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Standardization of *In-Situ* Propagation Technique for *Taxus baccata* Linn. an Endangered Medicinal Plant of Western Himalayas

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ABSTRACT The objective of this study was to standardize the *in-situ* vegetative propagation protocol for *Taxus baccata* Linn. Therefore, a field tested FRI-Wire Technique, Air Layering and root promoting hormones such as Indole 3 - Butyric Acid (IBA), Indole Acetic Acid (IAA) and Napthelene Acetic Acid (NAA) at concentration of 1000 parts per million (ppm), 2500 ppm and 5000 ppm for each were evaluated. Air layering performed poorly on this species, whereas, FRI-Wire technique showed a remarkable success for rooting with hormones IBA and IAA at 1000 ppm and 2500 ppm concentrations, on all the three branch length sizes 22-28 cm, 30-36 cm and 45-51 cm. However, the branch sizes of 30-36 cm, when treated with IBA at 2500 ppm concentration performed significantly better and produced 86.66 percent rooting in comparison to other treatments with the technique. Henceforth, FRI-Wire technique can be effectively adopted for mass multiplication of this valuable species.

INTRODUCTION

Taxus baccata Linnaeus ssp. wallichiana (Zucc.). Pilger, commonly known as *Himalayan Yew*, belongs to family Taxaceae. Genus Taxus has been reported throughout the world (Farjon 2001) particularly in Central and Southern Europe, North America, North Africa, Iran, China, Japan, and North and Temperate Asia (Khan et al. 2006; Benham et al. 2016). In India, *Taxus baccata* is indigenous to the Himalayan states of Jammu and Kashmir, Himachal Pradesh, Uttarakhand and North-Eastern region (Sahni 1990; Garawal 2016). It grows mostly as a second canopy crop in moist, shady and sheltered places at an elevation between 1800 m to 3300 m above msl (Khan et al. 2006). *Taxus baccata* has assumed great importance in recent decades, due to the presence of taxol, an anti-cancer agent used for the treatment of breast and ovarian cancer (Kovacs et al. 2007; Asif et al. 2016). The anti-cancer 10-deacity baccatin III (DAB) extracted from its needles and bark sells between US $ 0.1-13 million per kg, whereas, the demand exceeds US $ 100 million per year (Biswas et al. 2003). The global annual demand of taxol is 800 kg to 1000 kg, out of which around 400 kg is estimated to be required in North America and Europe alone (Cameron and Smith 2002). To produce one kg taxol, 10000 kg bark (Isah 2015) or around 3000 trees of *Taxus baccata* are required (Phillips et al. 1999) whereas, about three tons of needles are required to produce one kg of paclitaxel (McCoy 2004). The excessive extraction of needles and bark however, causes death of the trees (Purohit et al. 2001; Peerzada and Sharma 2007), which has negatively impacted its natural populations throughout the Himalayan range. Consequently, this species has been included in the Appendix II of CITES (CITES 2009) and in the endangered category of IUCN Red data list (Thomas and Farjon 2011).

Taxus baccata is a slow growing species and attains maturity in the 70th year (Zarek 2009). It can reach more than 1000 years of age with over one meter in diameter (Brzeziecki and Kienast 1994). Natural regeneration of *Taxus baccata* has been of great concern worldwide (Krol 1978). The reasons of the poor regeneration includes,

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low seed production (Lanker et al. 2010) due to its dioecious nature, which reduces the chances of seed setting in its natural habitat. The over-exploitation, cattle grazing, changes in forest management, habitat destruction and climatic change (Pant and Samant 2008; Juyal et al. 2014; Thomas and Garcia-Marti 2015) has further impacted the regeneration of *Taxus baccata*, thus restricting its population to the critically low. Artificial regeneration from seeds is very poor, due to hard seed coat and the growth of seedlings is extremely slow (Thomas and Polwart 2003; Zarek 2007). Some work has been done to propagate *Taxus baccata* through rooting of stem cuttings with varying degrees of success by the application of growth regulators such as IBA, IAA, NAA and GA_3 alone and/or in combinations (Khali and Sharma 2003; Mohd et al. 2007; Kaul 2008; Modanloo et al. 2008; Benham et al. 2016). However, most of the works have been carried out using small cuttings (usually 7.5 to 20 cm in length) only, with high failure rates and insufficient responses (Luna and Kumar 2006). The existing propagation methods namely, grafting, air-layering, vegetative cuttings and tissue culture techniques are sometimes more expensive, and are not so easy (Perez and Gradaille 2011) to be carried out and handled by unskilled or semi-skilled individuals. It is a specialist's job, requires special training (Surendran et al. 2000), knowledge and experience. In certain tree species, excessive phenolic substances leach out from injured surfaces, which inhibits root initiation (Tiwari and Chauhan 2006). Moreover, propagation material taken as a cutting, graft, layer or explant for tissue culture are more prone to desiccation (Leon and Withers 1986) and transmission of viruses or diseases (Sastry 2012) which can become systemic and spread infection to the fresh crops. Therefore, new methods of vegetative propagation are immediately required, which will surpass such inconveniences, must be easy to apply, cost effective and also convenient to adopt *in-situ*. This will encourage commercial plantation of these species and reduce the pressure on natural forest resource base. Keeping in view the above mentioned facts, this study was carried out to evaluate the phytohormonal treatments and FRI-Wire technique for *in-situ* multiplication of *Taxus baccata* Linn. to provide more insight into its propagation paradigm.

