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Propagation studies on threatened and medicinally important *Rauvolfia serpentina* (L.) Benth. ex Kurz through stem and root cuttings

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Abstract

Rauvolfia serpentina (L.) Benth. ex Kurz is one of the most important medicinal plants and is listed as critically endangered species by the IUCN. The dry roots of the *R. serpentina* is the economic component containing a variety of alkaloids such as reserpine, rescinnamine, deserpidine, ajamalacine, ajmaline, neoajmaline, serpentine, a-yohimbine and etc. It is majorly used as antihypertensive and as sedative. The species has become endangered through the exploitation and extraction of these roots and therefore, immediate steps need to be taken for conservation. So, in this context the present study on the propagation of *R. serpentina* was conducted by treating semi-hard wood stem and root cuttings for rooting with different indole-3-butyric acid (IBA) concentrations. The 18-24 months old plants were used as propagating material which had resulted in maximum survival percentage of 65% in semi hard wood cutting whereas, 78% survival observed in the root cuttings treated with 100 ppm of IBA. Over the last two decades, demand for the Serpentine roots has increased by many folds. However, the availability of the planting material is very limited for large scale cultivation. So, in order to increase the availability of the Quality Planting Materials (QPMs), we have studied the propagation through stem and root cuttings.

Keywords: propagation, threatened, medicinal, *Rauvolfia serpentina*

Introduction

Rauvolfia serpentina (L.) Benth. ex Kurz (Serpentine root, *Sarpagandha*) is an endangered woody perennial plant belonging to the family Apocynaceae. It normally grows up to 15-45 cm, but it can also show growth up to 90 cm under cultivation. Roots are vertical, tapering up to 15 cm thick at the crown and long giving a serpent-like appearance with greenish-yellow in colour externally and pale-yellow inside and are extremely bitter in taste. Leaves are in whorls of 3-4, elliptic-lanceolate or obovate, pointed at apex. Flowers are numerous borne on terminal or axillary cymose inflorescence, corolla is tubular, 5-lobed, 1-3 cm long, whitish-pink in colour, fruit drupe, obliquely ovoid and purplish black in colour at maturity with stone containing 1-2 ovoid wrinkled seeds. The plant is cross-pollinated (Sulochana, 1959) [27]. The species has become endangered through exploitation and extraction of these plants for its roots which has given rise to urgent need of conservation. In addition, the plant produces flavonoids, phenols, tannins and vitamins such as ascorbic acid, riboflavin, thiamin and niacin. Yet, medicinal plant cultivation *in vitro* is still not normal. They are mostly collected from the wild and their overuse poses a major threat to the resource. *R. serpentina* is also seriously threatened with extinction and seed or vegetative propagation cannot meet the growing demand (Kataria & Shekhawat, 2005 [12]; Dutta *et al.*, 1963 [6]). In this context, in order to increase the availability of *R. serpentina*, efforts are made through the application of other techniques like plant tissue culture, stem and root cutting (Mathur *et al.*, 1987 [14]; Mukhopadhyay *et al.*, 1991 [17]; Roy *et al.*, 1995 [23]; Roja & Heble 1996 [22]; Tiwari *et al.*, 2003(32); Kataria & Shekhawat 2005(12); Baksha *et al.*, 2007 [3]; Bhatt *et al.*, 2008 [4]; Salma *et al.*, 2008 [24]; Mishra *et al.*, 2010) [15]. Thus, it is very essential to conserve *R. serpentina* to fulfill the demand of herbal industry. Global statistics shows that 80% of approximately 4 million people are unable to afford western pharmaceutical industry products and must rely on the use of conventional medicines mainly derived from plants (Taley *et al.*, 2012) [30]. Its dried roots are economically important. It contains a range of alkaloids that are pharmacologically essential including reserpine, rescinnamine, deserpidine, ajamalacine, ajmaline, neoajmaline, serpentine, a-yohimbine. The root is sedative and can control high blood pressure and insanity. In Ayurveda, it is also used for curing various diseases like insomnia, epilepsy, asthma, acute stomachache and painful delivery. It is used in the treatment of snake-bite, insect stings and mental disorders (Joy *et al.*, 1998) [11]. In pharmaceutical industries, roots of *Sarpagandha* and its products are in boundless demand because of its valuable medicinal properties. However, the chemical synthesis of reserpine is possible but it costs more than extracting it from natural resources (Farooqi & Sreeramu

2001) [7]. *R. serpentina* shows very poor seed germination. Seeds are not easily available from wild sources (Paul *et al.*, 2008) [20]. This may be the limitation for the development of its population. The increasing demand of the roots of *Sarpagandha* and its alkaloids has led to studies of domestication which have brought it into cultivation. Several research institutions in India have carried out such studies making its cultivation available to the farmers. At present, it is cultivated in small areas, scattered far and wide in the states of Uttar Pradesh, Bihar, Tamil Nadu, Orissa, Kerala, Assam, Maharashtra, West Bengal and Madhya Pradesh (Dutta *et al.*, 1963) [6]. By the considerations, the present study was conducted to standardize propagation methods by semi - hard wood stem and root cuttings for production of QPMs.

