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Seed Germination Studies in *Rauvolfia serpentina* (L.) Benth. ex Kurz.

P. R. Salvi[1*], Rohan R. Patil[1] and D. N. Mokat[2]

[1]Dr. B.S. Konkan Krishi Vidyapeeth, Dapoli 415712, Dist-Ratnagiri (Maharashtra), India.
[2]Department of Botany, Savitribai Phule Pune University, Ganesh Khind, Pune-411007 (Maharashtra), India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. All authors managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The roots of *Rauvolfia serpentina* are under use in traditional and modern systems of medicine. The wild population of this valued medicinal plant is in dwindling stage in the natural habitats due to over exploitation and different anthropogenic activities. Cross pollination, poor seed germination, niche habitat, etc. are also responsible for shrinking the population of plants. Due to its potential to cure different diseases the demand for its roots has increased in several folds in national and international market, it is therefore essential to cultivate this drug yielding plant on large scale in the agricultural fields. For the mass multiplication and production of quality planting material (QPMs) of this valuable drug there is prerequisite to develop techniques through sexual and asexual methods. In the present investigation effect of pre sowing treatments of GA_3 on seed germination was studied and presented in the paper. The seed of pre-treated with scarification (T1) performed superior in respect of percent germination, growth rate index, mean daily germination, peak value and germination value as compared to other treatments.

**Corresponding author: E-mail: parimalsai@gmail.com;*

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1. INTRODUCTION

Indian snake root, *Rauvolfia serpentine* (L.) Benth. Ex Kurz. (Family: *Apocynaceae*) is most popular drug used for the treatment of mental illness, hypertension, blood pressure, insomnia, snake and reptile bites, rheumatism, etc. [1]. The demand for the root drug has been increased in traditional and modern system of medicine in recent days [2,3].

However, natural population of this species is under dwindling stage. The plant is categorised as vulnerable [4] and ethno-botanically important in the study area [5,6]. Unfortunately scanty information is exists in the literature on propagation through seed in *R. serpentina* for the production of QPMs for large scale production. Nursery and agro technique of this valuable plant are barely available. Hence, present research work was taken to find suitable treatment for production of seedlings through seeds.

2. MATERIALS AND METHODS

Field experiments were conducted in shed net house (50%) during the year 2012-13 at the experimental farm of College of Forestry, Dapoli, District-Ratnagiri 415 712 (Maharashtra). The healthy seeds of *R. serpentina* were collected from the 3 years old plants growing in research farm of College of Forestry, Dapoli. The individual seed was rubbed against sand paper and soaked in hot water at 80ºC for 5 minutes and then allowed to cool. The adequate number of seeds were pre-treated with five different treatments which includes scarification (control), soaking seeds in GA_3 solution @100 ppm, 200 ppm, 300 ppm, and 400 ppm each for the duration of 30 minutes. The treated seeds were allowed to dry before sowing them in seed beds. The statistically designed an experiment was laid out with five treatments replicated four time consisting 25 seeds in each treatment. One seed was placed in root trainer cup at its centre followed by other nursery management.

The total numbers of seeds germinated were counted every day up to 80 days from the date of sowing. The emergence of plumule above the soil was considered as indicator of seed germination. Data collected was computed into percent seed germination using the formula-

% seed germination = Number of Seeds germinated x 100/ Total number of seeds sown

Various germination attributes like germination rate index (GRI), mean daily germination (MDG), peak value of germination (PV), and germination vigour (GV) were calculated by using standard formula [7].

3. RESULTS AND DISCUSSION

Data recorded on effect of pre sowing treatments on seed germination is presented in Table 1. Treatment T4 scarification + soaking seeds in 300 ppm GA_3 for 30 minute required minimum number of days (16.00) followed by treatment T3 (Scarification + soaking seeds in 200 ppm GA_3 for 30 min. and (16.75) for first germination as compared to other treatments. Treatment T5 comprising of scarification + soaking of seeds in 400 ppm GA_3 for 30 min. required 17.00 days for the first germination. However, the treatment T1 consisting scarification (Control) as well as T2 (scarification + soaking seed in 100 ppm GA_3 for 30 min.) required more number of days (18.58) for the first germination.