METHODOLOGY

Study Area

The experiments were carried out in Gulmarg Forest Range of Jammu and Kashmir, India. Gulmarg is situated at an elevation of 1550 - 2730 m amsl, between 34°052 N latitude and 74°202 E longitude. The study area has temperate climatic conditions, with an average rainfall of 2012.2 mm during summer months. The main form of precipitation is however snow in winter and some stray rains in other seasons particularly during spring. The temperature ranges between -7.3°C minimum and 12 to 22°C maximum. The humidity varies between forty-three percent minimum and ninety-three percent maximum. The soils of the study area are derived from a variety of parent materials but grey sandstone, and shale are dominant. Comparatively shallow on steep slopes and deep on moderate slopes, these soils are medium to heavy in texture, but the moisture retention on sloping lands is very poor (Siddique 1989). Vegetation of the study area falls under the *Himalayan Moist Temperate* forest type as per the Champion and Seth (1968) classification of Indian Forests.

Methods

The experimental trials on *Taxus baccata* with FRI-Wire technique (Peerzada and Puni 2008) and Air layering (as control) were conducted in the beginning of summer season that is, during March - April. Phenotypically superior trees growing naturally in the forest and having maximum number of branches were selected for applying the techniques through ocular judgment following Kedharnath (1982). Two to three year old healthy branches receiving sufficient sunlight were selected from lower, middle and upper positions of the trees. Three different length sizes of branches viz; 22 - 28 cm designated as (S1), 30 - 36 cm as (S2) and 45 - 51 cm as (S3), having diameter of 1 to 2 cm, were selected for employing the techniques. The experiments were carried out in three replications for each hormone type (IBA, IAA, and NAA) and dose (1000 ppm, 2500 ppm, 5000 ppm); with ten branches in each replication.

Air layering was carried out by removing bark of the branch (1.25 cm wide ring) with the help of grafting knife. The portion of air layer from where the bark was removed was smeared with root inducing hormones and covered with handful of sphagnum moss and then wrapped with 17×12 cm wide polythene strip. Whereas, FRI-Wire technique was employed by tying the wire around the branch and applying a tightening pressure using pliers, till a visible depression on the bark was noticed. Rooting hormone was applied around the portion of the branch, where, wire was tied and covered with a handful of sphagnum moss, which was already soaked and washed in water. The whole mass was covered with a 17×12 cm wide polythene strip to hold the moss and to provide moisture for callus formation and rooting. The experimental branches were observed for root initiation and observations continued every week, till sufficient root formation took place. After successful rooting, the branches were detached from the mother plants at 5 cm below the rooted area and planted up in poly-bags containing potting media viz: soil + sand + vermicompost (at 2:1:1 ratio) for development of adequate roots. After one growing season in the poly bags, rooted branches were transplanted in the field.

The data obtained was analyzed statistically using computer programmes (SPSS 21.0 version). Data was subjected to analysis of variance (ANOVA; $P<0.05$) to find out the significance of differences between the treatments for each studied factors following the procedure adopted by Lopes et al. (2005). Data on percentage of rooting was subjected to arcsine transformation before analysis of variance and then converted back to percentage for presentation, following Zar (1984).

