EFFECT OF PHYTOHORMONES ON PROPAGATION OF HIMALAYAN YEW (*TAXUS BACCATA* L.) THROUGH STEM CUTTINGS

RAKESH PRASAD KHALI* AND AVINASH. K. SHARMA

Non-Wood Forest Products Division,
Forest Research Institute, Dehra Dun (India)

Introduction

Taxol, a drug for the treatment of ovarian and breast cancers is obtained from *Taxus baccata* L. sub spp. *wallichiana* (Zucc.) Pilger. In recent years, the extensive and reckless collection of *Taxus baccata* has posed a serious threat to this important Himalayan tree (Nandi *et al.*, 1996). This trend is affecting the genetic resource base of the species. Natural regeneration of *Taxus baccata* is poor and its growth rate is very slow. Therefore, to counter the continuous degradation of this valuable Himalayan tree species and augment its natural regeneration, attempts for artificial regeneration are urgently called for. Moreover, since the species is valued for its "Taxol" content, it becomes essential that the plantable seedlings be raised from elite plants that have higher active principle contents. Since seed germination in *Taxus baccata* is very poor even after application of different techniques of breaking the dormancy (Khali, 2001), vegetative propagation through stem cuttings may possibly be the only viable option for augmenting its natural regeneration and also for large-scale propagation and cultivation. This technique would potentially provide adequate supply of superior quality planting material with desirable characteristics of elite trees. Therefore, the present study was conducted to understand the development of suitable vegetative propagation methods for mass multiplication of *Taxus baccata*.

Material and Methods

Collection of Taxus baccata cuttings : New juvenile shoots from lower part of the canopy of mature *Taxus baccata* trees were used to obtain the cuttings. The experimental material was collected from Lokhandi area in the Kanasar Forest Range of Chakrata Forest Division, Dehra Dun in Uttaranchal State of India. Lokhandi is situated between 20° 26' and 31° 2' N-latitude and 77° 38' and 78° 4' E-longitude. The area experiences a wide range of temperature variations ranging from below 0°C in winters to a maximum of 35°C during summers and a mean rainfall of 1,600mm per annum.

Treatments of cuttings: Fifteen to twenty centimeter (cm) long stem cuttings with 3-4 nodes and 0.5 to 1.0 cm in diameter were used in the present study. The cuttings were treated with different phyto-hormones viz. Indole-3-Acetic Acid (IAA), Indole Butyric Acid (IBA), Gibberellic Acid

* Presently at Plant Physiology Branch, Botany Division, FRI, Dehra Dun (Uttaranchal).

(GA_3) and Naphthalene Acetic Acid (NAA) in different concentrations (1,000, 2,500, 5,000, 10,000 and 12,500 ppm) and with two different modes of applications for induction of rooting. The mode-1 consisted of dipping the cuttings in aqueous hormonal solution(s) for 18 hours prior to planting, while the mode-2 comprised of application of dry hormone(s) mixed thoroughly with talcum powder immediately at the time of planting.

The treated cuttings were planted in sterile sand medium (mixed with concentrated Sulphuric Acid and washed with water until all the acid was washed out completely) in cemented tanks at the Non-Wood Forest Products Division, Forest Research Institute nursery at Chakrata, Dehra Dun, Uttaranchal. The sand around cuttings was kept firm but was not compacted. Cuttings were watered regularly depending upon the weather conditions and sand moisture status. The beds were kept free of weeds manually. The cuttings were protected from direct sunlight and to maintain high humidity (70 per cent) by a poly-sheet covering. The cuttings were observed periodically (at 15 days interval) for initiation of rooting until rooting was completed.

Results

Effect of various hormones on rooting percentage : Analysis of data (Table 1) reveals that the cuttings responded differentially to various phyto-hormones, their concentration levels and the modes of application. Application of IBA induced higher rooting (percentage) irrespective of the mode of application. The highest rooting was achieved with 10,000 ppm of IBA (Mode-2) that was significantly higher compared to their corresponding values for

Fig. 1

Rooting induced by IBA in *Taxus baccata* cuttings

Mode-1. IAA was the next suitable hormone (after IBA) followed by NAA and GA_3 respectively. Control cuttings were observed to have lowest rooting percentage. Mode-2 was significantly superior to Mode-1 in the induction of higher rooting irrespective of the hormones and their concentrations used.

Effect of various hormones on number of primary roots : IBA was observed to be the best hormone for the induction of higher number of primary roots in the rooted cuttings (Table 2). The highest number of primary roots was observed in cuttings treated with 10,000 ppm of IBA (Mode-1) that was significantly higher compared to their corresponding values for Mode-2. IAA was the next best hormone followed by NAA and GA_3 respectively for the induction of higher number of primary

Table 1

Mean values of rooting percentage of Taxus baccata cuttings in response to various hormonal levels and modes of application

