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EFFECTS OF HORMONES (IBA & IAA) ON THE PROPAGATION OF HIMALAYAN YEW IN PAKISTAN: A CONSERVATION APPROACH

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Abstract

Himalayan yew (*Taxus wallichiana*) is one of the most important medicinal plant species for cancer treatment. The tree contains anticancer drug 'Taxol' which is mainly used for the treatment of ovarian, breast, and AIDS-related cancers. However, Himalayan yew is an endangered tree species and requires high conservation attention due to declining population. This study was therefore conducted for the propagation of Himalayan yew by the regeneration of fresh stem cuttings using hormone treatments of Indole Butyric acid (IBA) and Indole acetic acid (IAA) at 2000 to 7000 ppm concentrations. A total of 3840 stem cuttings were treated with IBA and IAA and grown in polythene bags containing soil at Lalku valley, Swat, Khyber Pakhtunkhwa (KP), Pakistan. The influence of IBA treatment at 7000 ppm concentration showed survival of 85.22% (average number of roots=10.4, average length of roots=15.5 cm, average number of leaves=92.4 and average number of sprouts=3.3) while IAA showed survival of 81.11% (average number of roots=9.1, average length of roots=14.6 cm, average number of leaves=84.0 and average number of sprout=3.0) at the same concentration. The lowest survival of 40-45 % (average number of roots=4.2, average length of roots=8.0 cm, average number of leaves=32.2, average number of sprouts=1.7) was observed for the control stem cuttings. This study recommends the application of IBA (7000 ppm) as a better hormone for the conservation and propagation of Himalayan yew.

Key words: Vegetative propagation; Hormone treatment; Endangered species; Himalaya; *Taxus wallichiana*; Plant conservation.

Abbreviations: IBA: Indole Butyric acid; IAA: Indole acetic acid; KP: Khyber Pakhtunkhwa; TIFAC: Forecasting and Assessment Council; CITES: Convention for International Trade in Endangered Species; MANOVA: Multivariate Analysis of Variance; IAA: Indole acetic acid.

Introduction

T. wallichiana is one of the threatened medicinal plants of the Himalayan Region (Uniyal, 2013) commonly known as Himalayan yew and belongs to the family Taxaceae (Hussain *et al*., 2013). Other names given to the species are Banrya in Pushto, Barmi in Hindi and Urdu, Common yew and Himalayan yew in English (Mulliken *et al*., 2008). It is a coniferous, evergreen and slow-growing tree having needle-like leaves and bright red fruit (Fig. 1) (Hussain *et al*., 2013) with a lengthy seed dormancy period of about 1.5 to 2.0 years and shows a growth rate (increase in its circumference) of 0.4 to 1.3 cm per year (Anon., 1976; Chee, 1994). The species survive for an average life of approximately 600 years (Valis *et al*., 2014). It is found in Pakistan, Afghanistan, Bhutan, Indonesia, Nepal, China, Malaysia, Philippines Vietnam, Myanmar, and India (Rahman *et al*., 2013; Mulliken & Crofton, 2008). In Pakistan, it is present in moist temperate forests of Murree, Galliat, Kaghan, Kurram, Chitral, Kashmir, Swat, and Hazara (Shabir, 2009). Unlike other common coniferous species, the population of *Taxus* occurs in patches not continuous. Its habitat is mainly characterized by moist, mixed coniferous tree forests or cool broad-leaved forests. Due to shade demanding nature, *Taxus* is usually found in association with large tree species such as *Abiespindrow, Betula utilizes Pinus wallichiana, Acer cesium, Rhododendron arboreuand, Quercus semecarpifolia* (Rikhari *et al*., 1998).

Taxus is very important to treat many types of cancer and other diseases like bronchitis, snake and scorpion bites, epilepsy, asthma, aphrodisiac, internal injuries, diabetes, and the diseases of lungs (Rikhari *et al*., 1998; Sharma *et al*., 2014).