RESULTS

The experiments were carried out with FRI-Wire technique and Air layering (as control) on the branches of *Taxus baccata* to evaluate the effect of different branch length sizes that are, 22-28cm (S1), 30-36cm (S2) and 45-51cm (S3); root promoting hormones IBA (H1) IAA (H2) and NAA (H3) and their doses 1000 ppm (D1), 2500 ppm (D2) and 5000 ppm (D3). The results clearly reveal that the variation among different branch sizes, hormones and doses was highly significant (at 0.05%) for rooting percent, average root length and number of roots per branch with FRI-Wire Technique. The rooting percentage was recorded maximum (86.66%) in size S2 with Hormone H1 and Dose D2 followed by S1×H2×D2 (73.33%) and S3×H1×D2 (66.66 %) respectively (Table 1). The average root length was found maximum for the combination of S2×H1×D2 (12.42 cm) followed by S2×H2×D2 (9.22 cm) and S1×H1×D2 (9.01 cm) respectively. The length of roots was minimum in S3 with all the hormone and dose combinations (Table 2). The number of roots were recorded maximum for the combination of S2×H1×D2 (8.11) followed by S2×H2×D2 (8.11) and S1×H1×D2 (6.89) respectively. The number of roots were minimum in S3 in all the hormone and dose combinations (Table 3).

Air layering on *Taxus baccata* was not effective. Rooting occurred in 4.81 percent branches only, which was very low as compared to experiments carried out with FRI-wire technique. Even though the rooting initiated, it was poorly formed. The rooted branches died within one month of planting in the polybags. The variation among different branch sizes, hormones and doses was significant (at 0.05%) for rooting with Air-layering. However, the maximum rooting percentage was only 13.33 percent with the combination of S1×H1×D1 followed by S1×H2×D1 (10%) and S2×H1×D2 (10%) respectively (Table 4). The

Table 1: Variation in the percentage of rooting under different branch sizes, hormones with hormone doses through FRI-Wire technique

Hormone	*Dose*	*22- 28 cm (S1)*	*30- 36 cm (S2)*	*45-51 cm (S3)*
IBA (H1)	1000 ppm (D1)	70.00 (56.97)	66.66 (54.76)	43.33 (41.13)
	2500 ppm (D2)	66.66 (54.76)	86.66 (68.82)	66.66 (54.76)
	5000 ppm (D3)	26.66 (30.98)	40.00 (39.13)	26.66 (30.98)
IAA (H2)	1000 ppm (D1)	50.00 (44.98)	56.66 (48.82)	43.33 (41.13)
	2500 ppm (D2)	73.33 (58.98)	66.66 (54.76)	46.66 (43.06)
	5000 ppm (D3)	30.00 (32.99)	46.66 (43.06)	23.33 (28.77)
NAA (H3)	1000 ppm (D1)	0	0	6.66 (8.85)
	2500 ppm (D2)	0	0	16.66 (19.91)
	5000 ppm (D3)	0	0	6.66 (8.85)

$CD_{0.05}$= 10.52

Table 2: Variation in the average root length (cm) under different branch sizes, hormones with hormone doses through FRI-Wire technique

Hormone	*Dose*	*22- 28 cm (S1)*	*30- 36 cm (S2)*	*45-51 cm (S3)*
IBA (H1)	1000 ppm (D1)	6.41	4.29	3.21
	2500 ppm (D2)	9.01	12.42	2.79
	5000 ppm (D3)	4.45	3.74	2.23
IAA (H2)	1000 ppm (D1)	4.10	3.97	1.81
	2500 ppm (D2)	6.39	9.22	1.83
	5000 ppm (D3)	3.93	3.67	1.82
NAA (H3)	1000 ppm (D1)	0	0	0
	2500 ppm (D2)	0	0	0
	5000 ppm (D3)	0	0	0

$CD_{0.05}$= 1.88

Table 3: Variation in the number of roots per branch under different branch sizes, hormones with hormone doses through FRI-Wire technique

Hormone	*Dose*	*22- 28 cm (S1)*	*30- 36 cm (S2)*	*45-51 cm (S3)*
IBA (H1)	1000 ppm (D1)	5.89	5.00	2.11
	2500 ppm (D2)	6.89	8.11	2.00
	5000 ppm (D3)	3.89	2.78	1.33
IAA (H2)	1000 ppm (D1)	3.67	4.11	1.11
	2500 ppm (D2)	4.33	8.11	1.11
	5000 ppm (D3)	3.55	3.11	1.33
NAA (H3)	1000 ppm (D1)	0	0	0
	2500 ppm (D2)	0	0	0
	5000 ppm (D3)	0	0	0

$CD_{0.05}$= 1.28

variation among different branch sizes, hormones and doses was non-significant for average root length and number of roots per branch with Air-layering that is, not causing any significant difference (Tables 5 and 6). The data on survival percentage of different rooted and planted branches with their different doses revealed that the maximum survival percent of 66.66 was recorded when S2 cuttings were treated with H1 of 2500 ppm concentration followed by H1D1S1 after 12 months of out planting (Table 7).

