INDUCTION OF ADVENTITIOUS ROOTING IN STEM CUTTINGS OF *TAXUS BACCATA* L., A THREATENED ANTICANCER PLANT OF KASHMIR HIMALAYA

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ABSTRACT

The present investigation was undertaken to see the effect of IBA, NAA and their combination for in-vivo rooting in stem cuttings of *Taxus baccata* L. in four different seasons of the year. The best response was registered under the influence of IBA (I000 ppm) in the winter season (December–February) and the average number adventitious roots observed was 10 with 100 percent response. This paper reports the rooting capacity of stem cuttings of *T. baccata* for its propagation on a mass scale to meet the increasing demand.

Key words: *Taxus baccata*, stem cuttings, auxins, rooting, season

INTRODUCTION

Genus *Taxus* belongs to family Taxaceae and is represented by nine species which are widely distributed in N. America, Europe and Asia extending up to Malaysia. Only one species namely *T. baccata* L. occurs in India which is distributed all along the Himalaya at an elevation of 1,800 – 3,300 m.

Taxus baccata, L. commonly known as Himalayan yew (Vernacular name *Posthal*) is an evergreen densely branched medium sized tree. It is a slow growing tree species and the propagation occurs through seeds and not by vegetative means in natural habitat. However, regeneration through seeds under natural conditions is very meagre due to long seed dormancy, poor establishment of seedlings, human disturbances, trempling by cattle and some other unknown factors (Singh, *et al.*, 1996). Seedling propagation is little used because of variation in progeny, complicated seed dormancy condition and the slow growth of seedlings (c.f. Hartmann *et al*., 1997). Natural propagation through seed has been found to be insufficient for its propagation and germplasm conservation.

T. baccata L. is known for its medicinal importance. Taxol, an anticancerous compound, is obtained from the dried inner bark (phloem-cambial tissue) and from its needles. Besides this, the medicinal tincture made from the young shoots has been long in use for other ailments. Fleshly aril is credited with carminative expectorants and stomachache properties. It has a unique property or therapeutic qualities against the ovarian cancer, breast cancer and colon cancer. The drug taxol has been approved for the clinical treatment of ovarian and breast cancer by Food and Drug Administration (FDA) in the USA (Zhang *et al*., 1995). Very recently a new taxoid has been found in callus cultures of *Taxus caspidata* which is an MDR (Multidrug Resistance) reversal agent (Dai, *et al*., 2006.) Apart from this wood of *Taxus* is strong and durable and is used for furniture, cabinetwork, candlesticks, woodcarvings and other fancy items. The bark is brewed into a concoction like tea in Ladakh. Aril is edible and seeds are sedative.

T. baccata L., being highly valuable, is continuously being exploited at an alarming rate. Current status of tree species in the valley is "Endangered" (Kaul, 1997; Dar and Naqshi, 2001; Dar *et al*., 2002). It has been shown as Critically Endangered by IUCN (BCPP Report, 1998 c.f. Singh and Panda, 2005). In view of this present work was conducted to see the possibility of using young stem cuttings for rooting purposes so that the propagation of the plant is achieved through vegetative means to meet the increasing demand.

MATERIAL AND METHODS

Young and newly born terminal shoot branches of *Taxus baccata* were collected from the mature trees growing at Tangmarg and Gulmarg areas of Kashmir in four different seasons viz. winter, spring, summer and autumn, to see the effect of auxins and seasons prevailing in the valley for inducing rooting in the cuttings. Stem cuttings were collected in polyethylene bags containing wet cotton to prevent their wilting and were brought to the laboratory for their immediate preparation for putting these into the soil. Nursery beds were prepared before hand by using sand and soil mixture in the ratio of 1:1 in the shady area of Naseem Bagh campus, University of Kashmir, Srinagar. The twigs (10 cm long approx.) alongwith pieces of old wood were prepared for planting in the soil.

Before planting the twigs in the soil the basal end of cuttings were dipped in NAA (1000ppm), IBA (1000ppm) and NAA + IBA (1000ppm) solutions separately for about 24 hrs. For control, twigs were dipped in distilled water. The cuttings were planted in four rows of 10 twigs each. Each row was kept 15 cms apart from each other. First row was kept as control, second row was supplied with NAA (1000ppm), third row with IBA (1000ppm) and fourth row with NAA+IBA (1000ppm). 10ml of respective phytohormonal supplement was added after every 15 days for a period of 10 weeks. Distilled water (10ml) was supplied to control lot in a similar fashion.

Following the treatments, the cuttings were planted obliquely in the nursery beds and a sheet of polyethylene cover was placed over the cuttings to maintain high humidity. The cuttings were watered and misted for about 10 weeks. The experiment was conducted in all the four seasons i.e. winter (Dec.-Feb.), spring (Mar.–May), summer (Jun.–Aug.) and sutumn (Sep.–Nov.) during the year 2002-2003.

RESULTS

The data in each trial and season was recorded after 14 weeks of planting the twigs. Observations were recorded, on average basis, for various parameters like callusing, number and length of adventitious roots induced. The response of stem cuttings planted in four different seasons in depicted in Table 1.

