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Seed Germination Improvement in Two Threatened Medicinal Plants

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ABSTRACT

Rauvolfia serpentina (L.) Benth. ex Kurz. and *R. tetraphylla* L. are threatened because of extensive utilization for their wide ranging medicinal applications. The seed mediated propagation is unsatisfactory due to dormancy and poor germination percentage. This however, decelerates the conservation strategy of these species. Thus, efforts were made in this study to break dormancy and improve germination of seeds of the two species for boosting conservation. The viability of *R. serpentina* and *R. tetraphylla* were found to be 67% and 82% respectively. Germination percentage of treated *R. serpentina* seeds showed improved germination percentage of 34.94% (H_2O_2) and 48.65% (GA_3) over control (11.27%). The germination percentages of treated *R. tetraphylla* seeds were improved to 52.70% (KNO_3) and 56.66% (GA_3) as compared to untreated seeds (31.26%). Temperature also influenced the germination percentage and the highest germination percentage was obtained at 35°C and 30°C respectively in *R. serpentina* and *R. tetraphylla*. Results of the treatments indicate the presence of coat induced and non-deep physiological dormancy in both these species. The datas were statistically analyzed by analysis of variance ($P<0.01$).

Key words: *R. serpentina*, *R. tetraphylla*, Germination, Dormancy.

INTRODUCTION

Rauvolfia, a genus of family apocynaceae, are shrubs or under shrubs or occasionally trees. *R. serpentina* is a well-known drug yielding plant, commonly used as an antidote for snake bites. It has number of other medicinal properties because it contains numbers of alkaloids viz. ajmalicine, chandrine, rauwolfinine, reserpine, serpagine, serpentine, serpentinine, tetraphyllicine, yohimbine, etc. *R. tetraphylla* is also of high medicinal value and is used in high blood pressure, stomach ailments, fever, insomnia, etc. Alkaloids such as ajmaline, reserpine, serpentine, tetraphyllin, tetraphyllicine, yohimbine, etc. are present. Both of these species of *Rauvolfia* cannot be propagated immediately after harvest due to the dormancy of seeds[1]. Dormancy is not just associated with the absence of capacity of germination; rather it is a characteristic of the seed that determines the conditions required for germination[2]. There are five classes of dormancy viz. physiological, morphological, morphophysiological, physical and combinational[3].

As both *R. serpentina* and *R. tetraphylla* have high medicinal value, they are very much important for pharmaceutical industries. Seed germination studies on medicinal plants have proved to be useful in developing appropriate conservation strategies[4]. The demand for medicinal plants is increasing in both developing and developed countries because of products being non-narcotic, having no side effects, easily available at affordable prices and sometimes the only source of health care available to the poor. Thus, the objective of this study was to develop some effective methods that

would help to break dormancy in both the species of *Rauvolfia* and provide suitable conditions required for their germination and easy propagation.

MATERIALS AND METHODS

The study was conducted in the department of Botany, Gauhati University, Guwahati.

Seed source

Mature fruits of *R. serpentina* and *R. tetraphylla* were collected from the plants grown in botanical garden of the Department of Botany, Gauhati University, Guwahati during the month of July, 2011. These seeds were allowed to dry in shade and kept in sealed cellophane paper bags at room temperature for further experiments. A total of 500 seeds were randomly collected from each species for the experiment.

Viability of seeds

Viability of the collected seeds was determined by using tetrazolium technique[5] and by dissection microscope[6].