DISCUSSION

The results of this experiment revealed that, IBA is the best hormone for induction of higher rooting percentage, number of roots and length of roots in both the propagation methods that is, FRI-Wire technique and Air layering. The application of auxin, principally IBA is an established and effective means to augment rooting in cuttings (Hartman et al. 2002; Dirr and Heuser

Table 4: Variation in the percentage of rooting under different branch sizes, hormones and hormone doses through air layering

Hormone	*Dose*	*22- 28 cm (S1)*	*30- 36 cm (S2)*	*45-51 cm (S3)*
IBA (H1)	1000 ppm (D1)	13.33 (17.70)	6.66 (12.28)	6.66 (8.85)
	2500 ppm (D2)	3.33 (6.14)	10.00 (14.99)	0
	5000 ppm (D3)	3.33 (6.14)	0	0
IAA (H2)	1000 ppm (D1)	10.00 (14.99)	3.33 (6.14)	0
	2500 ppm (D2)	6.66 (12.28)	6.66 (8.85)	0
	5000 ppm (D3)	0	6.66 (8.85)	0
NAA (H3)	1000 ppm (D1)	0	0	0
	2500 ppm (D2)	0	0	0
	5000 ppm (D3)	0	6.66 (8.85)	0

$CD_{0.05}$= 14.51

Table 5: Variation in the average root length (cm) under different branch sizes, hormones and hormone doses through air layering

Hormone	*Dose*	*22- 28 cm (S1)*	*30- 36 cm (S2)*	*45-51 cm (S3)*
IBA (H1)	1000 ppm (D1)	1.73	0.83	0
	2500 ppm (D2)	0.42	1.23	0
	5000 ppm (D3)	0	0	0
IAA (H2)	1000 ppm (D1)	1.24	0.41	0
	2500 ppm (D2)	0.83	0.81	0
	5000 ppm (D3)	0	0	0
NAA (H3)	1000 ppm (D1)	0	0	0
	2500 ppm (D2)	0	0	0
	5000 ppm (D3)	0	0	0

$CD_{0.05}$= NS

Table 6: Variation in the number of roots per branch under different branch sizes, hormones and hormone doses through air layering

Hormone	*Dose*	*22- 28 cm (S1)*	*30- 36 cm (S2)*	*45-51 cm (S3)*
IBA (H1)	1000 ppm (D1)	1.22	0.44	0
	2500 ppm (D2)	0.22	0.66	0
	5000 ppm (D3)	0	0	0
IAA (H2)	1000 ppm (D1)	0.66	0.22	0
	2500 ppm (D2)	0.44	0.44	0
	5000 ppm (D3)	0	0	0
NAA (H3)	1000 ppm (D1)	0	0	0
	2500 ppm (D2)	0	0	0
	5000 ppm (D3)	0	0	0

$CD_{0.05}$= NS

Table 7: Variation in survival percentage of rooted and planted branches with sizes, hormones and hormone doses after 12 months of out planting

Hormone	*Dose*	*22- 28 cm (S1)*	*30- 36 cm (S2)*	*45-51 cm (S3)*
IBA (H1)	1000 ppm (D1)	66.66	66.66	53.33
	2500 ppm (D2)	66.66	66.66	50
	5000 ppm (D3)	40	46.66	40
IAA (H2)	1000 ppm (D1)	43.33	56.66	40
	2500 ppm (D2)	50	43.33	36.66
	5000 ppm (D3)	46.66	53.33	23.33

$CD_{0.05}$ = 12.43

2006; Kaur 2015). The enhanced rooting performance under IBA has been attributed to its higher concentration near the base, as IBA translocates poorly and retains near the site of application, thus it works as a superior root stimulator (Weaver 1972; Kebede et al. 2013). Promotion of rooting percentage, number of roots and length of roots with IBA in this study corresponds well with the studies of other authors (Schneck 1996; Kaul 2008; Modanloo et al. 2008; Yu-Ci-Ying et al. 2008; Mishra et al. 2010; San-Jose et al. 2012; Tagipoor et al. 2015; Okao et al. 2016). IBA is considered a stable auxin, while IAA and NAA as readily destroyed (DeKlerk et al. 1997). The fairly poor rooting with IAA in comparison to IBA could be elucidated by the sensitivity of IAA to light (Hartmann et al. 2002), and production of more ethylene is also believed to inhibit the rooting (Mullins 1972).

The concentration or dose of the rooting hormones had a significant effect on the percentage of rooting and size of roots. In this experiment, the higher concentration of root promoting hormones (that is, 5000 ppm) resulted in reduced rooting percent, number of roots and root length. This may be due to the auxin induced ethylene production, which prevents auxin transport. Auxins at higher concentrations may have a promotive or an inhibitory effect on root formation or elongation. The differential response to varying dose of a hormone observed in the present study finds support from the studies of Avanzato et al. (1988) and Webster et al. (2005), who suggested that optimum concentrations of auxin is favorable, while supra-optimum auxins are toxic to the root formation.

