



Effect of Different Rooting Media on Root Proliferation of *Taxus baccata* L. Stem Cuttings

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Abstract

Taxus baccata L., an endangered medicinal tree is highly valued for anti-cancer drug 'Paclitaxel' which is extracted from its leaves and barks. Reckless exploitation and habitat destruction have greatly reduced its natural population. Its regeneration through seed is also poor, so focus has been paid on vegetative propagation by stem cuttings to augment its natural regeneration. The present study was carried out to evaluate the effect of different rooting media on rooting behavior of *Taxus baccata* cuttings under poly house conditions. Effect of ten rooting media was studied on rooting of juvenile and mature stem cuttings of *Taxus baccata*. Six substrates i.e. field soil, forest soil, river sand, FYM, peat and vermi-compost were used for preparation of rooting media. The experiments were laid out in a randomized completely block design. The different rooting parameters were evaluated after six months of planting. The results revealed that the highest value of per cent survived, per cent callused, percent rooted, mean number of root and mean root length was obtained in both juvenile and mature cuttings planted in Forest soil+ Peat+ FYM (1:1:1) compared to the other rooting media. Not a single cutting survived which was planted in river sand. Mature cuttings planted in Forest soil + River sand + VC (1:1:1) also failed to survive. The media containing peat as substrate significantly improved the rooting of cuttings but due to optimum physico-chemical properties, Forest soil+ Peat+ FYM (1:1:1) proved ideal rooting media for stem propagation of *Taxus baccata*.



Article History

Received: 27 November 2017

Accepted: 13 February 2018

Keywords:

Paclitaxel,
Rooting media,
Stem cuttings,
Taxus baccata,
Vegetative propagation.


Introduction

Taxus baccata L. (Himalayan Yew; family Taxaceae) is a long lived and shade tolerant coniferous tree species. Usually found in cool temperate forest,

Taxus baccata occurred in patches in association with *Betula utilis*, *Abies pindrow*, *Acer cesium*, *Pinus wallichiana*, *Quercus semecarpifolia* and *Rhododendron arboretum*¹. The species is widely

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To Link to this Article: <http://dx.doi.org/10.12944/CARJ.6.1.12>

but sparsely distributed throughout Himalayas from Ladakh eastward to Khasi and Jaintia Hills, Naga Hills and Manipur between 2,300 and 3,400 m amsl². This species is primarily valued for 'paclitaxel' a mitotic inhibitor, extracted from its leaves and barks, used in the treatment of patients with lung, breast and ovarian cancer^{3,4}. As such, this valuable tree is being over exploited mostly by various collectors, traders and industrialists; its regenerative capability has been highly compromised. The present rate of exploitation has rendered it endangered⁵. Meghalaya, India is also blessed with few good stocks of *Taxus baccata*, is also facing similar threat due to degradation of its natural habitat for various reasons. The effects of overutilization and habitat degradation are compounded by the species poor natural regeneration process, slow growth rate and long seed dormancy period of 1.5-2 years⁶. Due to poor regeneration through seed, *Taxus baccata* is generally propagated through stem cutting using plant growth regulators. The most crucial stage in cutting propagation is the ability of cuttings to survive for a period until it gets rooted. Production

of long and extensive root system and quick rooting also ensure the survivability of cuttings⁷. So, the provision of optimum rooting media is required. The present experiment was conducted keeping in view the development of an ideal rooting media for *Taxus baccata* L. cuttings particularly for a subtropical climate by using potting media which is locally available and cost effective.

Materials and Methods

Study Area

The experiment were carried out in Department of Environmental Studies, North-Eastern Hill University, Shillong (Meghalaya, India) situated 25°36' North and 91°53' East at an elevation of 1426 m above mean sea level. The climate of this region is typically humid sub-tropical which is characterised by warm wet summers with cool dry winters. The mean monthly weather data of study site during study period is presented in Table 1. The mean temperature and relative humidity inside poly house during study period is given in Table 2.