Pretreatments and experimental conditions

The experiment was conducted in two sets. In the first set, seeds were treated with H_2O_2, KNO_3, KH_2PO_4 and GA_3. These four chemicals were taken at three different doses i.e., 3%, 5% and 7% solutions of H_2O_2, KNO_3 and KH_2PO_4; whereas GA_3 was prepared as solutions of 100 ppm, 200 ppm and 300 ppm. The second set of experiments consisted of treatment with conc.H_2SO_4 and moist chilling. Seeds were treated with conc. H_2SO_4 for 5 minutes; whereas moist chilling was done for 7 days, 14 days and 21 days. Surface sterilization of seeds was done prior to experiment by soaking them in 3% sodium hypochlorite solution for 5 minutes and subsequently rinsing thoroughly with sterilized distilled water. Three replicates of 30 seeds were used for each treatment. The treated and untreated seeds were placed in sterile petridishes lined with two sterile Whatman no.1 filter papers moistened with sterile distilled water to ensure adequate moisture for the seeds. Further, each of these treatments were carried out at four controlled and constant temperature regimes viz., 25°C, 30°C, 35°C and 40°C. All the experimental sets of seeds were incubated at normal day/night photoperiod. The observations were recorded daily up to 60 days. Seeds with 2 mm radical emergence were considered germinated. Total germination percentage (Gt) was calculated by using the following formula-

$$G = \frac{n}{N} \times 100$$

Where, n= total no. of germinated seeds at the end of the experiment and N= total no. of seeds used for germination test[7].

Statistical analysis

All the experiments were conducted in a randomized block design (RBD) with three replications for each treatment combinations. The data collected were then statistically analyzed by two-way analysis of variance technique ($P<0.01$). The data of moist chilling and conc. H2SO4 treatment were not sufficient for ANOVA.

Table 1: Analysis of variance for seed germination in *R. serpentina*

Sources of variation	Degrees of freedom	Sum of squares	Mean square	Calculated 'F' value	Table value of 'F' at 1%
Chemical (A)	3	8039.97	2679.99	467.71**	5.09
Temperature (B)	3	381.26	127.08	22.18**	5.09
Dose (C)	2	142.85	71.42	12.46**	6.01
Chemical X Dose	6	1141.77	190.29	33.21**	4.01
Chemical X Temperature	9	886.35	98.48	17.18**	3.37
Temperature X Dose	6	59.6	9.93	1.73$^{n.s}$	4.01

* Highly significant ($P<0.01$); n.s = not significant

RESULTS AND DISCUSSION

Seed viability

The viability of seeds of *R. serpentina* and *R. tetraphylla* was 67% and 82% respetively. However, strong seed dormancy was observed in both the experimental plants. The results of tetrazolium test were not in accordance with the seed germination percentage of the two species of *Rauvolfia*.

Effect of pretreatments on germination percentage of *R. serpentina* and *R. tetraphylla* seeds:

The analysis of variance showed that different chemicals significantly affected seed germination in *R. serpentina* and *R. tetraphylla*. Similarly different temperatures and doses of chemicals also affected seed germination significantly. On the other hand, the interaction effect of chemical and dose and chemical and temperature were significant both in *R. serpentina* and *R. tetraphylla*. The interaction effect of temperature and dose was, however insignificant in *R. serpentina* but significant in *R. tetraphylla* (Table: 1 and 2).

The highest germination percentage of *R. serpentina* seeds in control was 11.27 at an incubation temperature of 30°C (Fig: I). The germination percentage at 30°C increased to 34.94 and at 35°C increased to 33.33 when the seeds were treated with 3% H_2O_2 (Fig: I). Further increase in dose of H_2O_2, decreased the germination percentage (Fig: I). On the other hand, *R. tetraphylla* seeds showed low rate of germination than the germination of seeds set for control (Fig: II). The excessive levels of oxidants may result in severe cellular damage[8]. Though, antioxidant enzymes (AOE) eliminate the effect of active oxygen species (AOS) [9]. But in this case, the activities of AOE may not be sufficient in scavenging of excess amount of AOS.

Seeds treated with KNO_3 in *R. serpentina* showed maximum germination percentage of 4.24 at a dose of 7% KNO_3 at 35°C which was low as compared to control sets (Fig: I). Whereas, *R. tetraphylla* seeds showed maximum germination percentage of 52.70 at a dose of 3% KNO_3 at 30°C which was higher than the control sets (Fig: II). But as the dose of KNO_3 increased to 5% and 7% the germination percentage decreased (Fig: II).