In Pakistan, lack of awareness, slow growth rate, agriculture, construction, habitat loss, forest fires, transformation, and grazing, over-harvesting, ornamental use, medicinal use, accidental mortality, lack of management policies, and illicit cuttings are the major threats to the species (Rikhari *et al*., 1998; Iqbal *et al*., 2020; Pant & Samant, 2008). Approximately 10 genera of Taxus are now declining in the Northern Hemisphere in temperate zones (Nhut *et al*., 2007). During 2001-2005, the Technology, Information, Forecasting, and Assessment Council (TIFAC) has reported 45 threatened medicinal plants with a specific recommendation for 7 plants including *Taxus* enlisted in Convention for International

Trade in Endangered Species (CITES) (Appendix II) in 1995 (Nimasow *et al*., 2016; Sharma & Thokchom, 2014; Yadav *et al*., 2013; Nimachow *et al*., 2010; Schippmann, 2001; Saqib *et al*., 2006; Lange, 2002). Recently the existence of Yew is receiving high conservation attention due to the high exploitation rate which has reduced its population by 87% (Haq, 2012; Joshi, 2009; Singh, 1992; Vishnu-Mittre, 1984). Poor regeneration process, slow growth rate, and lengthy seed dormancy of the species significantly contribute to hurdles in its conservation (Steinfield, 1992). Vegetative propagation could be one of the practical options to enhance its natural regeneration. The Taxus species has high regeneration potential by adventitious rooting of fresh stem cuttings (Schneck, 1996). Unlike other Taxus species, *T. wallichiana* is difficult to root and requires a longer time (Fordham *et al*., 1977). The rooting of Taxus stem cuttings is well documented (Nandi *et al*., 1996; Khali, 2001). The present study was aimed to enhance the potential of conservation and propagation of *T. wallichiana* using the stem cuttings of mature trees with the application of various doses of IBA and IAA.

Fig. 1. Leaves and fruit (bright red berries) of Himalayan Yew (*Taxus wallichiana*) from Kalam, Swat (Iqbal *et al*., 2020).

Material and Methods

Study area: This study was conducted in the Lalku valley of District Swat, KP, Pakistan from November 2016 to November 2017. Lalku valley lies at an altitude of 1963 meters above sea level, with a latitude of 35°.1375 and a longitude of 72°.38639. The valley has more Taxus density in the region. Total area of Lalku forest range is 8580 ha with a forest cover of 59.3%. In this region, annual temperature ranges from -2ºC to 34ºC, while average annual precipitation ranges from 1000 to1200 mm.

Collection, preparation, and planting of cuttings: The formal identification of the *T. wallichiana's* plant materials was undertaken by the Directorate of Non-Timber Forest Produce (NTFP), Forest Department, Peshawar; Department of Environmental Sciences, University of Peshawar. Proper permission was granted by the University of Peshawar, Ethical review board. Voucher specimens no. Bot. 20156 (PUP) were deposited in the herbarium of the Department of Botany, University of Peshawar.

Stem cuttings were collected from various mature patches of Himalayan yew. The cuttings were brought to the nursery, raised in Lalku Forest Research Station. The length of the final cuttings was kept 7 to 8 inches and 3 to 4 nodes were retained in each cutting. The needles at the basal portion (about 2 cm) of the stem cuttings were removed and sterilized using 2% benlate (fungicide) before planting. The cuttings were dipped in fungicidal solution for 5 minutes and dried for 20 to 25 minutes in an open environment. The dried cuttings were treated with 50% (Water: Ethanol) concentrated solution of the IBA and IAA (2000-7000 ppm) for 5 minutes and planted in the polythene bags containing soil. The soil was prepared by mixing forest soil, sand and, agriculture soil of the area in1:1:1 and sieved (2 mm) before filling into polythene bags.

Experimental design: The experiment was carried out in a randomized block design with a factorial treatment arrangement. The first trial treatment was carried out with 7 rows and each row contained 20 cuttings. A total of 140 stem cuttings were used in the first trial of IBA treatments (n =140; x 20 cuttings x 1 type of cuttings x 7 IBA treatments, 3 replications). Likewise, 140 stem cuttings were used in second trial of IAA treatments (Table 1). The trials were evaluated for the number of roots, length of roots, number of leaves, sprouts, and survival percentages of cuttings after 20 weeks in 2016-17of planting.

Table 1. Experimental Design of Hormones applications.

Trials	No. of rows	No. of cuttings in each row	Total cuttings	Length of each cutting (inches)	Nos of replications
1st_IBA	7	20	140	7-8	3
2nd_IAA	7	20	140	7-8	3

Statistical analysis

Statistical analysis was carried out using SPSS version 25.0. Mean values of all the parameters (number of roots, length of roots (cm), number of leaves, number of sprouts) were calculated to determine the highest growth of each trial for every applied concentration of hormones (2000 ppm- 7000 ppm).

