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Effect of different hormonal treatments on seed germination of *Rauvolfia serpentina* (L.) Benth. Ex Kurz

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Abstract
Rauwolfia serpentina (L.) Benth. ex Kurz is most important medicinal plant and its importance increasing day by day due to its higher medicinal value. However one of the major obstacles in popularizing its commercial cultivation is its poor seed germination. To improve the germination percentage of seeds, PGR's (Plant Growth Regulators) may impart significant role. In this regard, an experiment was conducted in Completely Randomized Design with thirteen treatments and three replications at nursery of research farm of BAU, Ranchi. Treatments were consisted of different concentration of GA_3, Kinetin and IAA (Indole Acetic Acid). For initiation of seed germination, maximum number of days (35.67) was observed in control and minimum in 200 ppm GA_3 (18.66 days). After the initiation of seed germination, nearly fourteen days was required to complete seed germination process. Maximum germination was observed till 10^{th} day from its initiation. Maximum rate of germination was observed in 200 ppm GA_3 (0.032) and minimum in control (0.020). Maximum germination energy was recorded in 200 ppm Kinetin (5.66) which was *at par* with 200 ppm GA_3 (5.33). Nearly 4.0% germination was noticed during peak time of germination. The root length was found almost same to shoot length, so the root-shoot ratio was recorded near to 1.00 with maximum seedling vigour index in 100 ppm IAA (2074.64). Seedling vigour index was highly positively correlated with rate of germination. Little value of environmental variance was noticed on all seed germination parameters. Higher value of phenotypic and genotypic coefficient of variation was observed for germination energy (25.47 and 24.39 respectively) and seedling vigour index (22.13 and 21.76 respectively) had highlighted the significance of these parameters among various germination parameters of Sarpgandha under nursery conditions. Maximum genetic advance was shown by germination energy (61.66%) and seedling vigour index (56.47%), so selection of these parameters could lead to the improvement in seed germination activities and crop improvement programme of Sarpgandha.

Keywords: Sarpgandha, *Rauvolfia serpentina*, germination percentage, seedling vigour index.

Introduction
Rauvolfia serpentina commonly known as Sarpgandha belongs to family Apocynaceae is the high priority medicinal plants of India. It is evergreen, erect glabrous perennial shrub that normally grows up to a height of 60 cm. Roots of this herb is tuberous with a cork of a pale brown color. It has been widely utilized by ayurvedic system of medicine for centuries and its usage in allopathic medicine is only for a few decades primarily in hypertension, insomnia, anxiety and epilepsy (Ghani, 1998). The root of Sarpgandha is store house of therapeutically active alkaloids like reserpine, serpentine, ajmaline, ajmalicine, yohimbine etc, which are used as a pharmaceutical drug to treat high blood pressure and mental disorders including schizophrenia (Isharwal and Gupta, 2006; Monachino, 1954; Kirtikar and Basu, 1993). Very useful treatment in high blood sugar, having a special property for lowering blood pressure has been reported by Vakil (1955). It is also a suitable remedy for insomnia, hysteria and hypertensive patients (Von Poser *et al.*, 1990). *Rauvolfia serpentina* has found to be useful in treatment of anxiety, psychosis and epilepsy (Noce *et al.*, 1954). It can regularize menstruation when used as a mixture with ginger and black pepper (Chunekar, 1998).
Due to high commercial exploitation for a long time, the natural reserve of this plant has been dwindling alarmingly and as a consequence, it is included in the endangered category as well as in CITES list. Coupled with this, poor seed germination rate of the plant limits its natural propagation as well as cultivation and thereby magnifies the problem further. In *Rauvolfia serpentina*, the seed germination is very poor and varies from 25-74 % in case of fully matured heavy seeds (Badhwar *et al.*, 1955; Dutta *et al.*, 1963). The rate of germination depends on the percentage of fully matured, heavy seeds in a particular lot. Fresh seed, collected from ripe fruits and immediately sown, show higher percentage of germination. Germination starts after