The length size of a branch or cutting is one of the important factors for rooting in vegetative propagation of plants (Vigl and Rewald 2014; Szasz-Len et al. 2015). In this experiment all the different branch length sizes (S1, S2 and S3) showed rooting, although S2 proved best branch length size for induction of maximum rooting percentage. Higher rooting percentage in medium sized branches is supported by the findings of Desrochers and Thomas (2003) and Munoz-Gutierrez et al. (2009). The maximum rooting on standard cuttings has been related to the level of endogenous auxins and other root inducing factors, believed to be lower in small cuttings (Palanisamy and Kumar 1997). The growth of roots is also dependent on carbohydrates present in leaves and stem (Leaky 2014), and the larger cuttings store more carbohydrates (Tchoundjeu and Leaky 1996). The relationship between rooting efficiency and size of cuttings is well known (Leaky 2004) but no information is available on the effectiveness of different branch sizes in rooting of *Taxus baccata*.

Although the rooting was initiated in Air layering, the roots were poorly formed. The rooted and planted branches died within one month of planting in the polybags. Propagation through layering is difficult and does not always succeed (Perez and Gradaille 2011). The other reason for hard to root behaviour of *Taxus baccata* with Air layering may be due to its slow growing nature and less water storage in stems or bark. The injury to branches and subsequent release of phenolic substances are also believed to inhibit root initiation (Tiwari and Chauhan 2006). The release of phenolic content reduces the capacity of protein synthesis and changes the free amino acid pool in plants, which has been related to slow growth rate and poor proliferation by Dubravina et al. (2005).

A remarkable success was achieved by rooting the branches of *Taxus baccata* using FRI-Wire technique. The profuse rooting in this difficult to root species was attributed to the appropriate vegetative propagation technique and root promoting hormones. In the experiments of FRI-Wire Technique, wire was used to tie up around the selected tree branches to induce pressure on the bark. This tightening pressure applied around the bark of branch at a point causes interruption in the downward translocation of organic materials such as carbohydrates, auxin, and other growth factors from leaves and growing shoot tips. Therefore, there is an induced accumulation of synthesized food at this region which encourages multiplication of cells and callus formation takes place. Availability of root promoting hormones at this point induces initiation of rooting. The sphagnum moss provides moisture and the right environment for callus formation. The moss is relatively sterile and light in weight with high moisture holding capacity. The polythene strip holds the moss, retains moisture and allows gaseous exchange. It is also sufficiently tough to withstand the variations in weather conditions.

CONCLUSION

Considering the importance of propagation, the present study standardized the *in-situ* vegetative propagation protocol for *Taxus baccata* to meet out the escalating demand for this important medicinal tree species. Rooting hormone IBA at dose of 2500 ppm was found best for induction of rooting in this species. The FRI-Wire technique was found easy to apply, time saving, require only very simple tools/materials, and can be adopted with simple instructions. The technique surpasses many inconveniences faced with other vegetative multiplication methods viz., reduction in time to get selected or improved material into production, decrease in establishment cost, and increase in uniformity. The FRI-wire technique can be effectively used for producing bigger sized plants of valuable species as compared to air layering or any other existing vegetative multiplication methods.

RECOMMENDATIONS

- *Taxus baccata* can be successfully multiplied by using FRI-wire technique of vegetative propagation during the months of March-April.

- Plants which are more than one and a half feet can be produced in a short time through FRI-wire technique which is not possible with normal cuttings.
- Direct planting of the rooted and detached branches in the field is possible.
- Rooting hormone IBA at dose of 2500 ppm is the best for induction of rooting and also vegetative growth of plants after field plantation.
- Bright colored polythene strips should not be used; it should be preferably camouflaged with moss to keep away the monkeys from destruction.

REFERENCES

Asif M, Rizwani GH, Zahid H, Zahid K, Rao Q 2016. Pharmacognostic studies on Taxus baccata L.: A brilliant source of anti-cancer agents. *Pak J Pharm Sci,* 29(1): 105-109.

Avantzato DG, Couvillon A, Pokorny FA 1988. The influence of P-IIB (Phenyle indole 3-thiolobutyrate), a Aryle ester of IBA, on the rooting of “Redhaven” peach. *Acta Hort,* 227: 197-201.

