Bangladesh J. Bot. **34**(1): 7-10, 2005 (June)

GROWTH AND PHYTOCHEMICAL INVESTIGATION OF *RAUVOLFIA SERPENTINA* BENTH. PROPAGULE

A.N.M. ALAMGIR AND MOMTAZ AHAMED

Department of Botany, Chittagong University, Chittagong-4331, Bangladesh

Key words: Rauvolfia serpentina, Propagule, NPK fertilizers, Biomass, Alkaloid

Abstract

For propagule development 15 cm cuttings from different regions of 1.5-2 years old plants of *Rauvolfia serpentina* Benth. were used. Root formation and successful propagule development from cuttings of root, stem and root-stem junction were 62, 42 and 78%, respectively. IBA, NAA and 2,4-D at low concentrations stimulated root formation and propagule development from stem cuttings. 2,4-D at 5 ppm had the highest positive impact on root formation and propagule development (100%), followed by IBA (83% at 50 ppm) and NAA (66% at 10 ppm). IBA and NAA in combination had little positive effect on rootings. *R. serpentina* revealed better growth activities under double or triple doses NPK, especially under increased N nutrition. Crude alkaloid contents of the roots increased significantly only under the increased N level. Increase of N, P and K all at a time, however, had little impact on alkaloid contents of roots.

Introduction

Rauvolfia serpentina Benth. is a good source of many (>50) important alkaloids of medicinal value. Its natural source is now almost exhausted due to indiscriminate recurrent harvest and other anthorpogenic pressure and activities. In Bangladesh it is now mainly confined to some virgin vallies of Chittagong and adjacent hilly districts. For the fulfilment of the present and future demand this plant needs to be cultivated scientifically at a commercial scale. But its cultivation through seeds is difficult due to low germination % for different structural and physiological reasons. Nair (1955) observed 15-20% seed germination, but only 10-13% plant development from the germinated seeds of *R. serpentina*. According to Hedayatullah (1959) seed germination of *R. serpentina* was quite erratic, ranged from 8-48% with an average of 19%. Thus, propagation through cuttings alone and/or in combination with hormone treatment may be helpful. Badhwar *et al.* (1956) tried to develop propagules of *R. serpentina* from stem and root cuttings with little success whereas Chandra (1956) was partially successful in developing propagules with stem cuttings of 12-15 em length and 0.4-1.0 cm diameter of *R. conescens* L. by using growth regulators.

In the present work attempts were made to develop propagules from root, stem and root-stem junction cuttings alone and with hormone treatment and also to investigate the effect of NPK on growth activities and alkaloid contents in *R. serpentina* Benth.

Materials and Methods

R. serpentina Benth. was used as test plant in the present work. Samples of *R. serpentina* were collected from different hill slopes and adjacent plain land of Sitakunda and Barabkunda of Chittagong district. Age of the collected samples was 1.5-2 years. For propagules development 15 cm long and 0.8-1.0 cm diameter cuttings of root, stems and root-stem junctions were taken. They were planted in the soil in perforated polythene bag (10 cm diam. 15 cm ht.) by inserting the basal end of the cutting at 45º angle upto 5 cm beneath the soil. Initially, the soil was mixed with decomposed cow dung (3 soil : 1 cow dung). NPK fertilizers were given at the rate as per

Arnon and Hoagland nutrient solution (1940) in the form of urea, TSP and MP 2 days before bag filling and plantation. Two cuttings were placed in a bag. After plantation polythene bags with cuttings were kept under shade and irrigated by water.

In another set of experiment for hormonal treatment: only stem cuttings were taken for exploring their potential for propagule development because stem cuttings without hormone treatment revealed poor performance in propagule development activity compared to others and besides, stem cuttings was considered less destructive to the parent bush than either of root or root-stem junction. Growth regulators like IBA, NAA and 2,4-D in different concentrations and combinations were used. Basal side (5 cm) of the cuttings were dipped in hormone solution of different strengths for 48 hours at 20°C. After 48 hours the cuttings were planted in soil in polythene bags (two in each bag) as described above.