Data in respect of days required for highest germination of seeds indicated that the treatment with scarification + soaking of seeds in 400 ppm GA_3 for 30 min. (T5) took minimum number of days (72.50). It was closely followed by T1 and T4 with 75.00 and 76.75 days respectively. The maximum per cent seed germination 68.25 was recorded in treatment T1 embracing scarification (Control). However, the lowest per cent seed germination (38.25) was recorded in treatment (T5) consisting of scarification + soaking of seed in 400 ppm $_{GA3}$ for 30 min. The second highest per cent seed germination of 52.00 was registered in the treatment T3 with scarification + soaking of seeds in 200 ppm GA_3 for 30 min. Perusal of data revealed that the treatment T1 with scarification was found better in getting maximum per cent seed germination. GRI was observed minimum (1.77) in treatment T3 (scarification + soaking seed in 400 ppm GA_3 for 30 min. and maximum growth index of 3.18 was recorded in untreated control which was statistically significant than all other treatments except T3 which was at par with each other. Effect of pre-sowing treatment on mean daily germination (MDG) showed significant variations in all treatments. The maximum MDG of 2.24 was recorded in untreated control (T1) while minimum of it (1.23) was found in treatment T5 consisting of scarification + soaking seeds in 400 ppm GA_3 for 30 min. The peak value and the germination values were varied between 1.46 to

2.24 and 1.94 to 5.08 respectively. However, these values were not affected due to any of the test treatments. The overall results showed that the seed of pre-treated with T1 scarification (control) performed better in respect of percent germination, growth rate index, mean daily germination, peak value and germination value as compared to all other treatments *viz.* Scarification + Soaking seeds in GA_3 @ 100 ppm for 30 min.; Scarification + Soaking seeds in GA_3 @ 200 ppm for 30 min.; Scarification + Soaking seeds in GA_3 @ 300 ppm for 30 min.; Scarification + Soaking seeds in GA_3 @ 400 ppm for 30 min (Table 1).

Present findings corroborates with the results of research work conducted by [8] who reported that the seed germination was as low as 20.47 per cent in case of intact seeds and was improved up to 40.2 percent after removal of seed coat. They achieved 52 percent seed germination with treatment of 500 ppm GA_3 in case of intact seeds. Similar findings were also reported by Singh and Motilal [9]; Paul et al. [10] recorded 32 percent germination in treatment with scarification (sand paper). In the present investigation maximum percent germination (68.25) was observed in the treatment T1 (scarification-control) which is not match with the report of Paul et al. [10]. The germination percentage was ranged from 38.25 to 68.25 per cent with mean value of 50.15 in respect of all pre sowing treatment. The responses of growth regulators like Cytokinin, IAA, Gibberlic acid, etc. has been demonstrated by research workers for to enhancing the germination of seed [11].

Data recorded on effect of GA_3 on growth attributes and biomass of *R. serpentina* propagated through seed at 60 days after sowing indicated that the treatment T5 consisting of scarification + soaking seed in 400 ppm of GA_3 for 30 minutes recorded significantly maximum shoot length of 6.98 cm except treatment T4 with scarification + soaking seed in 300 ppm GA_3 for 30 minutes. All other growth attributes like root, length, number of roots, number of leaves, diameter and biomass parameters like fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, total fresh weight and total dry weight were not influenced by various treatments among other growth parameters *viz.* root length, number of roots, number of leaves and diameter were ranged between 4.55 to 5.44 cm, 17.88 to 22.23, 5.15 to 5.65 and 1.44 to 1.56 mm, respectively. Among all biomass parameters, fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, total fresh weight and total dry weight were found to range between 0.32 to 0.39 g, 0.80 to 0.10 g, 0.05 to 0.07g, 0.02 to 0.02 g, 0.40 to 0.49 g and 0,07 to 0.08 g, respectively (Table 2).

In the second experiment conducted to record effect of GA_3 on growth attributes and biomass parameters propagated through seed at 90 days after sowing indicated more or less similar trend in respect of growth and biomass parameters recorded earlier except shoot height. All growth parameters *viz.* shoot height; root length, number of roots, number of leaves and the diameter of seedling were ranged from 5.76 to 6.23 cm, 6.14 to 7.84 cm, 20.85 to 25.65, 6.28 to 7.20 and 1.64 to 1.73 mm, respectively (Table 3). As regards the biomass parameters *viz.* shoot height, root length, number of roots, number of leaves and diameter of seedlings were ranged from 5.76 to 6.23 cm, 6.14 to 7.84 cm, 20.85 to 25.65, 6.28 to 7.20 and 1.64 to 1.73 mm, respectively (Table 3). As regards the biomass parameters *viz.* fresh shoot weight, fresh root weight, dry shoot weight, dry root weight, total fresh weight and total dry weight were ranged from 0.47 to 0.55 g, o.17 to 0.26 g, 0.08 to 0.09 g, 0.03 to 0.04 g, 0.64 to 0.76 g and 0.10 to 0.13 g respectively.

