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Adventitious Rooting in Shoot Cuttings of *Taxus wallichiana* Zucc., an Endangered Medicinally Important Conifer of Kashmir Himalaya

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Abstract

Taxus wallichiana Zucc. (Himalayan yew), is precious for taxol or paclitaxel removal used in the research of anti-cancer drugs (ovarian cancers, AIDS related cancers and other indications). It is a slow growing tree species due to its very poor natural regeneration and very low seed germination. The present investigation tested the consequence of different plant growth regulators such as, Indole Butyric Acid (IBA), Indole-3- Acetic Acid (IAA) and Naphthalene Acetic Acid (NAA) on adventitious rooting of Himalayan yew shoot cuttings in four different seasons (spring, summer, autumn and winter) under nursery conditions. Shoot cuttings were treated with diverse concentrations of IBA, IAA and NAA. However, IBA at 1000 ppm in spring season (Mar-May) showed best response in stipulations of root length, root number and rooting percentage. The present investigation reveals that this method has the potentiality of using the young shoot cuttings of Himalayan yew in presence of plant growth regulating hormone IBA for its propagation and production on large extent to congregate the increasing stipulate.

Keywords: Adventitious rooting; IBA; Plant growth regulators; Propagation; Shoot cuttings; *Taxus wallichiana*

Introduction

A superior indulgent of the dependencies among abiotic, biotic and human induced impacts on spatial forest composition is essential for the forest executive actions and protection as well as conservation measures, particularly for endangered tree species [1]. The forest ecosystems of Kashmir Himalaya have showed incredible decline because of improved anthropogenic interferences [2]. The decline in forest area is countered frequently by afforestation concerning planting of the trees in deforested areas. But there are several foremost difficulties in flourishing afforestation programs as of less percentage of adjustment and acclimatization of planted trees. Thus, some alternative strategies have to be carried out that can ensure enhanced growth of tree species in forest ecosystems, which will provide protection and environmental safety in order to maintain long expression ecological balance in forest ecosystems. Clonal propagation of stem cuttings provides the improvement of larger genetic consistency and accessibility of advanced supply in a short phase for afforestation works. Adventitious root configuration has been effectively used in a plan of management of genetic possessions of some medicinal timbered plants [3]. A proficient rooting management can direct to a high percentage of rooting and an elevated eminence of root system [4]. Therefore, the rooting competence of shoot cuttings of tree species for its propagation on a mass scale will meet the increasing demand.

Taxus wallichiana Zucc. is an evergreen small to medium-sized conifer, with red berries (seed covered by arils) is native to the Himalaya from Afghanistan to China. It grows up to 10–20 m tall at an elevation of about 1800-3300 m above the mean sea level. In some exceptional cases, it can grow upto 28 meters. Its leaves are dark green, flat, set spirally on the stem [5]. It grows in various soil types from acidic to neutral soils. As the species are extremely similar, they are frequently easier to split geographically than morphologically [6]. Typically, ten species are documented: *T. baccata* (European or English yew), *T. brevifolia* (Pacific yew or Western yew), *T. canadensis* (Canadian yew), *T. chinensis* (Chinese yew), *T. cuspidata* (Japanese yew), *T. floridana* (Florida yew), *T. globosa* (Mexican yew), *T. sumatrana* (Sumatran yew), *T. celebica* (Celebes yew) and *T. wallichiana* (Himalayan yew) [7]. It is found in temperate, moderate temperate, and tropical submontane to high montane forests and is extensively distributed in the areas of Afghanistan, China, Bhutan, India, Malaysia, Indonesia, Pakistan, Nepal, Philippines and Vietnam [8]. In the Indian Himalayas, the species occurs in the Himachal Pradesh, Uttarakhand, Northern-Western States of Jammu and Kashmir, Sikkim, Assam, and Arunachal Pradesh at altitudes connecting 1800 and 3300 m (amsl) and in the hills of Manipur and Meghalaya at an altitude of 1500 m (amsl) [9,10]. In Kashmir Himalaya, the species shows its dominance more in Tangmarg, Gulmarg, Pahalgam and Lolab regions and as well grows expansively in the inside areas of Vaastoorwon forests at Tral, of Awantipora Forest Division [11]. Similarly, the species furthermore grows in Dudu Basantgarh areas of Jammu Province and Sudhmahandev, Patnitop, Sannasar, Sarthal [12]. The species is unisexual and owing to its lengthy seed dormancy phase and speedy loss of feasibility coupled with stumpy endurance percentage, its natural rejuvenation from seeds is very pitiable [13].