For plant growth activities under different NPK fertilizers propagules from stem cuttings of first set of experiment were used. They were grown in earthen pots (10 L volume) containing soil mixed with cowdung (3:1) without or with NPK fertilizers which were selected as before and then different NPK doses were made and used. Irrigation and weedings were done as per requirement. Plants were grown upto 1.5 years in the open space of the Botanical Garden: Chittagong University, Bangladesh and at this age they were harvested for biomass estimation and alkaloid analysis. For dry wt. estimation plant samples were separated into leaf, stem and root, put in brown paper packets, dried in the oven at 65º C for several days until a constant wt. was attained. Alkaloid contents in the dried powdered plant materials were estimated following Bisset and Phillipson (1971). There were at least 3 replications for each set of experiment. Standard error of the mean was calculated and the mean values were assessed for their significance.

Results and Discussion

Importance of *R. serpentina* as medicinal plant, especially as a source for ajmaline, ajmalicine, serpentine, rauvolfine and reserpine is known to plant scientists for a long time. As propagation through seeds is rather difficult, cuttings have been practised for the purpose, but, cuttings from stem and other parts got little success with Badhwar *et al.* (1956). Results of the present work given in Table 1 show that cuttings from root-stem junction revealed the highest root formation and propagule development activity (78%) and it was followed by root cuttings (62%) and stem cuttings (42%).

Table 1. Root formation and propagule development potential of different cuttings of *Rauvolfia serpentina* Benth.

Type of cuttings	No. of cuttings	Number in which root formed and propagule developed	% of rooting and propagule development
Root	70	44.05 ± 1.84	62..92
Stem	70	30.01 ± 2.25	42.87
Root-stem junction	70	55.11 ± 2.11	78.73

In all the cases number of cuttings treated per replication was 70. So, cuttings from root-stem junction may be considered very effective for the development of propagules. Nair (1955), however, got 90-95% success in propagule development from stem cuttings with 0.25-0.5" of the root, planted between 15 June and 15 July, and only 30-50% success even after treatment with plant hormone Seradix B with stem cuttings. Hedayatullah (1959) noted 12% success with stem cuttings and 36% success with root cuttings of *R. serpentina*.

To increase the potential further only stem cuttings were tried with growth regulators. Effect of IBA, NAA, their combinations and 2,4-D in different concentrations on root formation in stem cuttings leading to propagule development of *R. serpentina* was investigated (Table 2.). It is evident that IBA at low concentration stimulated root formation, and 50 ppm IBA gave maximum root formation in cuttings (83%). With the further increase of concentration of IBA (100, 200 ppm) root formation decreased and at 500 ppm an inhibitory effect on propagule development, compared to control was noted. NAA only at low concentration (10 ppm) stimulated root formation and propagule development (66%). At higher concentrations (50,100,500 ppm) NAA inhibited the rooting activity of the cuttings. IBA and NAA were used in combination and at low concentration no synergistic effect was observed. IBA and NAA in combination at low concentration range (5 + 5, 25 + 25, 50 + 50 ppm) revealed only 50% root formation and propagule development activity, but at higher concentrations (250 +250 ppm) IBA and NAA had inhibitory effect. The highest activity (100%) was observed at 5 ppm 2,4-D, and at 50 ppm it decreased root formation down to 33% and at 100 ppm it totally inhibited rooting activity of the cuttings and propagule development. Chandra (1956) used different concentrations of IAA and NAA solutions on stem cuttings of *R. canescens* and noted stimulatory effect of these growth regulators on propagule development from those stem cuttings.

Table 2. Effect of growth regulators on root formation in stem cuttings of *Rauvolfia serpentina* Benth. and their propagule development.

Growth regulators	Concentration of growth regulators (ppm)	Mean value of cuttings where root formed and propagule developed[1]	% of rooting and propagule development
Control (H_2O)	-	30.01 ± 1.2	42.85
IBA	10	30.01 ± 1.0	50.00
	50	50.17 ± 1.5	83.53
	100	40.11 ± 1.4	66.83
	200	30.33 ± 1.1	50.56
	500	10.05 ± 0.5	16.75
NAA	10	40.02 ± 0.8	66.70
	50	20.23 ± 1.4	33.72
	100	10.14 ± 0.9	16.90
	500	10.13 ± 0.5	16.88
IBA + NAA	5 + 5	30.22 ± 0.6	50.35
	25 + 25	30.19 ± 1.1	50.23
	50 + 50	30.20 ± 0.8	50.33
	250 + 250	20.15 ± 1.2	33.58
2,4-D	1	30.30 ± 1.5	50.50
	5	60.00 ± 0.0	100.00
	50	20.15 ± 1.2	33.58
	100	0.00	0.00

[1]Mean of three replications.