Materials and Methods

The study was carried out in the research farm of College of Forestry, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (DBSKKV), Dapoli, Ratnagiri district of Maharashtra located between $17^0 45'$ N latitude and $73^0 12'$ E longitude with an elevation of 250 m MSL and Medicinal Plants Garden, Savitribai Phule Pune University (SPPU), Pune. The relative humidity (RH) ranged from 64.5 to 98.5 per cent. The monthly weather data during the study period from September 2012 to January, 2014 has been obtained from meteorological observatory, DBSKKV, Dapoli and Department of Environmental Science, SPPU, Pune. The study area is located in the lateritic soil belt and black basalt (Fig.1). Planting material of *R. serpentina* was procured from authentic sources. Cutting and rooting material was collected from about 18-24 months old mother plants in the month of September, 2012. The apical soft portion and the lower semi hard wood zones were separated. Each cutting was retained with only one pair of completely unfolded leaves. The lamina of each leaf was prunned with the help of sharp scissors to half its size. The lower portions of the branches were defoliated and semi hard wood cuttings of size 10-15 cm long were prepared. In case of roots, larger than pencil size roots were selected for propagation studies. Root cuttings at 3 cm in length having 2 nodes were used for study. All cuttings were soaked in 0.1 percent Bavistin suspension in water for 15 minutes. The upper ends of cuttings were sealed by wax followed by different treatments *viz*. Indole Butyric Acid (IBA) with concentrations of 25, 50, 75 and 100 ppm for 30 minutes including control. Immediately, the treated cuttings were planted in poly bags of size 4 X 7 cm. Before that, the polybags were filled with a mixture of soil and FYM in the ratio of 2:1. The polybags were kept under partial shed in net house and watered regularly to avoid desiccation. The same method was followed for root cuttings (Fig. 2A & B). The experiment consisted of 5 treatments *viz*. 25, 50, 75 and 100 ppm Indole Butyric Acid (IBA) including control with 4 replications consisting of 25 seedlings each arranged in Randomized Block Design (RBD) under 50 percent shade net. Observations on seedling growth parameters and biomass parameters were recorded up to 90 days after planting (DAP).

Statistical Analysis

The experiments are conducted in randomized block design (RBD) to assess the effect of number of day and five treatments on various variables related to plant growth. The two factors used for RBD. The number of days as one factor having three levels 30 days, 60 Days, and 90 Days whereas another factor was treatment having five levels (T1, T2 T3, T4, T5). The five response variables were measured for all these treatments and after 30, 60 and 90 days. We have carried out two ways ANOVA and the p-values for these factors are reported in the table. We have also reported the R-squared (adjusted) values which gives the degree of agreement of the actual data with the model used (Montgomery, 2017[16]).

Results and Discussion

Growth parameters like root length, number of roots, shoot length, number of leaves number of shoots, shoot height, number of leaves, root length, number of roots, diameter and biomass parameters like fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, total fresh weight and total dry weight of *R. serpentina* propagation through semi hard wood (stem) cuttings at 90 (DAP) were recorded and are depicted in Table 1, Fig. 3A & B; Fig. 4. Macropropagation techniques have been researched for the propagation of many commercially valuable species, notably uncommon, endangered, and threatened plant species, and are an important part of tree improvement efforts (Shekhawat & Manokari, 2016; Jamir *et al.*, 2016) [26, 10]. The data given in Table 1 showed that, the parameters like shoot height, number of leaves and number of roots showed significant variation among treatments. Similarly, the biomass parameters like fresh root weight and total dry weight varied significantly. Considering the data of plant growth parameters, number of shoots are having maximum value in T_1 (control) 2.30 and minimum value in T_5 (soaking cuttings in IBA @ 100 ppm for 30 min) 1.78. However, the root length was in the range of (T_5) 9 cm to 3.13 cm (T_1) whereas, the diameter measured maximum in T_5 (4.50 mm) and minimum (4.03 mm) in T_2 (soaking cuttings in IBA @ 25 ppm for 30 min) respectively (Table 1). The average decreased shoot height was observed as 2. 10 ±0.22 cm. It has been observed that shoot height decreased with respect to increase in concentration of IBA than that of control. Similar findings were observed by Vamil *et al.*, (2010) [34]; Vamil *et al.*, (2011) [33] in *Bambusa arundinacea*, Choe, (1972) [5] in *Pisum sativum*, and Sharma and Tyagi, (2013) [25] in *Rauvolfia serpentina* that IAA and IBA treatments enhanced seedling growth whereas, Prakash (1998) [21], have found out that after addition of IIA (100 ppm) and IBA (100 ppm) increased the shoot length and root length respectively at seedling stages of *Artocarpus heterophyllus.* However, the shoot height showed maximum value in treatment T_4 (soaking cuttings in IBA @ 75 ppm for 30 min) 16.27 cm while minimum value in T_2 observed at (13.06 cm) with mean value of 14.64 ± 0.53 cm. Treatment T_4, T_5 and T_3