The low germination percentage observed in *R. serpentina* may be due to high percentage of KNO_3 treatment. Paul *et al.*[10] treated *R. serpentina* seeds with 1% KNO_3 for 24 hours which was effective in improving the germination percentage and thus high dose of KNO_3 was not effective in this case. On the other hand, germination percentage improved in *R. tetraphylla* seeds to some extent but as the dose of KNO_3 increased germination percentage decreased. High dose of KNO_3 was not effective in breaking seed dormancy of *Chenopodium album* seeds as compared to low dose[11]. 0.5% KNO_3 treatment gave better results as compared to high dose of this chemical in chickpea seeds[12]. KH_2PO_4 was not effective in either of the two species (Fig: I and II). The results of this work are in accordance with the results obtained by Paul et al.[10] in case of *R. serpentina* seeds. Seed priming with 0.5% KH_2PO_4 gave better results as compared to its high dose in chickpea seeds[12]. Corn seeds primed with

Table 2: Analysis of variance for seed germination in *R. tetraphylla*

Sources of variation	Degrees of freedom	Sum of squares	Mean square	Calculated 'F' value	Table value of 'F' at 1%
Chemical (A)	3	10410.98	3470.33	13993.27*	5.09
Temperature (B)	3	171.10	57.03	229.96*	5.09
Dose (C)	2	508.73	254.36	1025.65*	6.01
Chemical X Dose	6	947.51	157.92	636.77*	4.01
Chemical X Temperature	9	59.96	6.66	26.85*	3.37
Temperature X Dose	6	14.38	2.40	9.68*	4.81

* Highly significant (P<0.01); n.s = not significant

KH_2PO_4 resulted in advanced metabolic processes and higher germination percentage and germination rate as compared with unprimed seed[13].

The seeds of *R. serpentina* and *R. tetraphylla* achieved maximum germination percentage when these were treated with GA_3 (Fig: I and II). A maximum germination of 48.65% in *R. serpentina* and 56.66% in *R. tetraphylla* was achieved when treated with a dose of 300ppm of GA_3 respectively at 35°C and 30°C of incubation temperature. GA_3 was the most effective chemical that showed good response in increasing the germination percentage of both the species. The results of our work are similar to those of Baskin and Baskin[14] where they found positive effect of soaking of *Osmorhiza claytonia* seeds in GA_3 solution. It is reported that GA_3 is effective in breaking the non-deep physiological dormancy, but it does not overcome the deep physiological dormancy[3].

Two functions for gibberellins (GA) during seed germination have been proposed. First, GA increases the growth potential of the embryo.