The twigs which were planted in (winter season) Dec.–Feb. responded best in terms of root induction, root number, length of roots and percent response. In winter season, the twigs which were treated with IBA showed excellent root inducing response, and the average number of roots recorded after 14 weeks was 10 (Pl. I., Fig. 1), while as cuttings treated with NAA+IBA also showed good rooting response but both average number of adventitious roots and length of roots was lesser than that of IBA treated cuttings (Pl. I., Fig. 2). Least affect amongst the trials was observed with NAA (1000ppm) where although length of root was better than NAA+IBA but the number of adventitious roots and percent response was low (Pl. I., Fig. 3). Control failed to show any such response (Pl. I., Fig.4). The trials carried in the months of spring season (Mar.–May) and summer season (Jun.–Aug.) showed no signs of callusing and root formation. However, the twigs which were planted in the months of

autumn season (Sep.–Nov.) showed low response of callusing and root initiation (Pl. II., Figs. 1-3). But the number and percent response was lesser as compared to winter season cuttings. In all the four seasons, the twigs which were kept as control showed no response of either callusing or root induction.

DISCUSSION

Most clonal selections of yews are propagated by cuttings which root without much difficulty (Sobo, 1976; Shugert, 1985). Present investigation carried on rooting of cuttings of *Taxus baccata* reveal that winter months are the best amongst all other months (seasons) for vegetative propagation trials. The response of twigs in winter season is followed by autumn season being lesser than winter season. This is in conformity with the earlier studies of Snyder (1955) wherein it has been shown that generally for propagation of woody tree species, cuttings are taken in early winter when actual shoot growth is arrested. These studies are again in corroboration with Snyder (1955) who has also reported winter season as the best season for rooting in *Taxus cuspidata*. Very recently Gupta *et al*. (2003) also reported successful rooting in the cuttings of *T. baccata* planted in the months of December which confirms the present study. Spring rooted *Taxus* were found equal to or superior to cuttings taken in late fall, (McGuire *et al.*, 1987; Studebaker *et al.*, 1988) which is quite contradictory to present findings where spring months were not at all found to be favourable for the rooting of cuttings.

Although rooting rates have been found to be greater in cuttings from younger and lateral shoots (Goo *et al*., 1990) but in the present study, since only young shoots were taken for trial, it has been found to be further influenced by other factor like season. In contrast to this Goo *et al*. (1990) reported relations between age, type of cuttings and IBA treatment in root or shoot growth in *T. cuspidata* cuttings.

Table 1: Response of stem cuttings to *in vivo* rooting in different seasons under the influence of two different auxins and their combination

ıs	Treatment	Response*			% Response
		Average callusing	Average No. of Adventitious roots	Average Length of Roots (cm)	
	NAA 1000ppm	+	4	1.5	40
eb.)	IBA 1000ppm	++	10	2.5	100
	NAA+IBA 1000ppm	++	6	0.5	70
	Control	---	0	0	0
	NAA 1000ppm	---	---	---	0
May)	IBA 1000ppm	---	---	---	0
	NAA+IBA 1000ppm	---	---	---	0
	Control	---	---	---	0
ır	NAA 1000ppm	---	---	---	0
Aug.)	IBA 1000ppm	---	---	---	0
	NAA+IBA 1000ppm	---	---	---	0
	Control	---	---	---	0
n	NAA 1000ppm	+	1	2.0	20
Nov.)	IBA 1000ppm	++	1	1.5	20
	NAA+IBA 1000ppm	+	---	---	0
	Control	---	---	---	0

* *Data recorded after 14 weeks of planting the cuttings into substrate; results are mean of ten replicates/treatment; --- no response.*

Effect of auxins on rooting in *Taxus baccata* cuttings (autumn season)
PLATE II. (Fig. 1-3)
Fig. 1. Root formation with NAA (1000ppm) after 14 weeks
Fig. 2. Root formation with IBA (1000ppm) after 14 weeks
Fig. 3. Only callus formation induced with NAA +IBA (1000ppm) after 14 weeks

It seems that temperature, humidity and physiological condition of the plant are of prime importance followed by hormonal treatment. In earlier studies Eccher (1988) has registered high relative humidity to be essential for proper rooting of *Taxus* cuttings which confirms the present case study, where high humidity was maintained during all

confirm over results for auxin IBA, although Ewald and Stauber (1994) at the same time also reported rooting of yews without auxin which is in contrast to the present studies, where control failed to show any results. Recently Gupta *et al*. (2003) reported 5cm long roots in cuttings of *T. baccata* L. after three months by using non-hormonal

treatment technology planted in the months of December which is again in disagreement with the present studies. The possible explanation of this phenomenon can be attributed to the variation in environmental conditions prevailing at these two different experimental sites.

The present investigation on rooting capacity of young cuttings of *Taxus baccata* reveal that this method has the potentiality of using at a large scale during winter under high humidity conditions and in presence of rooting hormone IBA, thus augmenting the natural regeneration through seeds which is very poor besides, being accompanied by the exploitation of the tree at an alarming rate.

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