccata* shoots, in which natural regeneration via seeds are very poor. Thus clonally raised plants through air layering method could be boon for *T.baccata* conservation in Himalaya. Moreover, this method is inexpensive and easy to perform to *in situ* conditions in tough terrains of Himalaya. This method is superior to tissue culture and cutting raised techniques and don't need hardening process, during which most of plants generally fails to survive. The regeneration and conservation could be improved by propagation through air layering of this valuable Himalayan species. Further, well rooted plants thought air layering could be obtain within short time period and will be helpful for cultivation and conservation of this medicinally valuable plant.

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