Mode	Level (ppm)	Growth Regulator					Control
		IAA	IBA	GA_3	NAA	Mean	
1	12,500	30.00	38.33	16.67	23.33	27.08	18.33
	10,000	50.00	60.00	25.00	40.00	43.75	
	5,000	48.33	60.00	21.67	33.33	40.83	
	2,500	41.67	56.67	18.33	33.33	37.50	
	1,000	31.67	36.67	13.33	26.67	27.09	
	Mean	40.33	50.33	19.00	31.33		
2	12,500	36.67	43.33	16.67	26.67	30.84	16.67
	10,000	58.33	76.67	33.33	46.67	53.75	
	5,000	50.00	63.33	28.33	43.33	46.25	
	2,500	46.67	63.33	23.33	41.67	43.75	
	1,000	40.00	58.33	16.67	30.00	36.25	
	Mean	46.33	61.00	23.67	37.67		
Mean		43.33	55.67	21.33	34.50		
Level mean	12,500	10,000	5,000	2,500	1,000		
	28.96	48.75	43.54	40.63	31.67		

$CD_{(0.05)}$ Mode = 2.07, Regulator = 2.93, Level = 3.28, Regulator x Level = 6.57, Treatment x Mode = NS

roots. Control cuttings were observed to produce the lowest number of primary roots. Mode-1 was significantly superior to Mode-2 irrespective of hormone and their concentrations used for the successful induction of higher number of primary roots in the juvenile cuttings of *Taxus baccata*.

Effect of various hormones on primary root length : The results (Table 3) revealed that higher primary root length in rooted cuttings was induced by the application of IBA. The highest primary root length was observed in cuttings treated with 10,000-ppm concentration of IBA (Mode-1) that was significantly higher compared to their corresponding values for Mode-2. IAA was the second best hormone followed by NAA and GA_3 respectively for the production of higher root length. Control cuttings were observed to produce the lowest root length. Mode-1 was significantly superior to Mode-2 irrespective of hormones and their concentrations used for the production of higher primary root length in the juvenile

Table 2

Mean values of primary root numbers of Taxus baccata cuttings in response to various hormonal levels and modes of application

Mode	Level (ppm)	Growth Regulator					Control
		IAA	IBA	GA_3	NAA	Mean	
1	12,500	13.32	21.89	4.61	5.88	11.43	0.97
	10,000	19.81	56.12	5.62	8.13	22.42	
	5,000	13.12	29.09	3.45	6.31	13.00	
	2,500	11.90	34.27	3.67	5.43	13.82	
	1,000	5.67	24.43	1.61	4.94	9.16	
	Mean	12.76	33.16	3.79	6.14		
2	12,500	9.10	8.89	1.20	3.86	5.76	1.43
	10,000	11.18	16.13	2.42	7.25	9.25	
	5,000	6.56	11.18	2.93	3.91	6.15	
	2,500	7.63	10.06	2.46	4.94	6.27	
	1,000	7.31	13.94	2.24	3.34	6.71	
	Mean	8.36	12.04	2.25	4.66		
Mean	10.56	22.60	3.02	5.40			
Level mean	12,500	10,000	5,000	2,500	1,000		
	8.59	15.83	9.57	10.05	7.94		

$CD_{(0.05)}$ Mode = 0.95, Regulator = 0.92, Level = 1.50, Regulator x Level = 3.01, Treatment x Mode = 4.25

cuttings of *Taxus baccata*.

Discussion

The result of the present investigations revealed that amongst all the phyto-hormones tried IBA is the most effective hormone for the induction of higher rooting percentage in the juvenile cuttings of *Taxus baccata*. The present study further revealed that application of IBA at 10,000-ppm concentration is most suitable for induction of a better rooting percentage, higher number and greater length of primary roots in the *Taxus baccata* cuttings.

A varying degree of success in induction of rooting by IBA in juvenile explants and sturdy woody cuttings of *Taxus baccata* has been reported from other temperate regions of the country (Nandi *et al.*, 1996; Mitter and Sharma, 1999; Dubey, 1997). Though the rooting percentage in the present study is slightly lower compared to that reported by above authors, a higher

Table 3

Mean values of primary roots length (cm) of Taxus baccata cuttings in response to various hormonal levels and mode of application

Mode	Level (ppm)	Growth Regulator					Control
		IAA	IBA	GA_3	NAA	Mean	
1	12,500	5.66	8.45	2.41	3.51	5.01	1.45
	10,000	8.94	12.50	3.91	5.65	7.75	
	5,000	8.63	9.66	3.04	5.08	6.60	
	2,500	6.95	10.44	4.31	6.46	7.04	
	1,000	4.31	8.75	1.99	5.68	5.18	
	Mean	6.90	9.96	3.13	5.28		
2	12,500	5.08	5.44	2.24	3.11	3.97	2.41
	10,000	6.73	8.05	3.67	4.86	5.83	
	5,000	5.86	6.14	2.84	6.70	5.39	
	2,500	4.36	6.18	2.69	7.34	5.14	
	1,000	6.05	9.23	1.56	3.58	5.11	
	Mean	5.62	7.01	2.60	5.12		
	Mean	*6.26*	*8.48*	*2.87*	*5.20*		
Level	12,500	10,000	5,000	2,500	1,000		
mean	4.49	6.79	6.00	6.09	5.14		

$CD_{(0.05)}$ Mode = 0.61, Regulator = 0.86, Level = 0.96, Regulator x Level = 1.93, Treatment x Mode = 2.73

number of primary root formation and also higher primary root length under the influence of IBA application have been obtained in the present trials, which are much higher compared to above mentioned studies. It must be borne in mind that a higher root to shoot ratio is advisable for achieving a higher survival rate after field transplantation. It has also been suggested that optimum concentration of auxin is favourable, while supra optimum auxins are toxic to the root regeneration (Chauhan and Reddy, 1974; Avanzato *et al.*, 1988). The differential response to varying concentrations of hormones observed in the present study could be due similar action of auxin in *Taxus baccata* as well.