Table 1: Mean monthly weather data during study period

Month	No. of Rainy Days	Rainfall (mm)	Mean Temperature (°C)		Relative Humidity (%)		Radiation (Mj/m ²)	Wind velocity (Km/h)
			Max.	Min.	Max.	Min.		
Jun	22	368.9	30.2	14.3	84.2	74.1	15.1	1.7
Jul	22	368.1	29.0	18.4	88.4	72.2	16.1	1.7
Aug	22	433.0	28.0	18.1	89.1	77.9	16.5	1.5
Sep	21	307.3	27.6	17.0	86.6	78.4	13.5	1.7
Oct	8	166.1	26.4	16.0	85.1	69.3	15.5	1.8
Nov	3	10.5	23.5	6.5	83.4	52.1	15.3	1.8

Table 2: Mean monthly temperature and relative humidity inside poly house during study period

Month	Mean Air Temperature (°C)		Relative Humidity (%)	
	Max.	Min.	at 1000 hrs	at 1600 hrs
Jun	32.2	23.5	96.8	91.3
Jul	31.0	21.2	93.75	88.7
Aug	32.1	17.7	90.15	88.4
Sep	30.6	18.3	90.3	91.2
Oct	28.4	16.3	86.1	84.3
Nov	26.5	15.1	94.3	88.2

Collection and Preparation of Cuttings

The branches were collected from phenotypically superior tree of *Taxus baccata* growing in Botanical Survey of India (East Khasi Hill, Shillong) during the first week of June (2012). The cuttings were then stored in moist polythene bags in order to prevent desiccation during transportation to experimental site. The stem cuttings of 15-20 cm long with 3-4 nodes were clipped from given branches. The needles were removed up to 2 cm above the basal portion of cuttings and grouped according to physiological age of shoot. The cutting with diameter is between 0.3-0.5 cm and green or light grey was classified as 'Juvenile cutting'. 'Mature cutting' was taken as stem cutting with diameter more than 0.5 cm but less than 1.0 cm and dark brown or grey in colour. To prevent fungal decay, the basal portion of cutting was dipped into 0.05% bavistin solution for 5 minutes. The treated cuttings were planted in slightly slant angle at a depth of 4-6 cm perforated poly-pot (18 cm deep and 6.5 cm diameter) containing prepared potting mixtures.

Preparation and Analysis of Potting Mixtures

Field soil, forest soil, river sand, farmyard manure (FYM) along with two imported substrates i.e. peat and vermi-compost (VC) were used for making rooting media. A total of ten rooting media either individually or in combination were made from given substrates. Top soil of 2-3 cm layer from base of the donor tree was used for rooting media "Field Soil"⁸. All media were sieved and oven dried at 120 °C for 48 hours prior to planting. In case of mixture, the substrates are mixed thoroughly prior to oven dry. The sample of all rooting media was analysed separately for pH, water holding capacity, porosity, organic matter content, organic Carbon content (by titration method), Nitrogen content (by Kjeldahl method) and Carbon:Nitrogen ratio (C:N ratio) as prescribed in Tropical Soil Biology and Fertility by Anderson and Ingram (1993)⁹. The summary of analysis is presented in Table 3.

Experimental Design

The experiment was laid out in a completely randomized block design with a factorial arrangement of treatments. Each treatment was replicated three times and each replicate had 5 cuttings. A total of 300 cuttings were used in the experiment (n = 300;

3 replications x 5 cuttings x 10 potting mixtures x 2 types of cuttings).

After Care

The cuttings were watered regularly as when depending on weather conditions and moisture status of the rooting medium. A high humidity of 75-90% and the desired temperature was maintained inside the poly house. The door and ventilators of the polyhouse were left open, whenever the temperature rose to about 35 °C to facilitate the maintenance of a constant temperature of 25-28 °C. The medium was drenched with Bavistin (0.15%) at fortnightly intervals to check disease incidence¹⁰.