R. serpentina is a promising herbal option in the pharmaceutical world due to the presence of significant chemical compounds in roots [12]. For the production of seedling the pre sowing treatment T1 (scarification) is showed better results followed by treatment T3 (scarification + soaking seeds in GA_3 @ 200 ppm for 30 min. The growth rate index was observed maximum in treatment T1 (3.18) and minimum in treatment T5 (1.77). Overall results showed that seeds treated in T1 performed better with respect to per cent germination GRI, MDG, PV and GV as compared to all other pre sowing treatments used.

Plant growth and biomass of *R. serpentina* with respect to influence of different GA_3 concentrations after 60 Days of sowing on the seedlings growth at like shoot height, root length, number of roots, number of leaves, diameter as well as seedling biomass like fresh shoot, root weight and dry shoot, root weight at after 60 Days of sowing showed variation among five treatments. Treatment T5 showed highest shoot (6.98) and root height (5.44). Overall result showed that the pre-sowing seed treatment T5 (scarification+ soaking seeds in 400 ppm GA_3 for 30 min.) performed better results with respect to other treatments regarding growth and biomass parameters after 60 Days of sowing .

Table 1. Effect of pre-sowing treatments (GA_3 soaking) on seed germination of *R. serpentine*

Pre-sowing treatments		Day of first germination	Day of highest germination	Per cent germination	GRI	MDG	PV	GV
T_1	Scarification (Control)	18.50	75.00	68.25	3.18	2.24	2.24	5.08
T_2	Scarification + Soaking seeds in GA_3 @ 100 ppm for 30 min.	18.50	74.50	48.50	2.28	1.57	2.24	3.67
T_3	Scarification + Soaking seeds in GA_3 @ 200 ppm for 30 min.	16.75	74.50	52.00	2.56	1.76	2.14	3.75
T_4	Scarification + Soaking seeds in GA_3 @ 300 ppm for 30 min.	16.00	76.75	43.75	2.17	1.47	1.70	2.68
T_5	Scarification + Soaking seeds in GA_3 @ 400 ppm for 30 min.	17.00	72.50	38.25	1.77	1.23	1.46	1.94
Mean		17.35	74.65	50.15	2.39	1.65	1.95	3.42
SEm (±)		-	-	-	0.28	0.19	0.28	0.73
CD (P = 0.05)		-	-	-	0.87	0.58	NS	NS

CD=Critical Difference; GRI= Germination rate index; MDG = Mean daily germination; PV = Peak value; GV= Germination value

Table 2. Effect of GA_3 on growth parameters and biomass of *R. serpentina* propagated through seed after 60 days of sowing

Pre-Sowing treatment		Growth parameters					Biomass parameters				
		Shoot height (cm)	Root length (cm)	Number of roots	Number of leaves	Diameter (mm)	Fresh shoot Wt. (g)	Fresh root Wt. (g)	Dry shoot Wt. (g)	Dry root Wt. (g)	Total fresh Wt. (g)	Total dry Wt. (g)
T_1	Scarification (Control)	5.37	4.88	17.88	5.15	1.56	0.33	0.08	0.05	0.02	0.41	0.07
T_2	Scarification + Soaking seeds in GA_3 @ 100 ppm for 30 min.	5.93	5.02	22.18	5.48	1.56	0.37	0.09	0.06	0.02	0.45	0.08
T_3	Scarification + Soaking seeds in GA_3 @ 200 ppm for 30 min.	5.54	4.55	22.23	5.48	1.47	0.35	0.08	0.06	0.02	0.43	0.08
T_4	Scarification + Soaking seeds in GA_3 @ 300 ppm for 30 min.	6.33	4.94	20.55	5.65	1.44	0.32	0.08	0.06	0.02	0.40	0.07
T_5	Scarification + Soaking seeds in GA_3 @ 400 ppm for 30 min.	6.98	5.44	21.13	5.65	1.50	0.39	0.10	0.07	0.02	0.49	0.08
Mean		6.03	4.96	20.79	5.48	1.51	0.35	0.09	0.11	0.02	0.44	0.08
SEm(±)		0.33	0.37	1.22	0.29	0.06	0.03	0.01	0.01	0.11	0.04	0.01
CD (P = 0.05)		1.01	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Table 3. Effect of GA_3 on growth parameters and biomass of *R. serpentina* propagated through seed after 90 days of sowing