(soaking cuttings in IBA @ 50 ppm for 30 min) were at par. The treatment T_4 was significantly different from T_1 and the shoot height has shown a decrease in T_2. The maximum number of leaves were observed at treatment T_3 (11.64 cm) and minimum at T_2 (7.84 cm) with mean value 9.90 ± 0.44 cm. Treatment T_3, T_5 and T_4 were at par, T_3 was significantly different from T_1 and T_2. Similarly, number of roots were observed highest in T_5 (13 cm) and lowest in T_1 (4 cm) with mean value 8.45 ± 1.46 cm. Treatment T_5 and T_4 were at par, T_5 was significantly different from T_2, T_3 and T_1 respectively. The observed values of root length ranged between 3.13 cm to 9.00 cm. The maximum value was observed at T_5 and minimum at control. The mean value was observed as 6.18 ± 1.46 cm. In case of diameter, the values ranged from 4.03 mm to 4.50 mm. The maximum value was observed at T_5 while the minimum value was observed at T_2 with mean value of 4.27 ± 0.16. It has shown increasing trend except T_2. Therefore, with an increase in concentration of IBA has shown an increase in diameter. Similar trend was also recorded in biomass parameters. Fresh shoot weight ranges from 2.55 gm to 5.45 gm in T_1 and T_5 respectively with mean value of 3.46 ± 0.87 gm and dry shoot weight showed maximum value (1.86 gm) in T_5 and minimum in T_4 (0.76 gm) with mean value of 1.11 ± 0.27 gm. Whereas, the dry root weight recorded highest value (0.61 gm) in T_5 and lowest in control (0.12 gm) with mean value of 0.36 ± 0.16 gm. However, the fresh root weight ranges from 0.29 gm to 1.84 gm in (T_1) and (T_5) respectively with mean value of 0.86 ± 0.30 gm. T_5 and T_4 were at par with each other, T_5 was significantly different from T_2, T_3 and T_1 respectively. Similar study has been done and found out that for root induction in stem cuttings, the IBA was more successful (Pandey *et al.*, 2011[19]; Nandi *et al.*, 2002) [18]. In case of dry shoot weight, values range from 0.76 gm to 1.86 gm with mean value of 1.11 ± 0.27 gm. Similarly, total dry weight showed maximum in treatment T_5 (2.46 gm) while it was minimum in T_1 (0.94 gm) with mean value 1.42. Treatment T_5 showed significantly superior to all other treatments, 0.12 gm in T_1 similarly, total fresh weight ranges from 2.85 gm to 7.31 gm in T_1 and T_5 respectively. Whereas, the percent survival was recorded maximum in treatment T_5 (65%), followed by T_4 and T_2 (55%) each, whereas the minimum was observed in T_1 (43%) with mean value 53.60%. The overall results showed that, the pre-sowing treatment T_5 (soaking cuttings in IBA @ 100 ppm for 30 min.) performed better with respect to shoot height, number of roots, fresh root weight and total dry weight compared to all other treatments. The data presented in Table 2 showed that, the effect of IBA on growth parameters and biomass of *Rauvolfia serpentina* propagated through root cutting 90 DAP. In that table, the growth parameters *viz.* shoot height, number of leaves and diameter showed significant variations among treatment. In case of biomass parameters, all treatments were non- significant. Number of shoots were observed maximum (1.80) in control (T_1) treatment and minimum (1.44) in T_4 (Deeping cuttings in IBA @ 75 ppm for 30 min). Interestingly, the highest shoot height was observed in T_5 (10.97 cm) and lowest in T_2 (Deeping cuttings in IBA @ 25 ppm for 30 min) treatment (5.50 cm) with mean 7.42. T_5 (Deeping cuttings in IBA @ 100 ppm for 30 min) was significantly superior compared to all other treatments. However, the number of leaves was recorded maximum in T_5 (13.55) and minimum in T_1 (8.69) with mean 11.22. Treatment T_5, T_3 and T_4 were at par. T_5 was significantly different from T_2 and T_1. Whereas, the root length of *R. serpentina* having non-significant with highest value in treatment T_5 (10.88 cm) and lowest in T_1 (6.00 cm). However, the number of roots were recorded