The results of the present study indicate that specific responses to hormonal induction of rooting and mode of application may be carefully used by the nursery managers to achieve genetically superior planting stock of *Taxus baccata* to enhance resources availability for meeting the market demand of the species.

Acknowledgments

The authors are thankful to The Director, Forest Research Institute, Dehra Dun for the financial support and Shri Rajeev Pandey, Scientist, Statistical Branch, Forest Research Institute, Dehra Dun for statistical analysis and valuable suggestions.

SUMMARY

Like many other medicinal plants, *Taxus baccata* is also facing extinction in its natural habitats due to increased market demand and consequent excessive destructive harvesting. Vegetative propagation through stem cuttings was tried for germplasm of *Taxus baccata* collected from Lokhandi area of Dehra Dun District (Uttaranchal) with varying concentrations of different phyto-hormones (IAA, IBA, NAA and GA_3) and modes of application. IBA was the most suitable hormone, followed by IAA, NAA and GA_3 respectively for the induction of higher rooting percentage, number of roots and length of roots. The study revealed that IBA at 10,000 ppm is optimum for induction of better rooting percentage, higher number and greater length of primary roots in the juvenile stem cuttings of *Taxus baccata*.

स्तम्भ-कलमों द्वारा हिमालयी धनुर्वृक्ष (*टैक्सस बैक्काटा लि०*) के प्रवर्धन पर पादप हार्मोनों का प्रभाव

राकेश प्रसाद खली व अविनाश के० शर्मा

सारांश

अन्य औषध पादपों की भांति *टैक्सस बैक्काटा* को भी अपने प्राकृतिक आवासों में बाज़ार की बढ़ती जा रही मांग और उसके फलस्वरूप अत्याधिक विनाशक फसल-कटाई के कारण विलुप्त हो जाने का सामना करना पड़ रहा है । स्तम्भ से काटी कलमों द्वारा *टैक्सस बैक्काटा* का बीजप्ररस, भिन्न-भिन्न प्रकार के पादप हार्मोन (इण्डोल एसेटिक अम्ल, इण्डोल ब्यूटिरिक अम्ल, न्यूक्लिइक एसेटिक अम्ल और गिबैरैलिक अम्ल) के विभिन्न संकेन्द्रणों तथा उपयोग के तरह-तरह के तरीकों से तैयार करने का प्रयास किया गया । कलमें देहरादून जिले के लोखण्डी क्षेत्र से संग्रह की गई । इण्डोल ब्यूटिरिक अम्ल सबसे उपयुक्त हार्मोन रहा, इसके बाद क्रम से इण्डोल एसेटिक अम्ल, न्यूक्लिइम एसेटिक अम्ल और गिबैरेलिक अम्ल रहे जिससे जड़े निकलने का अधिक प्रतिशत जड़ों की अधिक संख्या और अधिक लम्बाई प्राप्त हुए । इस अध्ययन से पता लगा कि जड़ें निकलने का श्रेष्ठतर प्रतिशत, प्राथमिक जड़ों की अधिक संख्या और अधिक लम्बाई, *टैक्सस बैक्काटा* की बाल स्तम्भ कलमों में प्रेरित कराने के लिए इण्डोल ब्यूटिरिक अम्ल 10,000 भाग प्रति दस लाख भाग पानी में मिलाकर उपयोग करना इष्टतम फलदायी रहता है ।

References

Avanzato, D.G., A. Couvillon and F.A. Pokorny (1998). The influence of P-IIB (Phenyl indole 3-thiolobutyrate), Aryl ester of IBA, on the rooting of "Redhaven" peach. *Acta. Hort.*, **227**: 197-201.

Chauhan, K.S. and T.S. Reddy (1974). Effect of growth regulators and mist on rooting in stem cuttings of plum. *Ind. J. Hort.*, **32** : 229-231.

Dubey, K.P. (1997). Himalayan yew (*Taxus baccata*) conservation: A vegetative approach. *Indian Forester*, **123** (12): 1150-1154.

Khali, R.P. (2001). Ecological studies on *Taxus baccata* L. in relation to regeneration and conservation. *Ph.D. Thesis* FRI Deemed University, Dehra Dun.

Mitter, H. and A. Sharma (1999). Propagation of *Taxus baccata* Linn. by stem cuttings. *Indian Forester*, **125** (2): 159-162

Nandi, S.K., L.M.S. Palni and H.C. Rikhari (1996). Chemical induction of adventitious root formation in *Taxus baccata* cuttings. *Plant Growth Regulation*, **19**(2): 117-122.