Multivariate Analysis of Variance (MANOVA) was applied to compare the effect of treatments at different levels.

Results

Test for normality: Results of Kolmogorov-Smirnov and Shapiro-Wilk tests were not significant and the data fulfill normal assumption (Table 2).

The Q-Q plots were used to test the assumptions of normality for all the expected and observed values for the four parameters (number of roots, length of root, number of leaves, and number of sprouts).

Multi-collinearity: Table 3 shows that correlation is significant at the 0.01 level (2-tailed). Correlations were done between the growth parameters (number of roots, root length (cm), number of leaves, and number of sprouts).Strong to moderate correlation was found among No of roots-root lengths (0.736), No of roots-leaves (0.800) and No of roots-sprouts (0.519). A significant correlation was also found among root length-number of leaves (0.718), root length-number of sprouts (0.421). Similarly, a significant correlation was found between the numbers of leaves-number of sprouts (0.541). Hence the data contain no multi-collinearity and fulfil all the assumptions for applying the MONOVA technique.

Since the p-value is <0.05 which means different treatments at the different levels are significant to each other. To check the performance of different concentration levels (2000 ppm – 7000 ppm) a post hoc test was applied. By having the same numbers of replications/ concentrations of hormones/number of plant cuttings, correlation analyses were done (Table 4).

Since two different hormones, IAA and IBA were used; therefore we interpret the differences by their mean values. The application of 7000 ppm hormone treatment showed highest marginal means for the number of roots (10.4, 9.1), roots length (11.82 cm, 10.27 cm), and the number of leaves (92.40, 88.30), sprout numbers (3.30, 3.0) and survival percentage (85.22%, 81.11%) throughout the trial. The next best dozes were 6000 ppm followed by 5000 ppm, 4000 ppm, 3000 ppm, and 2000 ppm. The lowest growth was observed in control cuttings (Figs. 2 to 6).

The results revealed that the stem cuttings respond differently to various concentration levels of hormones (2000 ppm-7000 ppm) and the mode of application. Comparisons of the various parameters (number of roots, length of roots cm, number of leaves, and number of sprouts) and marginal mean values are shown in (Table 4) which revealed that based on observed means the error term is Mean Square (Error) = 0.667 and (*) the mean difference is significant at the 0.05 level (*p*<0.05).

Table 2. Tests of normality.

Parameters	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
IAA number of roots	0.089	140	0.008	0.971	140	0.004
IAA root length (cm)	0.109	140	0.000	0.896	140	0.000
IAA number of leaves	0.150	140	0.000	0.933	140	0.000
IAA number of sprouts	0.209	140	0.000	0.898	140	0.000

a. Lilliefors significance correction

Table 3. Pearson correlations for the parameters i.e., number of roots, length of roots, number of leaves and number of sprouts of *T. wallichiana* stems cuttings.

Parameters		Number of roots	Root length (cm)	Number of leaves	Number of sprouts
Number of roots	Pearson Correlation	1	.736**	.800**	.519**
	Sig. (2-tailed)		.000	.000	.000
	N	140	140	140	140
Root length (cm)	Pearson Correlation	.736**	1	.718**	.421**
	Sig. (2-tailed)	.000		.000	.000
	N	140	140	140	140
Number of leaves	Pearson Correlation	.800**	.718**	1	.541**
	Sig. (2-tailed)	.000	.000		.000
	N	140	140	140	140
Number of sprouts	Pearson Correlation	.519**	.421**	.541**	1
	Sig. (2-tailed)	.000	.000	.000	
	N	140	140	140	140

**. Correlation is significant at the 0.01 level (2-tailed)

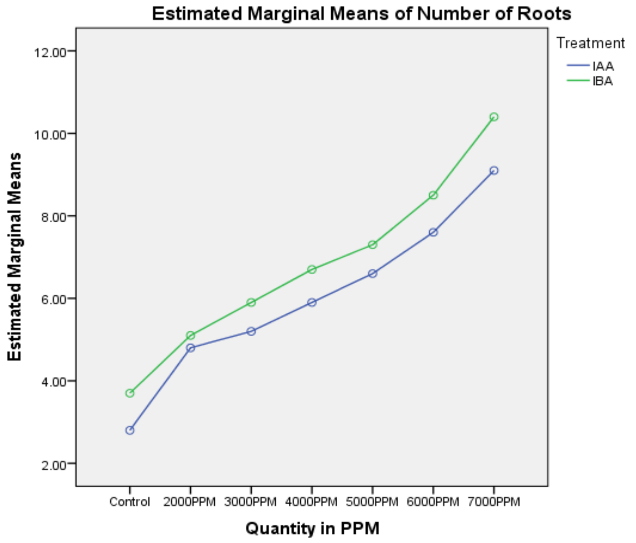

Fig. 2. Comparison of number of roots of *T. wallichiana* fresh stem cuttings by the application of various concentrations of hormones (IBA, IAA).