15-20 days and continuous up to 40-50 days after sowing. Propagation by means of seeds might prove ultimately even unwise, since variation in alkaloidal yield is apparently genetically controlled and might get reduced in successive progenies through adverse gene recombinations. Though Sarpgandha has a wide geographical distribution and edaphic tolerance, large-scale commercial cultivation is not feasible due to poor seed viability, low seed germination rate and low vegetative propagation rate through root cuttings (Chadha, 2007) so application of plant growth regulators to improve germination percentage should be explored to popularize its commercial cultivation.

Materials & Methods

The present investigation was conducted during March – June 2016 and experiment was laid out in Completely Randomized Design under nursery conditions with thirteen treatments and three replications. The seeds were collected from its natural habitat of Tropical dry deciduous forests of Jharkhand. The details of different hormonal treatments were T_1-Control, T_2 - 50 ppm GA_3, T_3 -100 ppm GA_3, T_4- 150 ppm GA_3, T_5 – 200 ppm GA_3, T_6- 50 ppm Kinetin, T_7-100 ppm Kinetin, T_8-150 ppm Kinetin, T_9-200 ppm Kinetin, T_{10}-50 ppm IAA, T_{11}-100 ppm IAA, T_{12}-150 ppm IAA and T_{13}-200 ppm IAA.

The germination study of Sarpgandha seeds was undertaken at nursery level in polythene tubes (13 cm × 7 cm) by establishing nursery. Number of polytubes kept in a treatment was 50 and in each polytubes two seeds of Sarpgandha were sown after giving respective treatments. Data on germination trend was recorded from initiation to completion of germination and based upon this different germination parameters was calculated. Parameters studied were days taken for initiation of seed germination, days taken for 50% of final seed germination, days taken for completion of seed germination, germination period (days), rate of germination, germination energy (%), germination percentage (%), shoot and root length (cm) of seedlings at time of transplantation, root-shoot ratio of seedlings and seedling vigour index. Data collected on various germination parameters were subjected to various statistical analysis like ANOVA, correlation test, multiple regression analysis, path value analysis and PCV & GCV analysis to draw meaningful conclusion.