Benham SE, Houston DT, Caudullo G, deRigo D 2016. Taxus baccata in Europe: Distribution, habitat, usage and threats. In: J San-Miguel-Ayanz, D deRigo, G Caudullo, T Houston Durrant, A Mauri (Eds.): *European Atlas of Forest Tree Soecies*. Luxembourg: Publ Office EU, pp. e015921.

Biswas S, Jain SS, Mohinder P 2003. Research needs and priorities for conservation of Indian medicinal flora. *Indian Forester,* 129(1): 86-92.

Brzeziecki B, Kienast F 1994. Classifying the life-history strategies of trees on the basis of the Grimian model. *Forest Ecology and Management,* 69: 167-187.

Cameron SI, Smith RF 2002. Bringing ‘Blue Sky Biology’ Down To Earth: Linking Natural Products Research with Commercialization. *Proceedings of the 29th Annual Meeting of the Plant Growth Regulation Society of America*, 28 July – 1 August, Halifax, Nova Scotia. LaGrange, Ga: Plant Growth Regulation Society of America, pp. 31-39.

Champion HG, Seth SK 1968. *A Revised Survey of Forest Types of India (Reprinted 2005)*. Dehradun, India: Natraj Publishers.

CITES 2009. Convention on International Trade in Endangered Species of Wild Fauna and Flora. Appendices I, II and III. From <http://www.cites.org> (Retrieved on 20 October 2010).

Desrochers A, Thomas BR 2003. A comparison of pre-planting treatments on hardwood cuttings of four hybrid poplar clones. *New Forests,* 26: 17-32.

DeKlerk G, Ter-Brugge J, Marinova S 1997. Effectiveness of IAA, ABA and NAA during adventitious roots formation in vitro in Malus ‘Jork 9’. *Plant Cell Tissue Organ Cult,* 49: 39-44.

Dirr AM, Heuser JR 2006. *The Reference Manual of Woody Plant Propagation: From Seed to Tissue Culture.* North Carolina, USA: Varsity Press Inc, P. 417.

Dubravina GA, Zaytseva SM, Zagoskina NV 2005. Changes in formation and localization of phenolic compounds in the tissues of European and Canadian yew during dedifferentiation in vitro. *Russian Journal of Plant Physiology,* 52(5): 672-678.

Farjon A 2001. *World Checklist and Bibliography of Conifers*. 2nd Edition. London, UK: Royal Botanic Gardens Press, Kew.

Garawal T 2016. Taxus: An endangered genus. *International Journal of Agricultural Science and Research,* 6(2): 175-178.

Hartmann HT, Kester DE, Davies FT, Geneve RL 2002. *Plant Propagation*. New Delhi: Prentice Hall of India.

Isah T 2015. Natural sources of Taxol. *British Journal of Pharmaceutical Research,* 6(4): 214-227.

Juyal D, Thawani V, Thaledi S, Joshi M 2014. Ethnomedical properties of *Taxus Wallichiana* Zucc. (Himalayan Yew). *Journal of Traditional and Complementary Medicine,* 4: 159-161.

Kaul K 2008. Variation in rooting behavior of stem cuttings in relation to their origin in *Taxus wallichiana* Zucc. *New Forests,* 36(3): 217-224.

Kaur S 2015. Effect of Different Treatment of Indole-3-Butyric Acid (IBA) on the Rooting and Growth Performance of Hardwood Cuttings of Peach (*Prunus persica* L. Batch). Agricultural Science Digest–A Research Journal, 35(1): 41-45. From <http://www .indianjournals.com/ijor.aspx?target=i jor: asd& volume=35&issue=1&article=008> (Retrieved on 25 November 2016).

Kebede M, Hulten H, Balcha G 2013. Vegetative propagation of juvenile leafy stem cuttings of *Prunus africana* (Hook. F.) Kalkm and *Syzgium guineense* (Willd.) DC. *International Journal of Botany,* 9(1): 30-36.

Kedharnath S 1982. Plus tree selection: A potential tool in forest tree improvement. In: PK Khosla (Ed.): *Improvement of Forest Biomass*. Solan, HP, India: Indian Society for Tree Scientist, pp.13-20.

Khali RP, Sharma AK 2003. Effect of phytohormones on propagation of himalayan yew (*Taxus baccata* L.) through stem cuttings. *Indian Forester,* 125(2): 289-294.

Khan M, Verma SC, Srivastava SK, Shawl AS, Syamsundar KV, Khanuja SP 2006. Essential oil composition of *Taxus wallichiana* Zucc. from the Northern Himalayan region of India. *Flavour Frag J,* 21: 772-775.

Kovacs P, Csaba G, Pallinger E, Czaker R 2007. Effects of taxol treatment on the microtubular system and mitochondria of Tetra hymena. *Cell Biol Int,* 31: 724-732.