maximum in treatment T_5 (14.25) and lowest in T_1 (5.50) interestingly, the diameter of *R. serpentina* having range between 5.78 to 8.07 mm in (T_1) and T_3 (Deeping cuttings in IBA @ 50 ppm for 30 min) respectively with mean 7.10. Treatment T_3, T_5 and T_4 were at par T_3 was significantly different from T_2 and T_1. In case of biomass parameters, fresh shoot weight showed highest value (5.50 gm) in treatment T_5 whereas treatment T_1 recorded lowest value (1.97 gm). Whereas, fresh root weight of plant biomass was recorded maximum (2.27 gm) in T_5 and minimum (0.70 gm) in T_1. Similar trend was observed in dry shoot weight ranges from 0.76 to 1.66 gm in (T_1) and (T_5). Whereas, dry root weight measured highest value (0.88 gm) in T_5 and lowest (0.25 gm) in T_1, Similarly, total fresh weight was recorded maximum (7.84 gm) in T_5 and minimum (2.67 gm) in T_1. Total dry weight was measured maximum in T_5 (2.55 gm) and minimum in T_1 (0.81 gm). Interestingly, in case of per cent survival, it was recorded maximum (78%) in T_5 (Deeping cuttings in IBA @ 100 ppm for 30 min) followed by T_4 (68%) and T_3 (61%) each, whereas minimum observed in T_1 (47%) with mean value 62.40. Furthermore, all 5 treatments, with the exception of control, exhibited an increase in all activities as the concentration of IBA increased. On the same line the result has been observed by the many researchers (Shekhawat & Manokari 2016; Jamir *et al.*, 2016) [26, 10]. Exogenous administration of IBA has been shown to increase the speed of sugar translocation and transport to stem cuttings, as well as boost root formation (Haissig 1974; Shekhawat & Manokari, 2016) [8, 26] used a greater concentration of 400 mg/L, but we merely employed a higher dosage of 100 mg/L in our study. The likely reason is that the IBA is a good growth promoter for all plants, which is why we saw growth in all of the measures evaluated. From the Table 4 it can be seen that number of days and treatments are significantly affect the response variables except, day do not affect much to survival (p-value 0.801) and number of leaves are not much affected by treatment (p value 0.099), when the stem material was used. All the model perform better except for survival (R-sq(adj)-62.05 % in this case. When root material is used Days and Treatments are significantly affecting the response variable except, Days do not affect much the survival (p value 0.479) and root length by treatments (p value-0.093). Here, model for number of roots do not perform well (R sq. (adj) 54.94%) but all others are pretty well. Moreover, in the comparison between root cutting and stem cutting, it can be seen that Root material performs better than the stem material (Fig. 4 A, B, C, D). The root cuttings showed more percentage than that of stem cuttings (Table 3). Several studies have found that the rooting potential of certain species varies significantly depending on the time of year stem cuttings are obtained (Swarts *et al.*, 2018) [29]. The physiological state of the plant is tied to the time of year or season, not just a calendar date (Hartmann *et al.*, 1997; Swamy *et al.*, 2001) [9, 28]. The growth media's job is to keep the cutting in place, provide moisture, and allow air exchange at the cutting's base. Lodama *et al.*, (2016)(13) found that growing media had a substantial impact on the rooting capacity of *L. fruticosus* cuttings in a study. Variations in rooting ability for cuttings taken from various points on the shoot are frequently overlooked (Al-Salem and Karam, 2001; Agbo and Obi, 2007) [2, 1]. The overall quality and subsequent growth habit of rooted stem cuttings can also be affected by different cut-ting positions from the shoot (Tchoundjeu and Leakey, 1995) [31]. Root cutting is simple, and root material is more efficient in acclimating to present conditions than stem material, as well as absorbing nutrients from the culture.