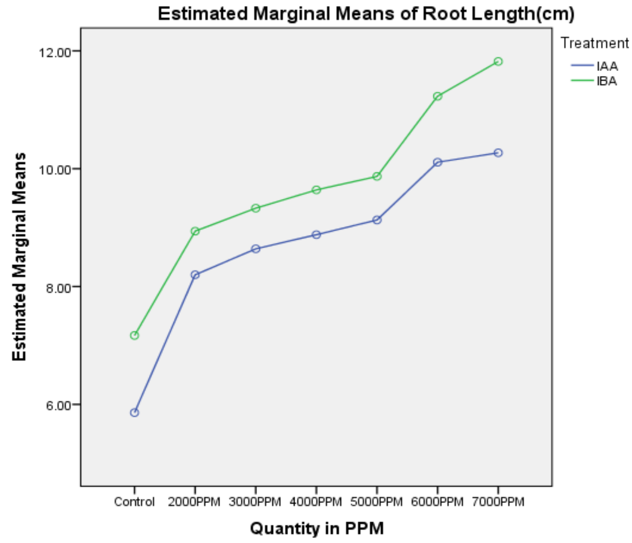

Fig. 3. Comparison of length of roots (cm) of *T. wallichiana* fresh stem cuttings by the application of various concentrations of hormones (IBA, IAA).

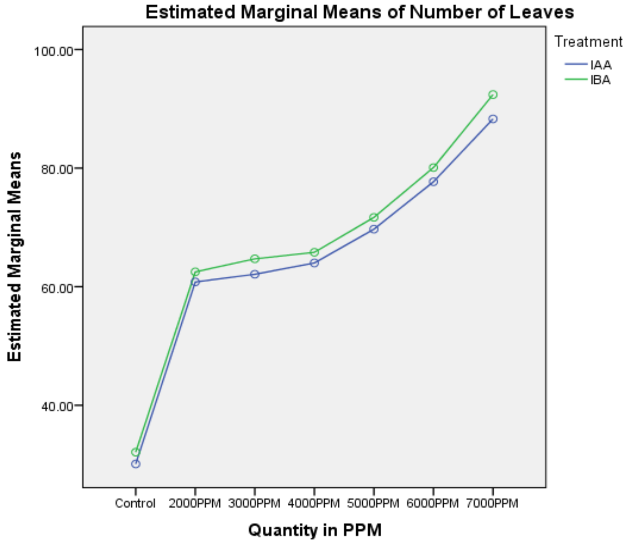

Fig. 4. Comparison of number of leaves of *T. wallichiana* fresh stem cuttings by the application of various concentrations of hormones (IBA, IAA).

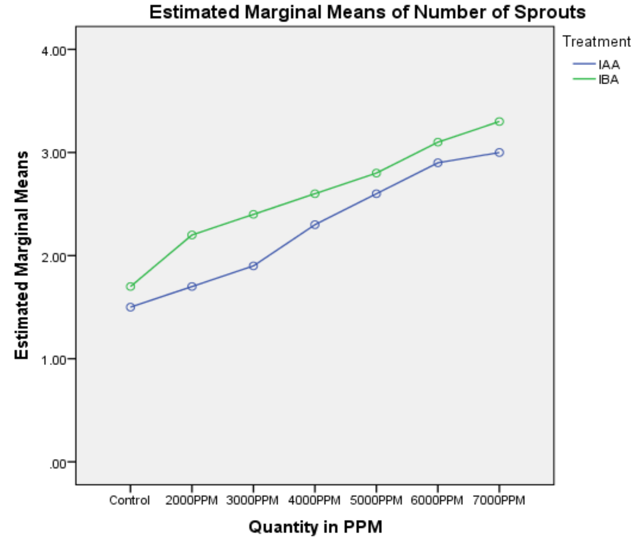

Fig. 5. Comparison of sprouts of *T. wallichiana* fresh stem cuttings by the application of various concentrations of hormones (IBA, IAA).