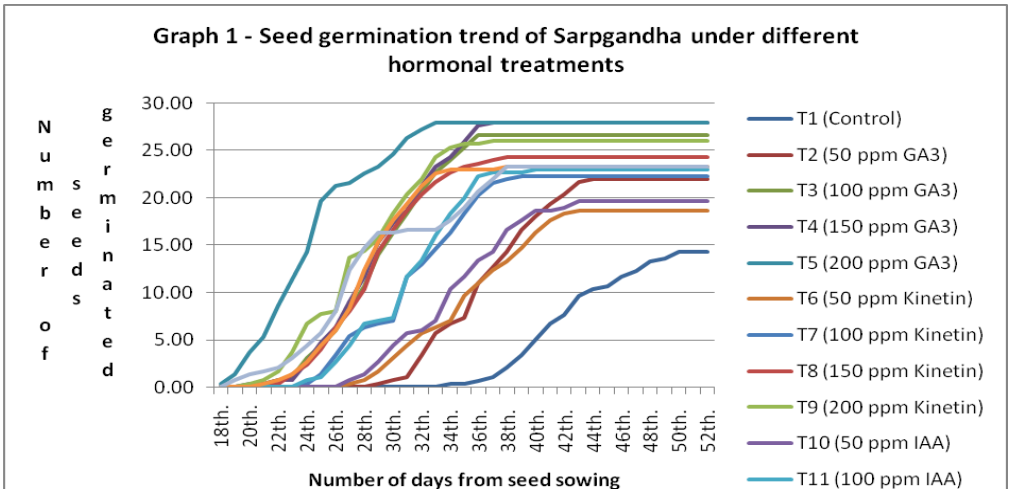
Results & Discussion

Results obtained on different germination parameters of Sarpgandha seeds are given below. For initiation of seed germination, mFVaximum number of days (35.67) was observed in control and minimum in 200 ppm GA_3 (18.66 days). Phatak *et al*., (2001) observed number of days for initiation of seed germination of Sarpgandha was 37.67 days in control. Seed treatment with GA3 treatment resulted in 38.94% reduction in number of days taken to first germination over control. In case of number of days taken for 50% of final seed germination, maximum number of days (41.67) was recorded in control, which was *at par* with 50 ppm IAA and minimum in 200 ppm GA_3 (23.33 days). Maximum number of days for completion of seed germination was recorded in control (48.67 days) and minimum in 200 ppm GA_3 (31.33 days). Different treatments gave highly significant difference for initiation and completion of Sarpgandha seed germination. From the mean values, it can be inferred that the initiation of seed germination was started during 25th days and after seven days from initiation of seed germination, 50% of final seed germination was observed.

Table 1: Effect of different hormonal treatments on number of days for initiation of germination, number of days for 50% of final germination, number of days for completion of germination, rate of germination and germination energy of seeds of Sarpgandha

Treatments	Number of days for initiation of germination	Number of days for 50% of final germination	Number of days for completion of germination	Rate of germination	Germination energy (%)
T_1 – Control	35.67	41.67	48.67	0.020	2.00
T_2 -50 ppm GA_3	30.00	37.00	43.00	0.023	3.67
T_3 -100 ppm GA_3	22.33	29.67	35.67	0.027	3.33
T_4- 150 ppm GA_3	22.00	28.67	35.00	0.028	3.66
T_5 – 200 ppm GA_3	18.66	23.33	31.33	0.032	5.33
T_6- 50 ppm Kinetin	28.00	34.00	41.33	0.024	2.66
T_7-100 ppm Kinetin	25.33	31.67	37.67	0.026	4.66
T_8-150 ppm Kinetin	23.00	31.33	36.00	0.027	4.11
T_9-200 ppm Kinetin	21.00	28.00	35.00	0.028	5.66
T_{10}-50 ppm IAA	27.66	40.00	41.33	0.024	3.33
T_{11}-100 ppm IAA	25.00	36.00	38.33	0.026	4.33
T_{12}-150 ppm IAA	22.66	28.67	35.33	0.029	4.22
T_{13}-200 ppm IAA	20.67	27.67	36.33	0.027	4.33
Grand mean	***24.76***	***32.12***	***38.07***	***0.026***	***3.94***
S.E.$_{(m)}$	***0.704***	***0.403***	***0.974***	***0.001***	***0.290***
C.D$_{5\%}$	***2.058***	***1.178***	***2.847***	***0.002***	***0.849***
C.V $_{(\%)}$	***4.923***	***2.224***	***4.431***	***5.551***	***12.743***

After the initiation of seed germination, nearly fourteen days was required to complete seed germination process. Maximum rate of germination was observed in 200 ppm GA_3 (0.032) and minimum in control (0.020). Maximum germination energy was recorded in 200 ppm Kinetin (5.66) which was *at par* with 200 ppm GA_3 (5.33). Nearly 4.0% germination was noticed during the peak time of germination.



Graph of germination trend of Sarpgandha showed that initiation of seed germination was started first on 18th day in 200 ppm GA3 and last in control (33th. day). Maximum germination was observed till 10th day from its initiation and later on it became more or less constant.