Plantation and Conservation Efforts

Sarpagandha (*R. serpentina*), is a highly demanded drug in the national and international market due to its astonishing therapeutic properties. Presently, it is categorized as critically endangered by IUCN. Hence, there are limitations to get the dried roots from natural habitat. The propagation through stem and root is the easiest method for mass multiplication and for production of Quality Planting Materials (QPMs). The QPMs of the *R. Serpentina* species have been produced on large scale through stem and root cuttings and planted in agricultural fields in about 25 hectares in the states like Maharashtra, Gujarat, Bihar and etc. by following Good Agricultural Practices (GAPs).

Conclusion

Herewith we conclude that, the propagation of *R. serpentina* can be done using root and stem cutting methods. Not only this technique is useful for the propagation but it is also beneficial to fulfill the current demand of herbal industries. This kind of projects must be initiated and proper research work should be carried to achieve sustainable yield of *R. serpentina.* IBA culture was a useful for the development of *R. serpentina*. It is also being observed that the root cutting method is more efficient than that of the stem cutting method. Root is a commercially important part hence preference through stem cutting should be given for large scale cultivation. This could be the best practice for the conservation of *R. serpentina.*

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Fig 1: The study site

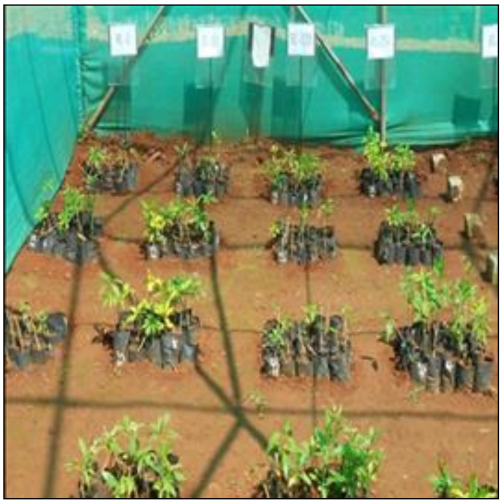

Fig 2: A Propagation through semi-hard wood cutting

Table 1: Effect of IBA on growth parameters and biomass of *Rauvolfia serpentina* propagated through semi-hard wood (stem) cuttings at 90 DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters						Survival (%)
		No. of Shoot	Shoot Height (cm)	No. of Leaves	Root Length (cm)	No. of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)	
T_1	Control	2.30	13.29	7.98	3.13	4.00	4.16	2.55	0.29	0.83	0.12	2.85	0.94	43.00
T_2	IBA @ 25 ppm for 30 min.	2.28	13.06	7.84	4.38	8.25	4.03	3.21	0.69	1.11	0.56	3.99	1.40	55.00
T_3	IBA @ 50 ppm for 30 min.	2.27	15.21	11.64	6.38	8.00	4.40	3.31	0.55	1.02	0.24	3.89	1.26	50.00
T_4	IBA @ 75 ppm for 30 min.	1.87	16.27	10.64	8.00	9.00	4.24	2.79	0.92	0.76	0.27	3.68	1.03	55.00
T_5	IBA @ 100 ppm for 30 min.	1.78	15.37	11.38	9.00	13.00	4.50	5.45	1.84	1.86	0.61	7.31	2.46	65.00
Mean		2.10	14.64	9.90	6.18	8.45	4.27	3.46	0.86	1.11	0.36	4.34	1.42	-
SEm(±)		0.22	0.53	0.44	1.46	1.46	0.16	0.87	0.30	0.27	0.16	1.04	0.32	-
CD (P = 0.05)		NS	1.64	1.36	NS	4.50	NS	NS	0.93	NS	NS	NS	0.98	-

Table 2: Effect of IBA on growth parameters and biomass of *Rauvolfia serpentina* propagated through root cuttings 90 at DAP

Pre-Sowing treatment		Growth parameters						Biomass parameters						Survival (%)
		Number of Shoot	Shoot Height (cm)	Number of Leaves	Root Length (cm)	Number of Roots	Diameter (mm)	Fresh Shoot Wt. (gm)	Fresh Root Wt. (gm)	Dry Shoot Wt. (gm)	Dry Root Wt. (gm)	Total Fresh Wt. (gm)	Total Dry Wt. (gm)	
T_1	Control	1.80	5.57	8.69	6.00	5.50	5.78	1.97	0.70	0.76	0.25	2.67	0.81	47.00
T_2	IBA @ 25 ppm for 30 min.	1.60	5.50	8.75	8.00	7.50	6.34	4.75	1.68	1.42	0.63	6.43	2.09	58.00
T_3	IBA @ 50 ppm for 30 min.	1.68	6.96	12.97	9.00	9.00	8.07	5.07	1.54	1.50	0.52	6.94	2.03	61.00
T_4	IBA @ 75 ppm for 30 min.	1.44	8.12	12.11	9.50	10.75	7.29	3.81	1.53	1.20	0.57	5.53	1.58	68.00
T_5	IBA @ 100 ppm for 30 min.	1.57	10.97	13.55	10.88	14.25	8.03	5.50	2.27	1.66	0.88	7.84	2.55	78.00
Mean		1.62	7.42	11.22	8.68	9.40	7.10	4.22	1.54	1.31	0.57	5.88	1.81	62.40
SEm(±)		0.08	0.30	0.63	1.09	2.17	0.51	1.17	0.45	0.42	0.17	1.55	0.55	-
CD (P = 0.05)		NS	0.93	1.95	NS	NS	1.56	NS	NS	NS	NS	NS	NS	-

Table 3: Survival percentage of both root cutting and stem cutting methods

Survival Root Cutting			Survival Stem Cutting		
Survival%	Survival%	Survival%	Survival%	Survival%	Survival%
30 days	60 days	90 days	30 days	60 days	90 days
60.00	61.00	61.00	55.00	58.00	56.00
63.00	63.00	63.00	65.00	61.00	63.00
71.00	70.00	70.00	51.00	55.00	45.00
65.00	60.00	62.00	55.00	55.00	48.00
73.00	73.00	75.00	63.00	61.00	70.00

Table 4: P values and the R-squared (adjusted) values for the two ways ANOVA.