Observation Recording

The cuttings were evaluated for following parameters after six months of planting: per cent survived, per cent callused, per cent rooted, number of cuttings rooted, mean number of roots formed per cutting and mean root length.

Statistical Analysis and Interpretation

Analysis of variance (ANOVA) was carried out for all the parameters and Least Significant Difference (LSD) test at 5% probability was used to compare significantly different means using GLM procedure in the SPSS (Statistical Package for Social Sciences version 16). To ensure normality and variance homogeneity, data of per cent survived, per cent callused and per cent rooted were converted into arc sine sqrt $((x + 0.5)/100)$, and data of root number was transformed into square root $(x + 0.5)$ ¹¹.

Results

The data on all the rooting parameters evaluated are furnished in Table 4.

Percent Survived

Cuttings planted in Forest soil + Peat + FYM recorded maximum rooting percentage (80.00 % in juvenile cuttings and 36.67 % in mature cuttings). Juvenile cuttings planted in Forest soil+ River sand (1:1) (13.33 %) and Forest soil + River sand + VC (1:1:1) (10.00 %) showed poor survivability. Not a single cutting able to survived which was planted in river sand. Mature cuttings planted in Forest soil + River sand + VC (1:1:1) also failed to survive.

Table 3. pH, Water holding capacity, Porosity, Organic matter content, Organic Carbon content, Nitrogen content and Carbon:Nitrogen ratio of Rooting Media

Rooting Media	Ph	Water Holding Capacity (%)	Porosity (%)	Organic Matter (%)	Organic Carbon Content (%)	Nitrogen Content (%)	C:N
1. Field soil (From the base of the mother plant)	4.35	69.44	53.47	4.83	1.63	0.13	13.02
2. Forest soil (From the pine forest inside NEHU)	4.83	63.37	54.37	5.47	1.84	0.17	10.64
3. Forest soil+ River sand (1:1)	6.03	51.48	78.50	2.38	0.80	0.12	6.87
4. Forest soil + Peat (1:1)	4.40	58.64	68.93	4.87	1.64	0.14	11.47
5. Forest soil + River sand (2:1)	5.47	54.82	64.10	3.77	1.27	0.14	9.29
6. Forest soil + Peat (2:1)	4.30	62.68	55.30	4.68	1.58	0.12	13.14
7. Forest soil + FYM + River sand	5.40	61.61	57.73	4.38	1.48	0.12	11.97
8. Forest soil + Peat + FYM	5.45	62.97	61.53	5.29	1.78	0.16	10.91
9. Forest soil + River sand + VC	5.97	71.00	51.00	5.87	1.98	0.41	4.83
10. River sand	5.90	30.20	91.33	0.78	0.26	0.12	2.1

Percent Callused

The stem cuttings of *Taxus baccata* planted in Forest soil + Peat + FYM showed highest callus formation as 73.33 % and 33.33 % was recorded in juvenile and mature cuttings respectively. Juvenile cuttings in River sand, and mature cutting in Forest soil+ River sand (1:1) did not exhibit any callusing. Poor callusing was seen juvenile cuttings planted in Forest soil+ River sand (1:1) (3.3%) and Forest soil + River sand + VC (1:1:1) (6.67%).

Percent Rooted

The mean value of per cent rooted cuttings was ranged between 0.00 to 63.33 % in juvenile cuttings while 0.00 to 30.00 % in mature cuttings (Figure 1). The highest percentage of rooting was noted in Forest soil + Peat + FYM (63.33 %) followed by Field soil (43.33 %). The least rooting success was observed in Forest soil + River sand + VC (1:1:1) (10.00 %) and Forest soil+ River sand (1:1) (13.33 %).

In mature cuttings, maximum rooting was observed in Forest soil + Peat + FYM (30.00 %). However, it was statistically at par with Forest soil + FYM + River sand (1:1:1) (26.67 %) and Forest soil + Peat (1:1)

(23.33 %). The poor rooting was observed in Forest soil+ River sand+ VC (1:1:1) and River sand alone where all cuttings failed to root.