A significant attention has been generated by the genus Taxus, owed to the occurrence of diterpene alkaloid content, mainly taxol which is also recognized like the generic drug paclitaxel and has a registered trade name Taxol® BMS (Bristol-Myers Squibb). Several taxoids of diverse structural types were inaccessible and recognized with five of them being novel molecules [14]. Taxiresinol, isotaxiresinol and secoisolariciresinol are the three lignans extracted from the heartwood of the plant which showed an anti-cancer activity on the foundation of their spectral distinctiveness [15]. Amid these compounds, the

complete configuration of taxiresinol showed notable anti-cancer activity against liver, colon, ovarian and breast cancer cell lines [14]. Presence of taxol in leaves and shoots has also an exhilarating prospective as an anti-cancer remedy for different cancer treatments viz ovarian cancer and breast cancer, kaposi's sarcoma etc. [16]. Taxene (alkaloid) is used as either in combination or monotherapy with other anti-cancer agents [17,18]. The worldwide demand of the taxol is 800 -1000 kg per annum. Approximately 3 to 4 million kg of taxol is harvested yearly while the expected harvesting rate is to be 0.7 million kg per annum [6].

The wood of *Taxus wallichiana* is durable and strong and is used in bow making and many other purposes, likewise it is burnt as incense in Nepal and parts of Tibet or used as fuelwood by the local communities [19]. *Taxus wallichiana* is medicinally used for the cure of high grade fever and aching inflammatory situation and many other diseases are treated including headaches, eruptions, cystitis, kidney and heart problems, rheumatism, bronchitis, asthma, indigestion and to treat viper bites, heart ailments and as an abortifascient [20]. The herbal tea is made by the leaves of the plant for epilepsy and indigestion. *T. wallichiana* has anti-bacterial, anti-fungal, immunomodulatory, antifertility, anti- convulsant and anti-pyretic properties [21]. In India, an Unani medicine (Zarnab- a supply of drug) is extracted from its leaves and bark, approved as an aphrodisiac, sedative and as a treatment for epilepsy, bronchitis, snake bites, asthma and scorpion stings [22]. Use of young shoots in Ayurvedic drug to prepare a curative tint for the cure of biliousness, diarrhoea, headache, hysteria, nervousness and is applied in steam baths to take care of rheumatism [23]. A paste prepared from its bark is used in treatment of fractures, coughs, colds and headaches [24].

Owing to its medicinal properties and other commercial values there is increased demands on the natural populations of this plant. The ended mistreatment coupled with pitiable natural regeneration impending, has led to the incredible decline in its populations [25] and has been placed in an endangered category of IUCN 2015 [26]. Thus, the requirement is to revitalize its population in nature via planting the seedlings in the natural setting. In this view, use of plant growth regulating hormones to persuade adventitious rooting in shoot cuttings of the majority forest tree species is well acknowledged [27], and might be a practicable way to accomplish preservation and refurbishment goals as well as to rally the stipulate for paclitaxel [28], intended for which the species is facing the peril of destruction and extinction [29]. In this view, the present study was conducted to achieve the rooting of young stem cuttings through vegetative means to congregate the growing demand of this medicinally important endangered conifer of Kashmir Himalaya.

Materials and Methods

Experimental site

The experiment has been carried out at the Nursery of Centre of Research for Development, University of Kashmir. The nursery is situated in Naseem Bagh campus of University of Kashmir Srinagar. The climate of the experimental site is temperate with moderately hot summers and very cold winters.

Collection and preparation of *T. wallichiana* stem cuttings

The cuttings of *T. wallichiana* were composed from the mature trees growing at Tangmarg region (J&K) in four different seasons (spring, summer, autumn and winter) to see the outcome of different plant growth regulating hormones to persuade adventitious rooting. Stem cuttings having 22.5 cm length and 1.4 cm mid diameter with a slanting cut at the bottom were taken from healthy yew trees and leaves from about 5 cm basal portion were removed and were brought to the Naseem Bagh nursery of University of Kashmir for immediate processing.

Treatment and planting of stem cuttings

The basal ends of the cuttings were treated with IAA (500 ppm and 1000 ppm), IBA (500 ppm and 1000 ppm) and NAA (500 ppm and 1000 ppm) solutions separately by dipping for 24 hrs. The cuttings dished in distilled water served as control. After treatment of 24 hrs with different plant growth regulating hormones (IAA, IBA and NAA) at 500 ppm and 1000 ppm concentrations, the shoot cuttings of *T. wallichiana* were planted in polybags already prepared with soil, sand and humus in the ratio of 2:1:1. The shoot cuttings planted in polybags were set aside under the shade of the net house in nursery conditions (Figure 1). The polybags with cuttings were regularly monitored and were watered and kept free of weeds as per need for about 10 weeks. The experiment was conducted in all the four seasons viz, spring (Mar-May), summer (June-August), autumn (Sep.-Nov.) and winter (Dec.-Feb.) throughout the year 2015-2016.