	Factors		
Response Variable	p-value for No. of Days	p-value for Treatment	R-sq(adjusted)
STEM			
Root length	0.001	0.014	86.80%
No. of Roots	0.001	0.008	91.53%
Shoot Length	0.001	0.001	91.13%
No. of Leaves	0.001	0.099	92.74%
Survival	0.801	0.010	62.05%
ROOT			
Root length	0.001	0.093	84.76%
No. of Roots	0.070	0.049	54.94%
Shoot Length	0.001	0.020	92.60%
No. of Leaves	0.001	0.026	94.75%
Survival	0.479	0.001	93.96%

Fig 2: B Propagation through root cutting

T_1	T_2	T_3	T_4	T_5

Fig 3: A Effect of IBA on growth parameters and biomass of *Rauvolfia serpentina* propagated through root cuttings

T_1	T_2	T_3	T_4	T_5

Fig 3: B Effect of IBA on growth parameters and biomass of *Rauvolfia serpentina* propagated through semi-hard wood leafy cuttings

Comparison for Root cutting and Stem cutting methods for 90 Days:

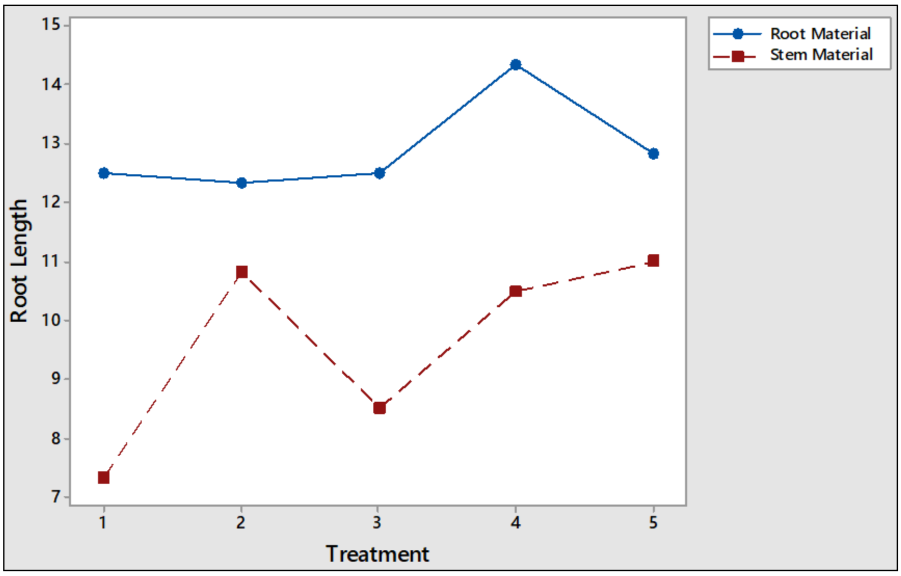

Fig 4: A - Comparison of root length

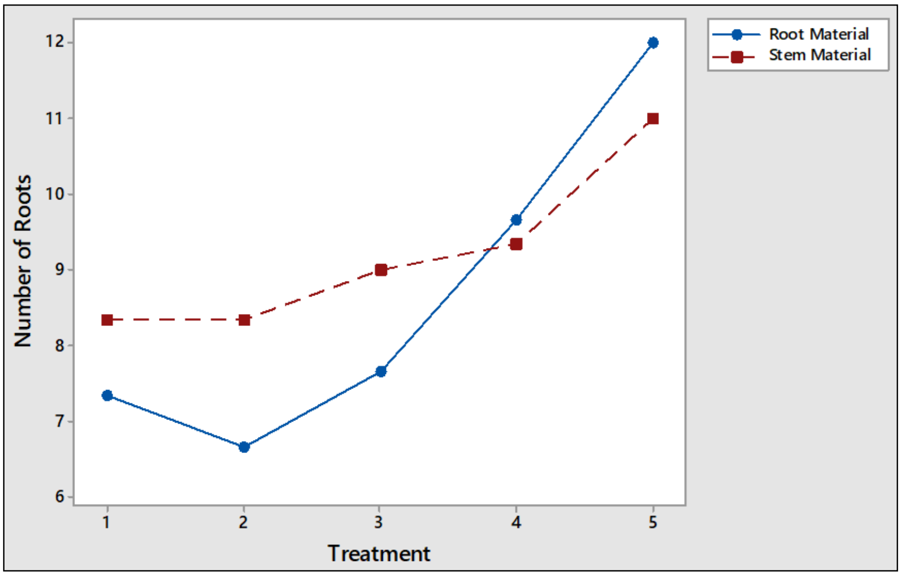

Fig 4: B comparison of Number of roots

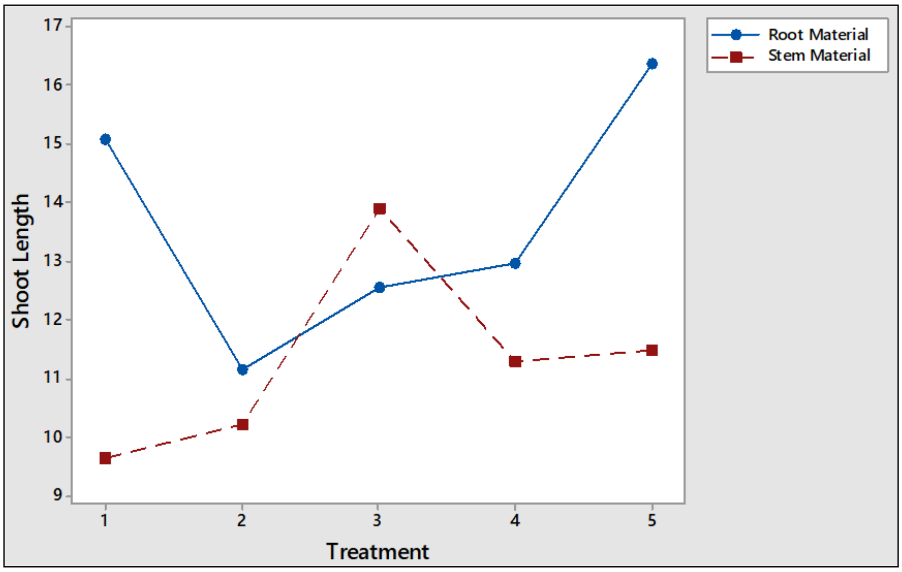

Fig 4: C – comparison of Shoot length

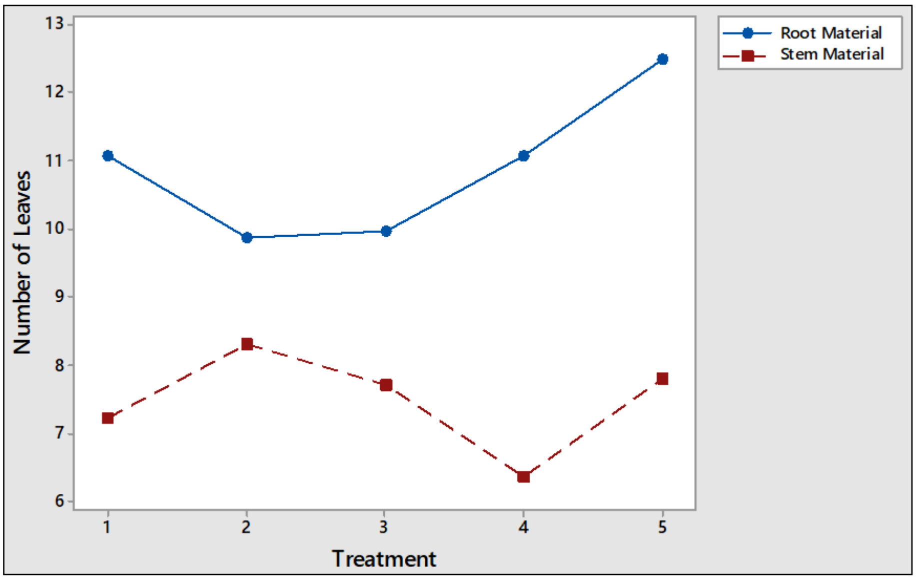

Fig 4: D- Comparison of Number of Leaves

References

1. Agbo CU, Obi IU. Variability in propagation potentials of stem cuttings of different physiological ages of *Gongronema latifolia* Benth. World Journal of Agricultural Sciences,2007:3:576-581.
2. Al-Salem MM, Karam NS. Auxin. wounding, and propagation medium affect rooting response of stem cuttings of *Arbatus andrachne*. Journal of the American Society for Horticultural Science,2001:36:976-978.
3. Baksha R, Jahan MA, Khatun R, Munshi JL. *In vitro* rapid clonal propagation of *Rauvolfia serpentina* (Linn.) Benth. - Bangladesh. Journal of Scientific and Industrial Research,2007:42:37-44.
4. Bhatt R, Arif M, Gaur AK, Rao PB. *Rauvolfia serpentina* L. Protocol optimization for *in vitro* propagation. African Journal of Biotechnology,2008:7:4265-4268.
5. Choe HT. Effects of presoaking seeds of *Pisum sativum* L. in GA3, IAA and KN solutions on seedling growth. Horticulture Science,1972:7(5)**:**476-478.
6. Dutta PK, Chopra IC, Kapoor PK. Cultivation of *Rauvolfia serpentina* in India. Economic Botany,1963:17:(4):243-251.
7. Farooqi AA, Sreeramu BS. Cultivation of Medicinal and Aromatic Crops. University Press Ltd. India, 2001, 210-211.