Mean Root Number Per Cutting

The mean value of the number of roots per cutting in juvenile shoot ranged between 0.00 - 9.83, where the highest number of roots were shown by the cuttings planted in Forest soil + Peat (1:1) and the lowest by Forest soil+ River sand (1:1). However, the number of roots exhibited by Forest soil + Peat (1:1) (9.83 no.) is not significantly different from that of Forest soil + Peat + FYM (1:1:1) (9.10 no.), Forest soil + FYM + River sand (1:1:1) (8.61 no.), Forest soil + Peat (2:1) (8.33 no.) and Field soil (7.92 no.).

In the mature cutting the variation due to various rooting media is comparatively less where five out of eight rooting media (excluding Forest soil + River sand + VC and River sand where no cuttings rooted) i.e. Forest soil + Peat (1:1) (4.78 no.), Forest soil + Peat (2:1) (4.67 no.), Forest soil + Peat + FYM (1:1:1) (4.67 no.), Field soil (4.33 no.) and Forest soil + FYM + River sand (4.00 no.) showed no significant difference for the number of root produced.

Mean Length of Root Per Cutting

The longest root in juvenile shoot was exhibited in Forest soil + Peat + FYM (1:1:1) (6.28 cm) which is statistically insignificant with that of Forest soil + Peat (1:1) (5.52 cm) and Field soil (5.12 cm). The lowest mean root length was recorded in forest soil (1.91 cm) and Forest soil+ River sand (1:1) (2.05

cm). In the mature cuttings the longest root length was exhibited by Forest soil + Peat + FYM (1:1:1) (4.03 cm) followed by Field soil (3.73 cm.), Forest soil + Peat (1:1) (3.43 cm) and Forest soil + Peat (2:1) (3.30 cm). Like juvenile cuttings, mature cuttings in forest soil also bore small root (1.81 cm).

Table 4: Mean values of percent survived, percent callused, percent rooted, number of root and mean root length of two types of cuttings of *Taxus baccata* in response to various rooting media