Maximum germination percentage was observed in 150 ppm GA_3 and 200 ppm GA_3 (56.00%), which was *at par* with 100 ppm GA_3 (53.33) and 200 ppm Kinetin (52.00%), while its minimum value was recorded in control (28.66%). Farooqui and Sreeramu (2001) observed variable germination percentage of Sarpgandha seeds ranging from 10-60%. Maximum shoot length was recorded in 100 ppm IAA (23.23 cm) which was *at par* with 150 ppm IAA (22.30 cm) and minimum in control (13.65 cm). Maximum root length was recorded in 100 ppm IAA (21.85 cm) and minimum in control (14.87 cm). Maximum root-shoot ratio was recorded in control (1.09) and minimum in 150 ppm IAA (0.80). Maximum seedling vigour index was recorded in 100 ppm IAA (2074.64), which was *at par* with 100 ppm GA_3 (1961.25), 150 ppm GA_3 (2053.05), 200 ppm GA_3 (2011.08). Minimum seedling vigour index was recorded in control (819.04). Maximum promotion in the length of radical was observed 34% at T_5 (IBA 25 ppm) treatment however, inhibition in the length of radical was observed 22% at T_4 (IAA 100 ppm) treatment as observed by (Sharma and Tyagi, 2003). Patel and Saxena (1994) observed that at seedling stage in *Vigna mungo and Vigna radiate*, the IBA at all concentrations was less effective as compared to control. The earliness of GA_3 treated seeds might be due to early induction of protein, α-amylase in aleurone layer by forming m-RNA for protein synthesis. The induction of mRNA might be in response to the gibberelin induced transcription (Bewley and Black, 1994)

Table 2: Effect of different hormonal treatments on germination percentage of seeds, shoot & root length, root-shoot ratio and seedling vigour index of Sarpgandha seedlings at the time of transplantation

Treatments	Germination percentage	Shoot length (cm) at the time of transplantation	Root length (cm) at the time of transplantation	Root-shoot ratio at the time of transplantation	Seedling Vigour Index
T_1 - Control	28.66	13.65	14.87	1.09	819.04
T_2 -50 ppm GA_3	44.00	17.25	18.23	1.06	1559.44
T_3 -100 ppm GA_3	53.33	18.34	18.48	1.01	1961.25
T_4- 150 ppm GA_3	56.00	18.29	18.35	1.01	2053.05
T_5 – 200 ppm GA_3	56.00	18.27	17.67	0.97	2011.08
T_6- 50 ppm Kinetin	37.33	17.66	17.67	1.00	1318.74
T_7-100 ppm Kinetin	44.66	18.09	18.30	1.02	1625.97
T_8-150 ppm Kinetin	48.66	18.24	18.50	1.02	1789.47
T_9-200 ppm Kinetin	52.00	19.61	19.77	1.01	2047.04
T_{10}-50 ppm IAA	39.33	15.87	16.85	0.97	1285.60
T_{11}-100 ppm IAA	46.00	23.23	21.85	0.94	2074.64
T_{12}-150 ppm IAA	46.66	22.30	17.88	0.80	1875.65
T_{13}-200 ppm IAA	46.66	20.31	18.25	0.90	1800.01
Grand mean	***46.10***	**18.55**	**18.21**	**0.98**	**1709.31**
S.E.(m)	***1.146***	***0.620***	***0.469***	***0.045***	***66.56***
C.D5%	***4.227***	***1.812***	***1.370***	***0.130***	***194.56***
C.V (%)	***5.433***	***5.791***	***4.460***	***7.840***	***6.745***

Correlation analysis - Germination percentage of seeds was found highly negatively correlated with days taken for initiation of seed germination (-0.898), days for 50% of final seed germination (-0.838) and days for completion of seed germination (-0.920), however it was highly positively correlated with rate of germination (0.875) and germination energy (0.707).