8. Haissig BE. Influences of auxin synergists on adventitious root primordium initiation and development. New Zealand Journal of Forestry Science,1974:4:311-323.
9. Hartmann HT, Kester DE, Davies FT, Geneve RL. Plant Propagation: Principles and Practices. Sixth ed. Prentice Hall, New Jersey, 1997.
10. Jamir SL, Deb CR, Jamir NS. Macropropagation and Production of Clonal Planting Materials of Panax pseudogin- seng Wall. Open Journal of Forestry,2016:06:135-141.
11. Joy PP, Thomas J, Mathew S, Skaria BP. *Medicinal Plants*. Kerala Agricultural University, India, 1998, 46-47.
12. Kataria V, Shekhawat NS. Cloning of *Rauvolfia serpentina* L. an endangered medicinal plant. Journal of Sustainable Forestry,2005:20:53-65.
13. Lodama KE, du Toit ES, Steyn JM, Araya HT, Prinsloo G, du Plooy CP *et al*. Improving rooting of *Lobostemon fruticosus* L. cuttings with delayed auxin treatment. South African Journal of Botany,2016:105:111-115.
14. Mathur A, Mathur AK, Kukreja AK, Ahuja S, Tyagi BR. Establishment and multiplication of colchiautotetraploids of *Rauvolfia serpentina* L. Benth. ex Kurz, through tissue culture. Plant Cell Tissue and Organ Culture,1987:10:129-134.
15. Mishra Y, Usmani G, Mandal AK. *In vitro* cloning of *Rauvolfia serpentina* L. Benth. var. CIM- Sheel and evaluation of its field performance. Journal of biological Research,2010:13:85-92.
16. Montgomery DC. *Design and Analysis of Experiments*, Ninth Ed., Wiley, India, 2017.
17. Mukhopadhyay S, Mukhopadhyay MJ, Sharma AK. *In vitro* multiplication and regeneration of cytological stable plants of *Rauvolfia serpentina* Benth. through shoot tip culture. Nucl Calcutta,1991:34:170-173.
18. Nandi SK, Tamta S, Palni LMS. Adventitious root formation in young shoots of *Cedrus deodara*. Biologia Plantarum,2002:45:473-476.
19. Pandey A, Tamta S, Giri D. Role of auxin on adventitious root formation and subsequent growth of cutting raised plantlets of *Ginkgo biloba* L. International Journal of Biodiversity and Conservation,2011:3(4):142-146.
20. Paul D, Paul NK, Basu PK. Seed germination response of *Rauvolfia serpentina* Benth. to certain physical and chemical treatments. Journal of Bioscience,2008:16:129-131.