Krol S 1978. *An Outline of Ecology. The Yew- Taxus baccata L.* Warsaw, Poland: Foreign Scientific Publications, Department of the National Center for Scientific and Technical, and Economic Information (for the Department of Agriculture and the National Science Foundation, Washington, DC).

Lanker U, Malik AR, Gupta NK, Butola JS 2010. Natural regeneration status of the endangered medicinal plant *Taxus baccata* Hook. F. syn. *T. wallichiana*, in northwest Himalaya. *International Journal of Biodiversity Science, Ecosystem Services and Management*, 6(1-2): 20-27.

Leakey RRB 2004. Physiology of vegetative reproduction. In: J Burley, J Evans, JA Youngquist (Eds.): *Encyclopedia of Forest Sciences*. London, UK: Academic Press, pp. 1655-1668.

Leakey RRB 2014. Plant Cloning: Macropropagation. In: N van Alfen (Ed.): Encyclopedia of Agriculture and Food Systems. San Diego, USA: Elsevier Publishers, pp. 349-359. From <http://dx.doi.org/10.1016/ B978-0-444-52512-3.00223-0> (Retrieved on 27 October 2016).

Leon J, Withers LA 1986. Guidelines for Seed Exchange and Plant Introductions in Tropical Crops. *FAO Plant Production and Protection Paper,* 76. Rome: FAO.

Lopes RL, Cavalcante IHL, Oliveira IV-de-M, Martins ABG 2005. Indol-butyric acid levels on cashew cloning by air-layering process. *Revista Brasileira de Fruticultura,* 27(3): 517-518.

Luna RK, Kumar S 2006. Vegetative propagation through juvenile shoot cuttings of Melia composite wild. *Indian Forester,* 132(12): 1561-1569.

McCoy M 2004. Lining up to make a cancer drug. *Chemical and Engineering News,* 82(35): 12-14.

Mishra Y, Usmani G, Chawhaanv and Mandal AK 2010. Propagation of *Tinospora cordifolia* (Wild.) Miers Ex-Hook. F. Thoms. through mature vine cuttings and their field performance. *Indian Forester,* 136(1): 88-93.

Modanloo S, Jalilvand H, Nasr MH 2008. Stimulation of rooting of *Taxus baccata* according to the kind of cutting and KIBA regulator treatment. *Iranian Journal of Forest and Poplar Research,* 16(2): 176-185.

Mohd A, Syed A, Rather MS, Salathia HS, Seth CM 2007. Auxin induced rooting in *Taxus baccata* Linn. stem cuttings. *Indian Journal of Forestry,* 30(2): 221-226.

Mullins MG 1972. Auxin and ethylene in adventitious root formation in *Phaseolus aureus* (Roxb.). In: DJ Carr (Ed.): *Plant Growth Substances*. New York: Springer, pp. 526-533.

Munoz-Gutierrez L, Vargas-Hernández JJ, López-Upton J, Soto-Hernández M 2009. Effect of cutting age and substrate temperature on rooting of *Taxus globosa*. *New Forests,* 38(2): 187-196.

Okao M, Ogwal L, Mutoni G, Alip SO, Okullo JBL, Okia CA 2016. Effect of mode of auxin application on rooting and bud break of Shea tree (*Vitellaria paradoxa*) cuttings. *American Journal of Plant Sciences,* (7): 2199-2208.

Pant S, Samant S 2008. Population ecology of the endangered Himalayan Yew in Khokan wildlife Sanctuary of North Western Himalaya. *J Mt Sci,* 5: 257-264.

Palanisamy K, Kumar P 1997. Effect of position, size of cuttings and environmental factors on adventitious rooting in neem (*Azadirachta indica* A. Juss). *For Ecol Manag,* 98(3): 277-280.

Peerzada I, Sharma SD 2007. Exploitation status of *Taxus baccata* Linn. in Chopal forest division of Himachal Pradesh, India. *International Journal of Forest Usufructs Management,* 8(2): 93-97.

Peerzada I, Puni L 2008. Development of new technique of vegetative propagation for tree species. *Indian Forester,* 134(6): 851-854.

Perez de Corcho MJ, Gradaille D 2011. In vitro propagation of *Callistemon speciosus* L. *Revista de la Facultad de Agronomia Universidad del Zulia (LUZ),* 28: 157-173.

Phillips LD, Dwyer DB, Dabur Research Foundation 1999. Sustainable Harvesting of Himalayan Yews. *Proceedings Of The First International Symposium on the Conservation of Medicinal of Plants Trade,* 22-23 June 1998, Royal Botanic Gardens, Kew, United Kingdom, Europe. Brussels, Belgium: TRAFFIC Europe, WWF-Belgium, pp.147-154.