Table 3: Correlation matrix between germination parameters of Sarpgandha seeds

	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
V_2	0.919**								
V_3	0.984**	0.923**							
V_4	-0.949**	-0.924**	-0.978**						
V_5	-0.777**	-0.709**	-0.781**	0.779**					
V_6	-0.898**	-0.838**	-0.920**	0.875**	0.707**				
V_7	-0.622*	-0.494NS	-0.605*	0.589*	0.581*	0.500NS			
V_8	-0.515NS	-0.314NS	-0.502NS	0.416NS	0.591*	0.543NS	0.809**		
V_9	0.514NS	0.416NS	0.484NS	-0.536NS	-0.344NS	-0.247NS	-0.749**	-0.268NS	
V_{10}	-0.884**	-0.775**	-0.892**	0.845**	0.757**	0.930**	0.768**	0.777**	-0.420NS

*- significant at 5% level, **- significant at 1% level, R-square value: 0.9972

Where V_1 - Days for initiation of seed germination, V_2 - Days for 50 % of final seed germination, V_3 - Days for completion of seed germination, V_4 - Rate of germination, V_5 - Germination energy, V_6 - Germination percentage, V_7 – Shoot length of seedlings at transplantation stage, V_8 – Root length of seedlings at transplantation stage, V_9 – Root-shoot ratio of seedlings at transplantation stage, V_{10} – Seedling vigour index of seedlings at transplantation stage.

Seedling vigour index was found highly negatively correlated with days taken for initiation of seed germination (-0.884), days for 50% of final seed germination (-0.775) and days for completion of seed germination (-0.892), however it was highly positively correlated with rate of germination (0.845), germination energy (0.757), germination percentage (0.930), shoot length (0.768) and root length (0.777), but shown non-significant relation with root-shoot ratio (-0.420).

Multiple regression analysis - Perusal of data indicated that rate of germination, days taken for 50% of final seed germination, completion of seed germination, germination energy, germination percentage, shoot length of seedlings and root-shoot ratio of seedlings had highly significant effect on seedlings vigour index of Sarpgandha.

Table 4: Regression coefficients and significance of germination parameters affecting Seedling vigour index of Sarpgandha seedlings

Variables	Coefficients	Standard Error	t-value
V_1	-26.120	16.252	-1.607
V_2	14.131**	9.355	1.511
V_3	23.309**	20.201	1.154
V_4	22,308.002**	19,986.226	1.116
V_5	1.124**	16.315	0.069
V_6	34.105**	3.576	9.537
V_7	102.120**	32.355	3.156
V_8	-27.144	40.763	-0.666
V_9	1,379.369**	720.093	1.916
Constant	-3,905.116		

R-square value: 0.9987, Multiple R-value: 0.9993

Path value analysis - Six parameters showed direct positive effect on seedling vigour index, in which maximum direct effect was observed by shoot length (2.825) followed by germination percentage (1.364) while minimum direct effect was showed by root-shoot ratio (0.273). Three parameters showed direct negative effect on seedling vigour index, in which maximum impact was shown by root length (-2.344) and minimum by rate of germination (-0.784). From the above findings, it can be inferred that for higher seedling vigour index, shoot length and germination percentage should be selected as important parameters as their higher values gave higher seedling vigour index.

Table 5: Path analysis of characters influencing seedling vigour index of Sarpgandha seedlings

V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
-1.649	0.927	1.116	0.762	-0.318	-1.276	-2.448	1.755	0.194
-1.540	**0.992**	1.061	0.748	-0.306	-1.220	-2.380	1.599	0.144
-1.642	0.939	**1.121**	0.769	-0.319	-1.279	-2.420	1.707	0.187
1.604	-0.947	-1.099	**-0.784**	0.322	1.238	2.213	-1.468	-0.187
1.290	-0.748	-0.878	-0.622	**0.406**	0.978	2.138	-1.683	-0.126
1.543	-0.887	-1.051	-0.711	0.291	**1.364**	2.368	-1.789	-0.141
1.430	-0.836	-0.960	-0.614	0.308	1.144	**2.825**	-2.251	-0.138
1.235	-0.677	-0.816	-0.491	0.292	1.041	2.712	**-2.344**	-0.103
-1.172	0.523	0.774	0.541	-0.187	-0.703	-1.426	0.884	**0.273**