21. Prakash M. Effect of plant growth regulators and chemicals on germination of Jack fruit. Annual Review of Plant Physiology,1998:12(1)**:**75-77.
22. Roja G, Heble MR. Indole alkaloids in clonal propagules of *Rauvolfia serpentina* L. Benth. ex Kurz. Plant Cell Tissue and Organ Culture,1996:44:111-115.
23. Roy SK, Roy PK, Rahman M, Hossain T. Clonal propagation of *Rauvolfia serpentina* L. through *in vitro* culture. Acta Horticulturae,1995:390:141-145.
24. Salma U, Rahman MSM, Islam S, Haque N, Khatun M, Jubair TA *et al*. Mass propagation of *Rauvolfia serpentina* L. Benth. Pakistan Journal of biological Sciences,2008:11:1273-1277.
25. Sharma N, Tyagi S. Effects of Plant Growth Regulators (Indoles) on Germination Percentage and Seedling Growth of *Rauvolfia Serpentina* (L.) Benth. ex Kurz. International Journal of Science and Research,2013:4:1234-1238.
26. Shekhawat MS, Manokari M. Impact of Auxins on Vegetative Propagationthrough Stem Cuttings of *Couroupita guianensis* Aubl.: A Conservation Approach. Hindawi Publishing Corporation Scientifica, 2016, 1-7.
27. Sulochana CB. Indian species of *Rauwolfia*. Journal of Indian Botany Society,1959:38:575-594.
28. Swamy SL, Puri S, Singh AK. Effects of auxins (IBA and NAA) and season on rooting of juvenile and mature hardwood cuttings of *Robinia pseudoacacia* and *Grewia optiva*. New Forests,2001:23:143-157.
29. Swarts A, Matsiliza-Mlathi B, Kleynhans R. Rooting and survival of *Lobostemon fruticosus* (L) H. Buek stem cuttings as affected by season, media and cutting position. South African Journal of Botany,2018:119:80-85.
30. Taley SL, Pethe AS, Rothe SP. Studies on antibacterial activity of some plant extracts. International Multidisciplinary Research Journal,2012:2(12):17-18.
31. Tchoundjeu Z, Leakey RRB. Vegetative propagation of African mahogany: effects of auxin, node position, leaf area and cutting length. *New Forests*,1995:11:125-136.
32. Tiwari S, Shah P, Singh K. Efficient *in vitro* clonal propagation of *Rauvolfia serpentina* L.: an important medicinal plant. Plant Cell Biotechology Moleular Biology,2003:4:157-162.
33. Vamil R, Haq A, Agnihotri RK, Sharma R. Effect of certain plant growth regulators on the seedling survival, biomass production and proline content of *Bambusa arundinacea.* Science Research Reporter,2011:1(2)**:**44-48.
34. Vamil R, Haq A, Agnihotri RK. Plant Growth Regulators as Effective Tool for Germination and Seedling Growth for *Bambusa arundinaceae.* Research Journal of Agricultural Sciences,2010:1(3):233-236.