Rooting media	Per cent Survived		Per cent Callused		Per cent Rooted		Mean number of root per cutting		Mean length of root per cutting	
	Juvenile cuttings	Mature cuttings	Juvenile cuttings	Mature cuttings	Juvenile cuttings	Mature cuttings	Juvenile cuttings	Mature cuttings	Juvenile cuttings	Mature cuttings
1. Field soil	66.67 ^{ab} (55.08)	16.67 ^{bc} (23.85)	56.67 ^{ab} (48.93)	13.33 ^b (21.15)	43.33 ^b (41.15)	13.33 ^{bc} (21.15)	7.92 ^{ab} (2.90)	4.33 ^a (2.18)	5.12 ^{ab}	3.73 ^a
2. Forest soil	46.67 ^{bc} (43.08)	6.67 ^{cd} (15.33)	36.67 ^{cd} (37.22)	6.67 ^{bc} (15.33)	23.33 (28.78)	6.67 ^{cd} (15.33)	5.06 ^{bc} (2.36)	2.00 ^b (1.48)	1.9 ^c	1.81 ^{bc}
3. Forest soil+ River sand (1:1)	13.33 ^{de} (20.74)	3.33 ^d (12.21)	3.33 ^g (12.21)	0.00 ^c (9.10)	3.33 ^e (12.21)	0.00 ^d (9.10)	1.67 ^{de} (1.25)	0.00 ^c (0.71)	2.05 ^c	0.00 ^d
4. Forest soil + Peat (1:1)	26.67 ^{cd} (30.29)	26.67 ^{ab} (30.99)	23.33 ^{de} (28.08)	26.67 ^a (30.99)	23.33 ^{cd} (28.08)	23.33 ^a (28.78)	9.83 ^a (3.21)	4.78 ^a (2.30)	5.52 ^{ab}	3.47 ^a
5. Forest soil + River sand (2:1)	13.33 ^{de} (20.74)	10.00 ^{cd} (18.03)	13.33 ^{ef} (20.74)	6.67 ^{bc} (15.33)	10.00 ^{de} (18.03)	3.33 ^d (12.21)	2.83 ^{cd} (1.68)	0.67 ^{ab} (1.00)	1.49 ^{cd}	0.75 ^{cd}
6. Forest soil + Peat (2:1)	46.67 ^{bc} (43.08)	30.00 ^a (33.21)	40.00 ^{bcd} (39.15)	23.33 ^a (28.78)	40.00 ^{bc} (39.15)	20.00 ^{ab} (26.07)	8.33 ^{ab} (2.97)	4.67 ^a (2.26)	4.54 ^b	3.30 ^a
7. Forest soil + FYM + River sand (1:1:1)	53.33 ^b (46.92)	33.33 ^a (35.22)	46.67 ^{bc} (43.08)	26.67 ^a (30.99)	23.33 ^{cd} (28.78)	26.67 ^a (30.99)	8.61 ^{ab} (3.00)	4.00 ^a (2.12)	4.24 ^b	2.88 ^{ab}
8. Forest soil + Peat + FYM	80.00 ^a (63.93)	36.67 ^a (37.14)	73.33 ^a (59.01)	33.33 ^a (35.22)	63.33 ^a (53.07)	30.00 ^a (33.21)	9.10 ^{ab} (3.09)	4.67 ^a (2.27)	6.28 ^a	4.03 ^a
9. Forest soil + River sand + VC (1:1:1)	10.00 ^e (18.03)	0.00 ^d (9.10)	6.67 ^g (15.33)	0.00 ^c (9.10)	0.00 ^e (9.10)	0.00 ^d (9.10)	0.00 ^e (0.71)	0.00 ^c (0.71)	0.00 ^d	0.00 ^d
10. River sand	0.00 ^d (9.10)	0.00 ^d (9.10)	0.00 ^g (9.10)	0.00 ^c (9.10)	0.00 ^e (9.10)	0.00 ^d (9.10)	0.00 ^e (0.71)	0.00 ^c (0.71)	0.00 ^d	0.00 ^d
Mean	35.67	16.33	31.28	20.51	23	12.33	2.19	1.57	3.12	2
SED	7.75	4.12	4.86	2.96	6.04	2.8	0.29	0.21	0.66	0.48
LSD @ 5%	17.95	8.17	10.64	6.3	10.51	6.92	0.74	0.51	1.61	1.17

Figures in parenthesis are mean of arc sine transformed value for percent survived, callused and rooted, and square root transformed value for root number. Mean followed by same alphabets in superscript are not statistically different @ p < 0.05.





Fig. 1: Effect of various rooting media on root proliferation of *Taxus baccata* stem cuttings: (a) Poorly developed root formation in juvenile cuttings; (b) Well developed root formation in juvenile cuttings; (c) callus formation in a mature cutting; (d) Poorly developed root formation in mature cuttings, (e) Well developed root formation in mature cuttings; (f) a profusely rooted mature cutting planted in Forest soil+ FYM + Peat (1:1:1)

Discussion

The results showed that there were significant differences among various rooting media on rooting efficiency. The differential rooting performance on various rooting media can be attributed to a direct effect of the substrate on the basal portion of the cuttings rather than the physiological change of cuttings.