Purohit A, Maikhuri RK, Rao KS, Nautiyal S 2001. Impact of bark removal on survival of *Taxus baccata* L. (Himalayan yew) in Nanda Devi biosphere reserve, Garhwal Himalaya, India. *Curr Sci,* 81(5): 586-590.

Sahni KC 1990. *Gymnosperms of India and Adjacent Countries*. 1st Edition. Dehradun, Uttarakhand, India: Bishen Singh Mahendra Pal Singh.

San-Jose MC, Romero L, Janeiro LV 2012. Effect of indole 3-butyric acid on root formation in *Alnus glutinosa* micro-cuttings. *Silva Fennica,* 46(5): 643-654.

Sastry KS 2012. Plant virus transmission through vegetative propagules (asexual reproduction). In: *Seedborne Plant Virus Diseases.* India: Springer, pp. 285-305.

Schneck V 1996. Investigation on the dependence of clone in rootability and the quality of the root system with propagation by cutting of 40-350 year old yew (*Taxus baccata* L.) Trees. *Silvae Genetica,* 45(5-6): 246-249.

Siddique H 1988. *Working Plan for Pirpanjal Forest Division (1988-89 to 1997-98)*. Jammu and Kashmir, India: Jammu and Kashmir Forest Department.

Surendran C, Parthiban KT, Vanangamudi K, Balaji S 2000. *Vegetative Propagation of Trees, Principles and Practices*. Coimbatore, India: TNAU Press.

Szasz-Len AM, Holonec L, Truta A, Rebrean FA 2015. Effect of cutting size on the rooting of *Thuja occidentalis* 'Columna'. *Bulletin UASVM Horticulture,* 72(1): 233-234.

Tagipoor L, Mahmodzadeh H, Jabarzadeh Z 2015. Effect of rooting beds, IBA concentrations and bottom heat on rooting of plane tree (*Plantanus orientalis* L.) cuttings. *Int Jr of Biosciences,* 6(3): 76-82.

Tchoundjeu Z, Leakey R 1996. Vegetative propagation of African mahogany: Effects of auxin, node position, leaf area and cutting length. *New Forest,* 11(2):125-136.

Thomas P, Farjon A 2011. *Taxus wallichiana*. The IUCN Red List of Threatened Species 2011. From <http://dx.doi.org/10.2305/IUCN.UK.2011-2.RLTS.T46171879A730085.en> (Retrieved on 15 December 2014).

Thomas PA, Garcia-Marti X 2015. Response of European yews to climate change: A review. *Forest Systems,* 24(3): 1-11. http://dx.doi.org/10.5424/fs/2015243-07465.

Thomas PA, Polwart A 2003. Biological flora of the British Isles: *Taxus baccata* L. *Journal of Ecology,* 91: 489-524.

Tiwari ON, Chauhan UK 2006. Rhododendron conservation in Sikkim Himalaya. *Current Science,* 90(4): 532-541.

Vigl F, Rewald B 2014. Size matters? The diverging influence of cutting length on growth and allometry of two Salicaceae clones. *Biomass Bioenergy,* 60: 130-136.

Weaver JR 1972. *Plant Growth Substances in Agriculture*. San Fransico: HW Freman and Co, pp. 119-145.

Webster L, Smith RF, Cameron SI 2005. Developing Improved Nursery Culture for the Production of Rooted Cuttings of Canada Yew (*Taxus canadensis* Marsh.). *Proceedings of the 32nd Annual Meeting of the Plant Growth Regulation Society of America*, 24-27 July, Newport Beach, California, USA, pp. 95-100.

Yu-Ci-Ying, Li-Xiu-Peng, Yuan-Yan-Fei, Chen-Ye-Ping, Li-Wan-Xing, Miao-Ling-Xia 2008. Ex-situ island cultivation and propagation techniques for *Taxus chinensis* var. mairei. *Journal of Zhejiang Forestry College,* 25(1): 60-64.

Zar JH 1984.*Biostatistical Analysis.* Englewood Cliffs, NJ: Prentice Hall.

Zarek M 2007. A practical method for overcoming the dormancy of *Taxus baccata* isolated embryos under in vitro conditions. *In Vitro Cell Dev Bio-Plant,* 43: 623-630.

Zarek M 2009. RAPD analysis of genetic structure in four natural populations of *Taxus baccata* from southern Poland. *Acta Biologica Cracoviensia-Series Botanica,* 51(2): 67-75.

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