Discussion

This study indicateed that the application of IBA and IAA hormone treatments on the stem cuttings favorably enhance the growth and vegetative propagation of Himalayan yew. The IBA was found better rooting hormone in comparison to the Indole acetic acid (IAA) since more primary root formations were observed under the influence of IBA application. The least root formation and survival was observed in control stem cuttings with no application of hormone treatments.

The hormone treatments produce roots in fresh stem cuttings by stimulating secondary plant growth that results in the reserve food material mobilization to the root initiation site (Haissig 1974; Philips 1971). The auxins application also increases the rooting and quality of roots in various tree species (Hartman & Kester, 1983).

Previously, various studies reported successful rooting in juvenile shoot cuttings of *T. wallichiana* with different hormone treatments from other temperate areas (Khali & Sharma, 2003; Mishra *et al*., 2000; Mitter & Sharma, 1999; Chandra & Verma, 1989; Dubey, 1997; Nandi, 1997).

Our study results are consistent with Nautiyal *et al*., (1991) who treated hormones on stem cutting of teak for roots induction and concluded that IBA is the favourable and best auxin. Likewise, the vital role of IBA application in the propagation of stem cuttings of *Taxus wallichiana* has been reported in various studies (Aslam *et al*., 2017; Nautiyal *et al*., 2004; Chandra & Verma, 1989; Gurumurti & Bhandari, 1988; Blazich, 1988; Pal, 1992; Singh & Chander, 2001; Mitter & Sharma, 1999; Nandi *et al*., 1996).

However, auxin application more than optimum concentration is toxic to the root regeneration (Chauhan & Reddy, 1974; Avanzato *et al*., 1998). In this study, highest concentration of 7000 ppm of both the auxins (IBA and IAA) was applied which showed favourable response to the growth of *T. wallichiana* cuttings.

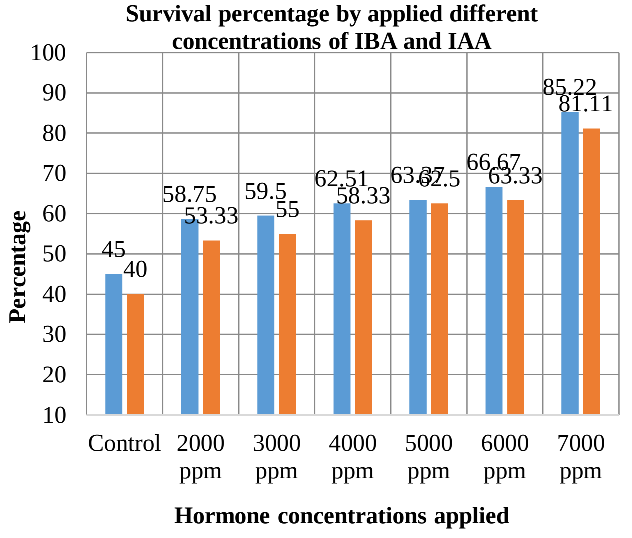

Fig. 6. Survival percentage of *T. wallichiana* cuttings by the application of various concentrations of hormones (IBA and IAA).

The differential response to changing concentrations of hormones for survivals of *T. wallichiana* was observed in the present study. The IBA application may have an indirect effect by increasing the translocation speed and the movement of sugar to the base of the cutting which results stimulate rooting (Haissig, 1974).

IBA application in the current study significantly increased the survival percentage (number of roots, length of roots, number of leaves, and number of sprouts). These results are consistent with the findings of Aslam & Rather (2008). The time duration for this study was kept 20 weeks after plantation of the stem cuttings since the effects of hormone treatments on the growth and induction of adventitious roots can be fully observed after 19 weeks. In general, it has been found that among the various auxins, IBA is more effective for root germination, showed maximum percentage of survival, rooting percentage, percentage of callusing roots per cutting, and length of root per cutting (Chandra and Verma 1989; Aslam *et al*., 2007; Gurumurti & Bhandari, 1988; Singh & Chander, 2001; Blazich, 1988; Pal, 1992). Furthermore, Nasir *et al*., (2018) studied the application of 1000 ppm and 500 ppm of IBA on the *T. wallichiana* cuttings and observed that IBA 500 ppm has slightly less response than IBA 1000 ppm. The IBA 1000 ppm showed the best rooting response on shoot cuttings of *T. wallichiana* in the spring season with a rooting percentage (95%) compared to other treatments in other seasons. In our study, the best response was given by 7000 ppm in the case of both IBA and IAA. These results indicate that by increasing the concentration up to 7000 ppm the growth and propagation of *T. wallichiana* will increase.