The results showed that the rooting media containing the mixture of equal proportion of forest soil, peat and FYM has the overall best rooting performance. The values obtained for various parameters for forest soil, peat and FYM were statistically par above or equal to that of field soil. The improved root formation and vegetative growth on Forest soil + Peat + FYM (1:1:1) can be attributed to its well balanced physico-chemical properties as revealed from the physicochemical analysis of rooting media (Table 3). The other two rooting media: Forest soil + Peat (2:1) and Forest soil + FYM + River sand (1:1:1) also had a positive effect on the rooting of cuttings. The formulation of rooting media requires two considerations: the retention of sufficient moisture to help prevent desiccation of the cutting and the provision of an aerating agent so that air can circulate within the medium. Moreover, the higher nutrient status was better in the vegetative growth. A similar result was observed in *Delonix regia* studied by Waziri *et al.*, (2015)¹², who reported that the mixture of cow dung + top soil + washed river sand in 1:1:1 ratio had better rooting success. It was also found that the media having water holding capacity ranged between of 60-70 % had better rooting percentage for *Taxus* cuttings (Field soil, Forest soil, Forest soil + Peat (1:1), Forest soil + River sand + FYM (1:1:1). Too low (less than 55 %) or too high (more than 70 %) WHC proved detrimental to rooting of *Taxus* cutting. In addition, the coarseness of rooting media determines the quality of root developed by cuttings¹³. The good drained rooting media improved the adventitious root formation. River sand is coarser than other media and has good water drained property¹⁴, resulted in the highest mortality of cuttings in the present experiment due to poor water holding capacity. The river sand produces good results when used in combination with other components with rooting media or when there is a provision of intermittent mist and where there is no limiting factor.

It is also revealed from the experiment that the combination of Forest soil and Peat in equal proportions resulted in the highest number of roots per cuttings followed by media having equal proportion, Forest soil, peat and FYM (1:1:1) and Forest soil, sand and FYM (1:1:1). The root also showed an increased growth in media with an adequate water drainage as seen in soil + Peat (1:1) and Forest soil + River sand + FYM (1:1:1). It is also evident from results that the cuttings raised in Forest soil had poor root formation despite having a fairly good rooting percentage. It can be attributed to poor drainage of water in forest soil which might have created an anaerobic condition around the rooting zone of cuttings resulting in poor root development.

It is revealed from results that the cuttings planted in rooting media having an organic component (FYM and peat) had resulted in better root development. Sabina (2006) reported that 55 % leaf earth + 15 % sand + 30 % limestone is appropriate for propagation of *Taxus baccata*¹⁵. Bashir *et al.*, (2007) reported that the decomposed organic material improved the root zone by increasing soil aeration, water holding capacity and water infiltration and lower surface crusting¹⁶. It is evident from results that the rooting media having FYM as a component resulted in early rooting. However, it was also found during experiment that only few cuttings planted in media containing vermi-compost as an organic component was able to root. This unusual result could be related to the Carbon Nitrogen ratio. From the chemical analysis, it was found that FYM and/or peat containing media had a relatively high C:N content (Carbon: Nitrogen ratio) which might be responsible for improved root initiation. Many workers found the positive correlation between C:N ratio and rooting percentage of cuttings^{17,18,19}. High C:N ratio implies high carbohydrates content in the tissue of cuttings which is always considered as one of the important factor for rooting as carbohydrates serve as building blocks of complex macromolecules, structural elements and energy sources.

The role of nitrogen in adventitious rooting is questionable. According to Ling and Zhong (2010), nitrogen content has negative relationship with degree of rooting²⁰. From the chemical analysis it was seen that media having as a substrate approximately

two or three times higher than other rooting media. High nitrogen supply in form of fertilizer to seedlings or cuttings has often resulted in decrease of subsequent rooting of cuttings²¹. But optimum nitrogen concentration in relation to carbohydrates is necessary for root initiation because nitrogen is essential in synthesis for root differentiation^{22,23}.

Conclusion

From present experiment, it can be concluded that the rooting of *Taxus baccata*, can be improved by rooting media made up of soil, peat and FYM in equal proportions. In case if peat is not available or due to some financial constraints, one can go with

river sand as a replacement for peat. Apart from rooting media, other factors such as maintenance of propagation house, plant growth regulator treatment and moisture regulation should also be taken into account while propagating by cuttings.

Acknowledgement

We thank Dr. A. A. Mao, Scientist E & In charge, Botanical Survey of India, ERC, Shillong, Meghalaya and State Forest officials of Meghalaya for providing planting materials for research work. The financial assistance to Mr. Saumitro Das in the form of UGC NET fellowship from University Grants Commission (UGC), New Delhi, is gratefully acknowledged.

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