The data for each treatment and season was recorded after the termination of each experiment.

Statistical analysis

The data was analysed by ANOVA using Duncan's multiple range test (SPSS 17.0) with a significance level of $p<0.005$.

Results and Discussion

Phytohormones especially auxins, as a growth regulator, under nursery conditions is used to enhance the quantity of seedlings, to curtail the rooting time, raise in root production and number of roots per plant [30]. The outcome of the experiment conducted to study the cause of diverse auxins (IBA, IAA and NAA) on rooting of Taxus wallichiana. The data via view to effect of auxins on stimulation of rooting in *Taxus wallichiana* shoot cuttings was recorded after 16 weeks of treatment and planting. In present study it was clearly pragmatic that the use of auxins had a direct effect on rooting system on the shoot cuttings of *Taxus wallichiana* as we have seen that control showed no rooting response in every four seasons (Figure 2). However, it has been reported that in general all auxins initiate the rooting but in our experiment conducted for four different seasons, IBA 1000 ppm showed the best rooting response on shoot cuttings of *T. wallichiana* in spring season in conditions of root number (90.2 ± 0.30), root length (14.2 ± 0.14) and rooting percentage (95%) compared to other treatments in different seasons (Table 1). However, IBA 500 ppm showed slightly less response than IBA 1000 ppm. Spring season was followed by winter season with 75 ± 0.13 root number, 13 ± 0.10 cm root length and 69% rooting, followed by autumn season, moreover, summer season showed very less or no response in all the treatments (Table 1). While as shoot cuttings treated with auxins (IAA, NAA) also showed a good response in expressions of root number, root length and rooting percentage but was found lesser than that of IBA treated shoot cuttings (Tables 2 and 3). Therefore, among all the treatments of auxins IBA>IAA>NAA. It has been reported that exogenous appliance of IBA and other auxins in promotion of Adventitious Root Formation

(ARF) in shoot cuttings of many plants including Taxus [31]. The outcome of this study is in conformity with the Gudeva et al. [32], who showed best rooting response due to IBA followed by IAA and NAA in provisions of rooting percentage, height of cuttings, number of roots per cutting in Elderberry, Rosemary and Sage. The consequence of auxins (IBA, NAA and NAA) on root development was studied in many species like *Oryza sativa* [33], *Melissa officinalis* [34] and *Ficus benjamina* [35]. Our results are also in line with Aslam et al. [13] who showed root development in shoot cuttings of *T. wallichiana* using IBA followed by IAA and NAA. But our findings showed best response with IBA in spring season. Division of root preliminary cells is dependent on exogenous auxins [36] which leads to the synthesis of root primordial [37]. Thus, in the current study, in control where no auxin treatment was given no root formation was observed in any of the four seasons. In this case the survival becomes very difficult and ultimately the cuttings died (Tables 1-3). In this respect our results are similar as obtained by Dugma and Puri et al. [38,39]. Thus, our study suggests the utilization of auxins mainly IBA in spring season for regeneration of *Taxus wallichiana* under natural conditions.

Seasons	Treatment	Root number	Root length (cm)	Rooting %
Spring (Mar-May)	Control	-	-	-
	500 ppm	78.03 ± 0.37	11.9 ± 0.12	81
	1000 ppm	90.2 ± 0.30	14.2 ± 0.14	95
Summer (Jun- Aug)	Control	-	-	-
	500 ppm	6 ± 0.21	2 ± 0.25	9
	1000 ppm	9 ± 0.10	2 ± 0.18	10
Autumn (Sep- Nov)	Control	-	-	-
	500 ppm	15 ± 0.23	8 ± 0.16	15
	1000 ppm	27 ± 0.11	9.8 ± 0.22	34
Winter (Dec- Feb)	Control	-	-	-
	500 ppm	53 ± 0.20	11 ± 0.32	64
	1000 ppm	75 ± 0.13	13 ± 0.10	69
Values within columns are represented as mean ± SD (n=10), Data scored after 16 weeks of treatment and planting the cuttings.				

Table 1: Effect of IBA on initiation and growth of adventitious roots in shoot cuttings of *Taxus wallichiana* Zucc. in four different seasons.