Medicinal plants usually grow in wild in natural habitat, but scientific method would be helpful to increase their biomass yield as well as their alkaloid contents. In the present work effect of different doses of NPK fertilizers on growth activities and biomass production of *R. serpentina* was investigated (Table 3). NPK fertilizers increased plant height, leaf number, shoot and root dry weights and consequently total dry biomass of plant. The highest growth activity was observed where fertilizers level, especially N level was high. The highest biomass increase, 270%, was due to 1 PK + 3 N. The sequence of biomass increase was: 1 PK + 3 N > 1 PK + 2 N > 2 NPK > 1 NPK. This clearly indicates that growth attributes of *R. serpentina* was more sensitive to N fertilizer under constant (optimum) PK than increase of all three nutrients (NPK).

Table 3. Effect of NPK fertilizers on growth activities, biomass and alkaloid production of *Rauvolfia serpentina* Benth.

Fertilizer level NPK	Plant height (cm/plant)	Leaf No./plant	Dry biomass (g/plant)		Biomass increase (%)	Alkaloid content (mg/plant root)	Alkaloid content (%)
			Shoot	Root			
Control 0	43.25 ± 1.02	10.12 ± 1.1	6.22 ± 0.67	8.30 ± 0.44	-	30.74 ± 2.66	-
1	46.50 ± 1.33	14.33 ± 1.6	9.21 ± 1.06	16.44 ± 1.39	176.65	78.44 ± 2.18	255.17
2	49.25 ± 1.50	20.38 ± 1.6	12.46 ± 1.55	14.29 ± 1.03	184.22	95.99 ± 3.11	312.26
1 PK + 2 N	52.0 ± 1.16	20.44 ± 1.2	13.00 ± 0.28	18.95 ± 1.44	220.66	143.46 ± 5.69	466.68
1 PK + 3 N	51.00 ± 1.35	23.42 ± 1.9	14.4 ± 1.77	24.93 ± 2.00	270.17	466.12 ± 9.01	1516.33

In the present work the dry mass increase at the elevated NPK and N level was accompanied by the total crude alkaloid contents in *R. serpentina* (Table 3). Alkaloid contents were increased more when N level was increased under constant PK level and not with the simultaneous increase of all of NPK. The highest quantity, 1516.33 mg/plant noted under 1 PK + 3 N fertilizer dose. The sequence of different fertilizer combinations for the increase of alkaloids was: 1 PK + 3N > 1 PK + 2 N > 2 NPK > 1 NPK.

References

Arnon, D.I. and D.R. Hoagland, 1940. Crop production in artificial culture solution and in soil with special reference to factors influencing yield and absorption of inorganic nutrients. Soil Sci. **50**: 463-483.

Badhwar, R.L., G.V. Kasira and S. Ramaswami. 1956. *Rauwolfia serpentina*: methods of propagation and their effect on root production. Indian J. Pham. **18**: 170-175.

Bisset, N.G. and J.D. Phillipson. 1977. The African species of strychnos. Part II. The alkaloids. Lioydia. **37**: 10-59.

Chandra, V. 1956. Inducing rooting in stem cuttings of *Rauwolfia canescens* L. Sci. and Cult. **22**: 101.

Hedayatullah, S. 1959. Culture and propagation of *Rauwolfia serpentina* Benth. in East Pakistan. Pakistan J. Sci. Indust. Res. **2**: 118-122.

Nair, K.N.R. 1955. *Rauwolfia serpentina* Benth. - its importance and cultivation. Ind. Forester **81**: 168-171.

(Manuscript received on 21 June, 2004; revised on 19 February, 2005)