Pre-sowing treatment		Growth parameters					Biomass parameters						Vigour indices				
		Shoot height (cm)	Root length (cm)	Number of roots	Number of leaves	Diameter (mm)	Fresh shoot Wt. (g)	Fresh root Wt. (g)	Dry shoot Wt. (g)	Dry root Wt. (g)	Total fresh Wt. (g)	Total Dry Wt. (g)	SQ	QI	S:R	RGR	NAR
T_1	Scarification (Control)	6.23	6.84	20.85	6.38	1.68	0.53	0.25	0.08	0.04	0.76	0.12	3.76	2.90	1.02	0.01	0.00
T_2	Scarification + Soaking seeds in GA_3 @ 100 ppm for 30 min.	6.19	6.14	24.35	6.28	1.64	0.52	0.26	0.08	0.03	0.76	0.12	3.79	3.06	1.06	0.01	0.00
T_3	Scarification + Soaking seeds in GA_3 @ 200 ppm for 30 min.	6.10	7.84	22.10	7.00	1.73	0.55	0.22	0.09	0.04	0.76	0.13	3.56	3.03	0.93	0.01	0.01
T_4	Scarification + Soaking seeds in GA_3 @ 300 ppm for 30 min.	6.08	6.69	25.65	7.20	1.69	0.54	0.20	0.08	0.03	0.74	0.12	3.64	3.37	1.12	0.01	0.01
T_5	Scarification + Soaking seeds in GA_3 @ 400 ppm for 30 min.	5.76	6.46	24.08	6.33	1.64	0.47	0.17	0.08	0.03	0.64	0.10	3.56	3.09	0.98	0.00	0.00
Mean		6.07	6.79	23.41	6.64	1.67	0.52	0.22	0.08	0.03	0.73	0.12	3.66	3.09	1.02	0.01	0.00
SEm(±)		0.29	0.48	1.54	0.27	0.04	0.05	0.05	0.01	0.01	0.07	0.01	0.16	0.45	0.07	0.00	1.18
CD (P = 0.05)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

SQ= Sturdiness Quotient, QI= Quality index, S:R = Shoot to root ratio, RGR = Relative growth rate, NAR = Net assimilation rate.

Effect of GA_3 on growth parameters and biomass of *R. serpentina* propagated through seed after 90 Days of sowing in growth parameters like root length, number of roots, number of leaves, diameter showed non-significant variation. Similarly in case of seedling biomass i.e. fresh shoot weight, fresh root weight and dry shoot, dry root weight, total fresh and total dry weight showed non-significant variation. Considering in case of vigour indices like Sturdiness quotient, Quality index, Shoot to Root ratio, Relative Growth Rate, and Net Assimilation Rate showed non-significant variation.

4. CONCLUSION

To fulfil the gap in between demand and supply of the *R. serpentina* roots there is need to develop techniques for mass multiplication and production in agricultural fields. The field experiments were conducted to study seed germination percent, seedling growth, biomass attributes and survival per cent. Germination per cent 68.25 was recorded maximum in T1 (scarification) compared to other treatments and germination attributes *viz.* Germination Rate Index (3.18), Mean Daily Germination (2.24), Peak Value of Germination (2.24) and Germination Value (5.08) was observed maximum in T1 (scarification) followed by in T3 (scarification + soaking seeds in 200 ppm GA_3 for 30 min.) in which Germination Rate Index (2.56), Mean Daily Germination (1.76), Peak Value of Germination (2.14) and Germination Value (3.75). The shoot height 6.23 cm was recorded maximum in T1 (scarification) followed by 5.76 cm in T5 (scarification+ soaking seeds in 400 ppm GA_3 for 30 min.) and root length 7.84 cm recorded maximum in T3 (scarification+ soaking seeds in 200 ppm GA_3 for 30 min.) followed by 6.14 cm in T2 (scarification+ soaking seeds in 100 ppm GA_3 for 30 min.). The overall performance of treatment T1 (scarification) was found better in per cent germination and germination attributes as compared to other treatments. Therefore, treatment T1 (scarification) is recommended for mass multiplication of Indian snake root.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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