PCV and GCV analysis – It showed that the initiation of seed germination was started during 25th days and after seven days from initiation of seed germination, 50% of final seed germination was observed. After the initiation of seed germination, nearly fourteen days was required to complete seed germination process. Nearly 4.0% germination was noticed during the peak time of germination. The root length was found almost same to shoot length, so the root-shoot ratio was recorded near to 1.00 with seedling vigour index 1709.30. The phenotypic variance of various seed germination characters showed its higher values than its corresponding genotypic variance. Little value of environmental variance was noticed on all seed germination parameters. Higher value of phenotypic and genotypic coefficient of variation was observed for germination energy (25.47 and 24.39 respectively) and seedling vigour index (22.13 and 21.76 respectively) had highlighted the significance of these parameters among various germination parameters under field condition. All the seed germination parameters showed more than 90.00% heritability except root-shoot ratio, but two parameters such as days for seed germination initiation (99.70%) and days for 50% of final seed germination (99.80%) showed higher heritability. Maximum genetic advance was shown by germination energy (61.66%) and seedling vigour index (56.47%), so selection of these parameters could lead to the improvement in seed germination activities and crop improvement programme of Sarpgandha. Varadarajan (1958) reported differences in root bark and wood ratio of different ecotypes in *Rauvolfia serpentina*; the root samples from the North-Indian hills had a lower ratio than those of Peninsular India. There was a greater physiological activity of the species in the southern region due to the tropical climate than on the Himalayan tract where the leaves are shed in winter. He observed that there was no difference in general mean of all the populations for plant height and leaf traits. There was not much variation in populations from the three states for these traits.

Table 6: Components of genetic variability of seed germination characters of Sarpgandha under field conditions

Parameters	Grand Mean	Range	Variance			PCV	GCV	ECV	Heritability (%)	GA% of means (at 5%)	GA% of means (at 1%)
			Phenotypic	Genotypic	Environment						
Days taken for initiation of seed germination	24.76	18.66-35.66	21.229	21.15	0.073	18.60	18.57	1.89	99.70	38.18	48.93
Days taken for 50% of final seed germination	31.38	23.33-41.66	21.905	21.85	0.046	14.91	14.90	1.18	99.80	30.65	39.28
Completion of seed germination	38.07	31.33-48.66	20.244	19.60	0.637	11.82	11.63	3.63	96.90	23.57	30.21
Rate of germination	0.026	0.02-0.031	0.00	0.00	0.00	11.28	10.97	4.57	94.50	21.97	28.15
Germination energy	3.946	2.00-5.66	1.01	0.926	0.084	25.47	24.39	12.72	91.70	48.11	61.66
Germination percentage	46.09	28.66-55.99	60.283	58.065	2.219	16.84	16.53	5.60	96.30	33.41	42.82
Root length at time of transplantation	18.55	13.65-23.33	6.194	5.780	0.414	13.42	12.96	6.01	93.30	25.79	33.05
Shoot length at time of transplantation	18.20	14.86-21.84	2.462	2.231	0.231	8.62	8.21	4.58	90.60	16.08	20.61
Root-shoot ratio	0.984	0.80-1.09	0.005	0.003	0.002	7.84	5.82	8.14	60.60	9.33	11.96
Seedling vigour index	1709.30	819.04-2074.63	143050.03	138297.62	4752.41	22.13	21.76	6.99	96.70	44.06	56.47

Conclusion

Overall it can be concluded for higher germination percentage of Sarpgandha seeds, 200 ppm GA_3 or 150 ppm GA_3 is the best. However for maximum seedling vigour index, 100 ppm IAA is the best treatment. From present findings it can be concluded that low concentration of PGRs showed promotory effect on seed germination percentage, radical and plumule while high concentration of PGRs had inhibitory effect on seedling growth parameters of *Rauvolfia serpentina*, so this technique will be helpful for farmers to increase the production of Sarpgandha.

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