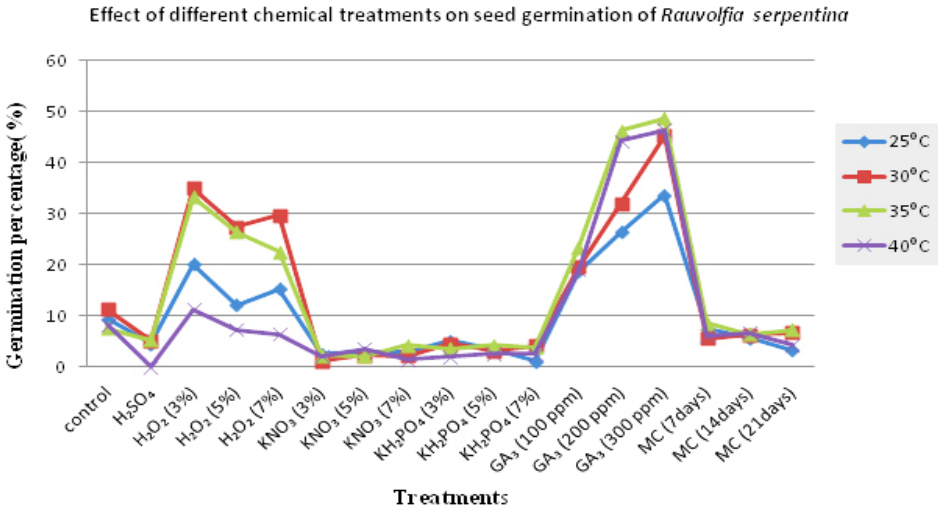

Fig. 1: Germination percentage of seeds of *Rauvolfia serpentina.*

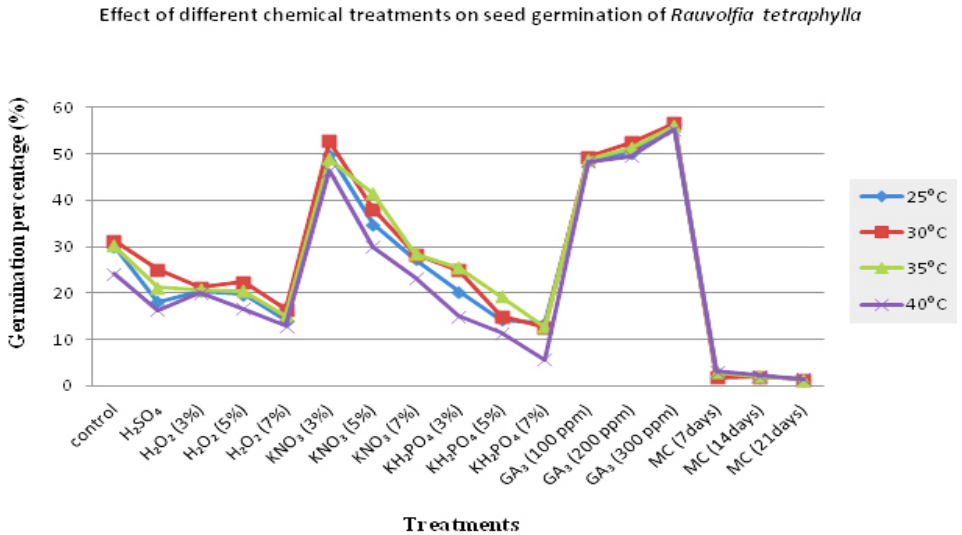

Fig. 2: Germination percentage of seeds of *Rauvolfia tetraphylla.*

Secondly, GA is necessary to overcome the mechanical restraint conferred by the seed-covering layers, by weakening of the tissues surrounding the radicle[15, 16]. Scarification of *R. serpentina* and *R. tetraphylla* seeds with concentrated sulphuric acid (conc. H_2SO_4) showed no improvement in germination (Fig: I and II). Trivedi and Kumari[17] also did not observe any germination improvement in *R. serpentina* seeds. In our experiment, acid scarified seeds reached a maximum level of germination of 5.27% in *R. serpentina* and 25.04% in *R. tetraphylla* which was low as compared to the untreated seeds i.e., respectively 11.27% and 31.26%. The failure of germination of acid scarified seeds was due to damage caused to the seeds or embryo of seeds for a longer period[18]. Cold stratification treatment for 7 days, 14 days and 21 days failed to show any improvement in the germination percentage of *R. serpentina* and *R. tetraphylla* seeds. The germination percentage decreases as compared to control and it was due to the lethal effect of cold stratification on the seeds. It has been reported by Ren and Tao[19] that cold stratification cause lethal effect on viable seeds. Favourable conditions of moisture and temperature directly affected embryo growth and ultimately germination of seeds of *Pastinaca sativa* and *Conium maculatum* which exhibited morphological dormancy[20]. The role of four different temperature regimes were also observed in addition to all other treatments and it was found that optimum seed germination temperatures were 35°C and 30°C for R. serpentina (Fig:I) and R. tetraphylla (Fig:II) respectively. Seeds of different species have different degrees of temperature requirements. Bewley and Black[21] stated that, the most optimum temperature for germination is generally the one at which a given number of seeds achieve their maximum per cent of germination, over a period of time.

From the above results of the present work it is concluded that *R. serpentina* and *R. tetraphylla* have non-deep physiological dormancy. Though GA_3 broke dormancy of seeds of these two species to a significant extent, but it could not break dormancy of all seeds. This indicated the presence of another kind of dormancy in addition to non-deep physiological dormancy. The other kind of dormancy might be the coat-induced dormancy, as the seed coats were very hard in both these species. Hence, further work is necessary to break the dormancy of all viable seeds and to determine the type of non-deep physiological dormancy.

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