Seasons	Treatment	Root number	Root length (cm)	Rooting %
Spring (Mar-May)	Control	-	-	-
	500 ppm	50 ± 0.23	6.6 ± 0.23	42
	1000 ppm	73 ± 0.22	10.2 ± 0.34	54
Summer (Jun- Aug)	Control	-	-	-
	500 ppm	-	-	-
	1000 ppm	-	-	-
Autumn (Sep- Nov)	Control	-	-	-
	500 ppm	11 ± 0.15	3 ± 0.22	8
	1000 ppm	18 ± 0.34	5.2 ± 0.15	9
Winter (Dec- Feb)	Control	-	-	-
	500 ppm	20 ± 0.22	5 ± 0.24	15
	1000 ppm	31 ± 0.15	7.9 ± 0.11	21

Values within columns are represented as mean ± SD (n=10), Data scored after 16 weeks of treatment and planting the cuttings.

Table 2: Effect of IAA on initiation and growth of adventitious roots in shoot cuttings of *Taxus wallichiana* Zucc. in four different seasons.

Seasons	Treatment	Root number	Root length (cm)	Rooting %
Spring (Mar- May)	Control	-	-	-
	500 ppm	-	-	-
	1000 ppm	17 ± 0.23	2.9 ± 0.32	20
Summer (Jun- Aug)	Control	-	-	-
	500 ppm	-	-	-
	1000 ppm	-	-	-
Autumn (Sep- Nov)	Control	-	-	-
	500 ppm	-	-	-
	1000 ppm	-	-	-
Winter (Dec-Feb)	Control	-	-	-
	500 ppm	-	-	-
	1000 ppm	10 ± 0.14	2.3 ± 0.14	7

Table 3: Effect of NAA on initiation and growth of adventitious roots in shoot cuttings of *Taxus wallichiana* Zucc. in four different seasons.

Figure 1: *Taxus wallichiana* shoot cuttings planted in polybags under the shade of the net house in nursery conditions.

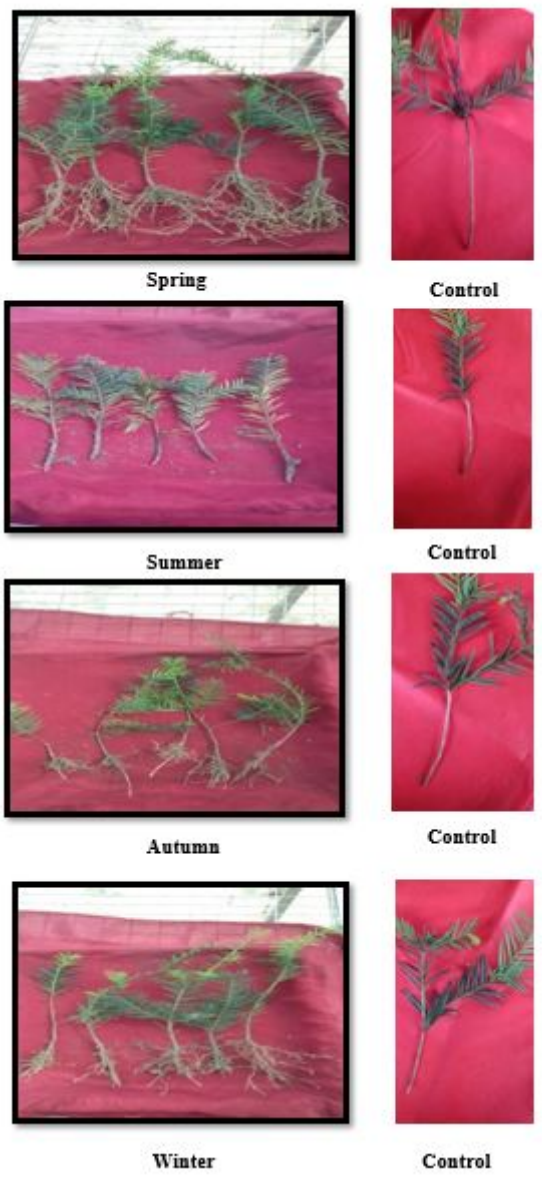

Figure 2: Rooting response on the shoot cuttings of *Taxus wallichiana* in every four seasons by application of Auxins.

Conclusion:

The adventitious rooting capability by the application of Indole Butyric Acid (IBA), the rooting hormone at a concentration of 1000 ppm revealed the highest potentiality of rooting as compared to other rooting hormones in *T. wallichiana* stem cuttings during spring season. Therefore, IBA a rooting hormone at 1000 ppm concentration is recommended to foster the rooting competence in young shoot cuttings of *T. wallichiana* by augmenting its very pitiable seed germination and natural regeneration as well as will compensate by its exploitation at an upsetting rate.

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