By using IBA, with some other cultivar of yew Eccher (1987) reported that the hormone treatment is necessary for the successful conservation and propagation of *T. wallichiana*. Similarly, Koleva *et al*., (2017) reported best response for rooting with IBA followed by IAA and NAA in Sage, Rosemary, and Elderberry. Similarly, the treatments of auxins (IBA, IAA, and NAA) on the root development were studied in species like *Melissa officinalis* (Sevik & Guney, 2013), *Ficus Benjamina* (Topacoglu *et al*., 2016), and *Oryza sativa* (Chhun *et al*., 2003).

Auxins are involved in the formation of the root, cambial cell activation, and lateral bud inhibition. They have been found as naturally existing compounds that promote the formation of root. Similarly, synthetic auxins also stimulate the emergence of roots on stem cuttings. It is well established that both natural and artificial auxins are needed for the initiation of adventitious roots on stem cuttings. Initial cell division of roots is dependent on either endogenous or applied auxins (Gill *et al*., 2006).

Thus, our study suggests the utilization of auxins mainly IBA for regeneration of *T. wallichiana* under natural conditions. The results of our research will be beneficial for developing propagation protocol of *T. wallichiana* species especially for the most temperate climate of the Himalayan Region.

Table 4. Multivariate analysis of variance.

Effect		Multivariate Tests[a]				
		Value	F	Hypothesis df	Error df	Sig.
Intercept	Pillai's Trace	0.988	2574.650[b]	4.000	123.000	0.000
	Wilks' Lambda	0.012	2574.650[b]	4.000	123.000	0.000
	Hotelling's Trace	83.728	2574.650[b]	4.000	123.000	0.000
	Roy's Largest Root	83.728	2574.650[b]	4.000	123.000	0.000
Harmons	Pillai's Trace	0.126	4.448[b]	4.000	123.000	0.002
	Wilks' Lambda	0.874	4.448[b]	4.000	123.000	0.002
	Hotelling's Trace	0.145	4.448[b]	4.000	123.000	0.002
	Roy's Largest Root	0.145	4.448[b]	4.000	123.000	0.002
Concentration	Pillai's Trace	1.082	7.785	24.000	504.000	0.000
	Wilks' Lambda	0.109	15.905	24.000	430.306	0.000
	Hotelling's Trace	6.468	32.742	24.000	486.000	0.000
	Roy's Largest Root	6.204	130.275[c]	6.000	126.000	0.000
Harmons * concentration	Pillai's Trace	0.025	0.130	24.000	504.000	1.000
	Wilks' Lambda	0.976	0.128	24.000	430.306	1.000
	Hotelling's Trace	0.025	0.126	24.000	486.000	1.000
	Roy's Largest Root	0.017	0.353[c]	6.000	126.000	0.907

a. Design: Intercept + Harmons + Concentration + Harmons * Concentration

b. Exact statistic

c. The statistic is an upper bound on F that yields a lower bound on the significance level

Conclusion

This study was aimed to conserve and propagate the Himalayan yew (*T. wallichiana*) by finding out the best rooting hormone (IBA and IAA) with suitable concentration applied (2000ppm-7000ppm) for the regeneration of the fresh stem cuttings. The application of IBA and IAA hormone treatments on the stem cuttings favorably enhance the growth and vegetative propagation of Himalayan yew. The IBA was found better rooting hormone as compared to IAA for producing more primary roots and sprouts on the stem cuttings of Himalayan yew. The survival percentage of the stem cuttings increased with increasing concentrations of both hormones from 2000 ppm to 7000 ppm. Highest survival percentage of the stem cuttings was observed for IBA (85.22%) and IAA (81.11%) at the application of 7000 ppm concentration. However, lower survival percentage of 66.67 % (IBA) and 63.3% (IAA) was observed at 6000 ppm concentration followed by 5000ppm>4000ppm> 3000ppm>2000ppm. The lowest survival percentages were observed for the control stem cuttings in both trials. This study recommends the use of IBA hormone treatment (7000 ppm) for the propagation and regeneration of Himalayan yew from fresh stem cuttings of the tree.

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